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SUMMARY

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VITICULTURE AND OENOLOGY

VEGETABLE GROWING

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THE RELATIONSHIP BETWEEN THE PHYSICAL ATTRIBUTES OF CHERRIES AND THEIR QUALITY TRAITS

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Abstract

The research question seeks to examine the extent to which the physical attributes and subsequent impact on quality traits of cherries may influence the sustainable choices made by both merchants and consumers. Seven varieties were selected to that purpose and measured for the retained investigated features. Two physical characteristics, diameter and height, and two quality traits, firmness and soluble substance were paired and studied for eventual correlation. The correlation coefficients are relevant for a limited number of varieties, almost half of the analysed ones, and they indicate an acceptable to moderate correlation between the screened features. A larger diameter or height does not necessarily mean a larger amount of sugar or soluble substance, as demonstrated by the absence of correlation for the richest analysed variety. Subsequently the diameter cannot be used as a quality indication and cannot support a sustainable choice. The physical appearance of the cherries cannot represent an element that supports the sustainable choice of merchants or consumers when the quality of cherries is a relevant reference.

Key words: *sustainable choice, cherries, firmness, soluble substances, diameter, height, correlation.*

INTRODUCTION

The growth of cherries is significant in the global agricultural industry, with notable focus on the fruit's quality and physical characteristics (Shahbazi et al., 2013). Grasping the link between physical properties and quality indicators of cherries is indispensable for improving production efficacy and fulfilling consumer expectations (Romano et al., 2006; Shewfelt, 2006). Investigation in this domain is pivotal to identify principal traits influencing cherries' overall quality, including parameters such as diameter, height, and firmness.

Through the examination of these variables, scholars can obtain crucial understanding regarding factors impacting the sensory perception and market valuation of cherries (Asănica et al., 2015).

Additionally, an exhaustive analysis of these connections can result in the formulation of methodologies to enhance cherry production workflows and ascertain uniform quality benchmarks (Pal et al., 2017).

Within this framework, examining the relationship between cherry physical characteristics and quality attributes holds considerable relevance for the agricultural domain and consumer contentment (Devasirvatham et al., 2022; Dudu et al., 2015). The exploration concerning the association amid cherry physical traits and quality metrics is grounded in the necessity to elevate the comprehension of elements swaying cherry quality determinants. As evidenced in the physical properties of cherries, comprising diameter, height, and firmness, significantly influence their general quality (Asănică et al., 2011). Investigating the distinctions discerned in these attributes across diverse cherry cultivars allows investigators to reveal vital discernments into elements inducing variations in fruit quality (Faniadis et al., 2010; Romano et al., 2006). Furthermore, scrutinizing the interactions between these physical traits and quality metrics such as taste, shelf longevity, and market worth is pivotal for formulating strategies to refine cherry production and satisfy consumer preferences (Paunovic et al., 2022). This research endeavours to augment the extant knowledge reservoir by elucidating the complex associations between cherry physical traits and quality metrics, ultimately guiding industry methodologies towards improving cherry quality and consumer contentment.

Comprehending the relationship between physical attributes and quality aspects of cherries manifests a noteworthy research quandary in agricultural science. Examination of variables such as diameter, stature, and firmness, along with their influence on the aggregate quality of cherries, holds importance for refining production techniques and catering to consumer expectations (Romano et al., 2006; Ricardo-Rodrigues et al., 2023). Prior inquiries have accentuated the relevance of said physical properties in ascertaining the commercial worth and sensory characteristics of cherries. Nonetheless, a lacuna exists in scholarly literature concerning the precise correlations among these attributes and quality benchmarks. Via an in-depth analysis of statistical data and experimental outcomes, the present study aspires to tackle this research quandary holistically (Esti et al., 2002; Chockchaisawasdee et al., 2016; Devasirvatham et al., 2022). By methodically scrutinizing the facts, this investigation seeks to elucidate the convoluted interrelations between cherry physical attributes and quality dimensions, proffering indispensable insights for both industry specialists and academicians.

Investigation into the association of cherry physical attributes with quality traits possesses notable implications for agricultural and consumer domains (Muskovics et al., 2016). By clarifying the linkages involving traits such as diameter, height, and firmness with quality markers, this research augments comprehension of cherry growth and development, also yielding pertinent insights for producers and consumers. The conclusions lay groundwork for precise agricultural approaches, aiding enhanced cultivation tactics which might elevate total crop yields and quality (Mateos-Fierro, 2020). Moreover, the identified associations could steer consumer choices, shaping marketing tactics and assuring buyer contentment. Additionally, the statistical examinations performed on the dataset highlight the robustness of the observed linkages, affirming the reliability and validity of the study's outcomes. These insights are critical for advancing cherry cultivation techniques and fine-tuning product quality, thus contributing meaningfully to the broader agricultural sector (Kappel et al., 2012; Tzouramani et al., 2014). Subsequent investigations may expand upon these findings to propel forward innovation and sustainability within the cherry sector, fostering economic enhancement and addressing consumer needs effectively (Janes et al., 2010; Holusova et al., 2023; Demirsoy, 2004; Kappel et al., 1996).

Consumer Preferences. Paunović et al. (2022) and Asănică et al. (2018) conducted a survey to evaluate consumer attitudes on the most important characteristics of cherry fruit, and found that “the attitude toward fruit firmness, fruit size, and presence of a stalk and the stalk length” depended on respondents’ residence and sex, and that “the attitude toward fruit firmness, fruit size, and presence of a stalk” were influenced by the age of the respondents (Paunović et al., 2022; Asănică et al., 2018).

The perception of fruit firmness varied considerably by place of residence and gender of respondents. Urban and rural consumers may favour firmness differently, perhaps due to differences in storage conditions or cultural preferences. Additionally, men and women may have distinct preferences, potentially influenced by factors such as taste or use in cooking compared to direct consumption (Thornsbury et al., 2012).

Older and younger consumers showed different attitudes towards firmness. Older respondents might prefer firmer cherries due to better texture retention during storage, while younger consumers might focus on the immediate consumption experience.

Like firmness, preferences for cherry size were influenced by the location and gender of respondents. This could be related to factors such as the typical use of cherries in local cuisines or aesthetic preferences that vary between genders.

Age also played a role in determining the preferred size of the fruit. Younger consumers may prefer larger cherries because they are often perceived as juicier and more satisfying, while older consumers may favour other factors over size.

The presence of a strain and its duration also depend on the demographic factors of the respondents. These attributes can be important for a variety of reasons, such as the convenience of food or the aesthetic appeal of fruit (Ross et al., 2009; Bujdosó et al., 2020).

It is interesting to note that although the presence of a strain influenced consumer attitudes in different age groups, the length of the strain did not show significant variation with age. This suggests that while the presence of the stem is a universal factor considered, its length may be more subjective.

Understanding these nuanced consumer preferences can help cherry growers and retailers tailor their products to better meet the demands of their target market segments (Calvo-Porral et al., 2018; Revell, 2008).

Marketing campaigns can be designed to highlight specific attributes preferred by certain demographics. For example, promoting the firmness and size of cherries in urban areas or to younger consumers (Correia et al., 2017).

Retailers can segment their products based on these preferences, offering different varieties or styles of packaging that cater to the distinct tastes of different consumer groups.

Growers can focus on selecting and growing cherry varieties that meet the specific preferences highlighted by the study, such as ensuring optimal firmness and desired size for the most demanding consumer segments.

By aligning product offerings with consumer preferences, stakeholders in the cherry industry can increase customer satisfaction and increase sales (Correia et al., 2017).

MATERIALS AND METHODS

The research question seeks to examine the extent to which the physical attributes and subsequent impact on quality traits of cherries may influence the sustainable choices made by both merchants and consumers. The sustainable choice is defined in the present context as representing a rule or a set of rules, applied systematically for supporting a decision or a set of decisions based on existing scientific evidence.

Sweet cherry (*Prunus avium* L.) cultivars are harvested in December and January in the Southern Hemisphere, "as this harvest time coincides with lucrative markets such (North America, Southeast and East Asia, Western Europe)"; are "in high demand in the late spring and early summer" because they are first on the fresh market; dominate the market when their fruit are red, "while cultivars of blush, white, or yellow colour are in less demand"; "mature from the end of April (in southern growing regions) to June-July (main season)" in temperate zones of the Northern Hemisphere, "while the picking season finishes in late August in Norway"; ripen first among stone fruits; span a long maturity period (Bujdoso et al., 2017).

The story about the measurements of the varieties started with the study carried out on seven cherries different varieties collected from their specific area, respectively Southwestern part of Romania, County of Timis. The cherry varieties studied according to the area of origin were: 'Ulster' (Variety 1), 'Kordia' (Variety 2), 'Rosii de Bistrita' (Variety 3), 'Bigarreau' (Variety 4), 'Black Star' (Variety 5), Royal Lady' (Variety 6), 'Wista' (Variety 7).

For the seven varieties, we selected two physical characteristics and two for quality. One of the two quality characteristics is also physical, but this is the most important physical characteristic sought especially by merchants, but also by consumers. For each of the seven varieties we chose normal fruits, in number of 25 per variety, which we later analysed for each variety, the four characteristics. The fruits have been collected for examination at their optimal ripening stage, eliminating the hit, misshapen, uneven fruits, or anything else that is determined by an accident, and not by a variety characteristic.

The characterization of the cherry fruits involved the determination of their height, diameter, firmness and the soluble substance (sugar content). The measurable elements were calculated using the digital caliper (Insize-1108, Loganville, GA, USA; unit: mm), the firmness was determined using the fruit hardness tester (Lutron FR-5105, unit: g/cm^2). The soluble substance (% Brix) was determined using the Atago Pal 3870 (Tokyo, Japan) portable refractometer. All these determined characteristics can be found in Appendix 1, at the end of the present paper, and their averages are introduced in Table 1.

The physical characteristics selected for analysis are the diameter and height of the fruit, while the quality traits are the firmness, which is a physical feature still one of the most important quality traits, and the soluble substance, which is indicative of the sugar content.

Table 1. Averages of the 4 characteristics of the 7 varieties analysed

Variety	Height (mm)	Diameter (mm)	Firmness (g/cm^2)	Sol. Substance (% Brix)
Average 1	23.49	25.58	408.4	20.16
Average 2	24.42	25.01	231.96	16.26
Average 3	22.63	25.07	231.68	14.75
Average 4	20.79	23.63	448.64	14.93
Average 5	22.72	24.42	398.8	18.75
Average 6	22.84	25.91	289.6	11.28
Average 7	23.82	28.29	374.2	23.89

Source: Own measurements and determinations.

The seven varieties investigated were screened for the four features and subsequently paired for determining the correlation coefficients, as following: diameter and firmness, diameter and soluble substance, height and firmness and respectively height and soluble substance.

Regarding the analyses of the physical characteristics and quality traits of the seven varieties, based on the data provided, including height, diameter, firmness and percentage of soluble substances (%Brix), we may notice that the variation between varieties is relatively small (a maximum difference of 3.63 mm), indicating a relatively similar size overall. No variety is distinguished by a particularly different size. Most varieties are close to a height of about 23 mm.

Height, being a fundamental physical characteristic, influences the visual appearance of varieties and can play a role in consumption preferences (such as handling, processing or presentation). Larger varieties, such as Variety 2 and Variety 7, may be perceived as more imposing or visually appealing in a market context.

In terms of diameter, there is a greater variation in diameter in relation to height (a maximum difference of 4.66 mm). Variety 7, with a much larger diameter, could be more imposing, while Variety 4, with the smaller diameter, is probably more compact.

Diameter is a key factor for the overall appearance and weight of varieties. A wider variety, such as Variety 7, could be perceived as having better value for the consumer, or more suitable for certain types of processing (such as wider cuts or slices). On the other hand, Variety 4, with its smaller diameter, could be suitable for uses requiring smaller or more concentrated products.

For firmness, we concluded that it varies widely, with some varieties (such as Variety 4 and Variety 1) exhibiting much higher firmness than others (Variety 3 and Variety 2). This suggests a marked difference in the texture or consistency of the varieties, which could be related to the maturity, density, or even freshness of the varieties measured.

Firmness is an important parameter for industrial processing and consumption. Varieties with higher firmness (such as Variety 4 and Variety 1) may be preferred for preservation or processing (e.g., slicing or cutting). On the other hand, softer varieties (Variety 3 and Variety 2) might be more suitable for immediate consumption or products that require a softer texture.

Concerning the soluble Substances, there is a very large variation in the percentages of soluble solids. Variety 7, with a content of 23.89% Brix, is by far the sweetest, while Variety 6, with 11.28% Brix, is the least sweet. These measurements are often used to assess the sugar content, which is crucial for the taste perception of fruit.

Varieties with high soluble contents, such as Variety 7 and Variety 1, are probably more popular for direct consumption due to their sweetness. Variety 6, with its much lower Brix content, may be less appealing to those seeking a sweet taste experience, but could have other industrial uses, such as for juices or processed products where the sugar can be adjusted.

Below we enclosed a comparison between the lowest and highest averages for each parameter.

The difference between the smallest and largest heights is 3.63 cm, which is a moderate variation. Variety 4 is about 15% smaller than Variety 2, the largest. This indicates that while the difference is noticeable, it is not extreme, suggesting that the varieties remain relatively comparable in terms of height.

In terms of diameter, the difference is 4.66 cm, or about 20%. Variety 7, with a much larger diameter, could be perceived as bulkier or more robust compared to Variety 4, the smallest in diameter.

Regarding firmness, there is a very significant difference here, with Variety 4 being almost twice as firm as Variety 3 (a difference of 216.96). This variation could reflect drastic differences in the texture of the varieties, with the firmer variety being more robust or dense, while the softer variety might be easier to bite or cut.

For soluble substances, the difference is very marked, with Variety 7 having a soluble substance content more than twice that of Variety 6 (12.61 difference). This suggests that Variety 7 is much sweeter or has a much higher dry matter content, which could make it more popular for consumption as a fresh fruit. Conversely, Variety 6 could be less sweet.

The most noticeable differences appear in terms of firmness and soluble substance content. Variety 4 and 7 are distinguished by extreme characteristics: Variety 4 is very firm and small, while Variety 7 is larger, with a larger diameter and a very high soluble substance content. Variety 6, on the other hand, is the opposite in terms of soluble substance content, with a much lower level.

We may conclude that this data shows that each variety has unique characteristics that influence their optimal use, whether for direct consumption, industrial processing or other specific applications. Varieties with larger heights and diameters, with high levels of soluble substances, will probably be preferred for fresh consumer markets. While firmer or less sweet varieties can have specific uses depending on their other physical qualities and texture.

The selection of tests identified the Kendall Tau b test as the most suitable approach for investigating the correlation coefficients between the paired physical characteristics and quality traits of the seven cherry varieties under consideration. The option pertains to the relatively limited number of fruits from each variety that were subjected to the selected characteristics, thereby enabling the extension of the number of varieties encompassed in the analysis. The pairs of features as introduced above were tested for the correlation coefficients using the selected test Kendall Tau b employing the SAS for Academics software platform and the results are presented and interpreted in the section bellow.

Figure 1. Cherry analysed varieties (from top left to bottom right, reading left to right: Var. 1, Var. 7, Var. 2, Var. 6, Var. 3, Var. 4, Var. 5)
(Source: Own determination and framing)

RESULTS AND DISCUSSIONS

The results of the first enquired pair of features respectively the potential link between the diameter and the firmness returns systematically p values above 0.05 indicating that null hypothesis cannot be rejected and therefore the correlation coefficients are irrelevant in the case of the analysed pair. The results are introduced in the Table 2 displaying the (absence of) correlation results for diameter and firmness using the Kendall Tau b correlation test.

Table 2. Correlation results for diameter and firmness using the Kendall Tau b correlation test

Firmness / Diameter	Value	Variety
Correlation coef.	0.14141	1
p value	0.3258	1
Correlation coef.	-0.01675	2
p value	0.9070	2
Correlation coef.	0.10385	3
p value	0.4687	3
Correlation coef.	-0.0802	4
p value	0.5749	4
Correlation coef.	-0.02007	5
p value	0.8885	5
Correlation coef.	-0.10385	6
p value	0.4687	6
Correlation coef.	0.06020	7
p value	0.6740	7

Source: computation of own determined data using SAS for Academics

Although a strictly physical approach could suspect that a larger diameter implies and lower firmness or the other way around, the seven screened varieties, the links between the two features has no statistical relevance and the correlation cannot be established.

Similar results are recorded testing for correlation the next paired features, firmness and height, where the *p* value for all the varieties systematically indicates that the null hypothesis cannot be rejected.

The results for all the tested varieties are introduced in Table 3 below where the correlation results, irrelevant for the purpose of the examination and the *p* values are presented.

Table 3. Correlation results for height and firmness using the Kendall Tau b correlation test

Height / Firmness	Value	Variety
Correlation coef.	0.13423	1
p value	0.3496	1
Correlation coef.	-0.06365	2
p value	0.6570	2
Correlation coef.	0.12395	3
p value	0.3871	3
Correlation coef.	-0.09015	4
p value	0.5282	4
Correlation coef.	0.01669	5
p value	0.9070	5
Correlation coef.	-0.22074	6
p value	0.1230	6
Correlation coef.	0.09683	7
p value	0.4981	7

Source: computation of own determined data using SAS for Academics

The results indicate that no correlation can be established between the height and the firmness of the fruits although, just like in the case of the diameter, the logic could suspect a link between the two features.

The situation regarding the results presents itself different when analysing the diameter and the soluble substance as screening for the sugar content. The results of the correlation test introduced in Table 4 indicate that for the Varieties 1, 2 and 5 we can reject the null hypothesis while for the remaining varieties the null hypothesis cannot be rejected. This reads as relevant correlation coefficients for the above-mentioned varieties while in the case of the others no link can be established between diameter and sugar content. Reading the correlation coefficients translates into an acceptable correlation since the values of the three varieties are all placed in the range 0.25-0.50, respectively 0.44 for Variety 1, 0.46 for Variety 2 and 0,56 for Variety 5. Although the last variety, the number 5, has a correlation coefficient higher than 0.50 could qualify for a moderate correlation, the determined value is too close to the boundary to interpret it as belonging to the upper category and relatively close to the other values to segregate them. The Varieties 3, 4, 6 and 7, are outside the correlation discussion given the high *p* values returned when compared in pairs.

Table 4. Crrelation results for diameter and soluble substance using the Kendall Tau b correlation test

Diameter / Sol. subst.	Value	Variety
Correlation coef.	0.44857	1
p value	**0.0019**	**1**
Correlation coef.	0.46723	2
p value	**0.0012**	**2**
Correlation coef.	0.23490	3
p value	0.1017	3
Correlation coef.	0.17696	4
p value	0.2157	4
Correlation coef.	0.56326	5
p value	**<.0001**	**5**
Correlation coef.	0.24284	6
p value	0.0920	6
Correlation coef.	0.13468	7
p value	0.3494	7

Source: computation of own determined data using SAS for Academics

Pairing and testing the height and the soluble substance for the correlation coefficients returns the results displayed in Table 5 for the seven selected varieties.

Table 5. Correlation results for height and soluble substance using the Kendall Tau b correlation test

Height / Sol. subst.	Value	Variety
Correlation coef.	0.54792	1
p value	**0.0001**	**1**
Correlation coef.	0.33278	2
p value	**0.0206**	**2**
Correlation coef.	0.24162	3
p value	0.0923	3
Correlation coef.	0.34667	4
p value	**0.0151**	**4**
Correlation coef.	0.57915	5
p value	**<.0001**	**5**
Correlation coef.	0.15489	6
p value	0.2819	6
Correlation coef.	0.05714	7
p value	0.6909	7

Source: computation of own determined data using SAS for Academics

Reading the correlation coefficients translates into an acceptable correlation since the values of the three varieties are all placed in the range 0.25-0.50, respectively 0.44 for Variety 1, 0.46 for Variety 2 and 0.56 for Variety 5.

Although the last variety, the number 5, has a correlation coefficient higher than 0.50 could qualify for a moderate correlation, the determined value is too close to the boundary to interpret it as belonging to the upper category and relatively close to the other values to segregate them. The Varieties 3, 4, 6 and 7, are outside the correlation discussion given the high *p* values returned when compared in pairs.

Pairing and testing the height and the soluble substance for the correlation coefficients returns the results displayed in Table 5 for the seven selected varieties. Like the examination for diameter and soluble substance, the Varieties 1, 2 and 5 return p values under 0.05 allowing the rejection of the null hypothesis and weighting the correlation coefficients. To these three variables a fourth one, the Variety 5, is added indicating that four out of the seven analysed varieties can display links between the analyse features for correlation. The strength of the correlation splits between acceptable correlation for the Varieties 2 and 4, and moderate correlation for the Varieties 1 and 5. The correlation coefficients for the moderate correlation are near the lower boundary and can be rather read as acceptable-moderate correlation.

The highest physical measurements are systematically recorded for the Variety 7, however the physical characteristics are not linked to the quality traits and no correlation can be established between them.

The analysis concerns the correlation links between the features and not the causality relations. In the case of the Variety number 6 analysed, the records for the soluble substance are the poorest among all varieties in absolute values and the firmness is the third lowest. With the second average largest diameter the Variety 6 is a good proof of lack of linkage between physical and quality traits. Varieties 1, 2, and 4, most likely as results of the breeding enhancements demonstrate an acceptable to moderate correlation for the soluble substance, respectively sugar content, with Variety 5 adding a moderate correlation for height yet not for diameter. The Variety 3 although acceptable for physical appearance for both diameter and height has low values recorded for firmness and soluble substance.

CONCLUSIONS

A correlation can be established between the physical characteristics of the fruits and their quality traits for selected varieties. Although the correlation coefficients do not indicate a strong correlation yet rather a moderate or acceptable one there is evidence indicating that certain physical features and quality traits are linked. The research question aimed to identify and measures these links, if existing, and make use of them in supporting the choice of both merchants and customers. Since a larger diameter or height does not necessarily means a larger amount of sugar or soluble substance, as the absence of correlation for richest analysed Variety (number 7) demonstrates, diameter cannot be used as a quality indication and cannot support the choice of either merchants or final consumers. The firmness, a quality trait of high importance for the merchants with direct impact on the shelves time and for the consumers as they can verify it and usually opt for a higher firmness as sign of a longer durability, is acceptable correlated for three of the analysed varieties. Similar to the diameter, the physical expression of the height is equally not a visual indication of any quality traits *per se*. Varieties with good physical records still uncertain and variating quality traits might still pass as a good choice for consumers. Perception could win as result of the visual impact of large fruits still there is no proof of any stable quality traits linked to the respective appealing fruits. The physical appearance of the cherries cannot represent an element that supports the sustainable choice of merchants or consumers when the quality of the cherries is a priority.

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Appendix 1. Dataset of physical characteristics and quality traits measurements of the seven selected varieties

Variety	Height (mm)	Diameter (mm)	Firmness (g/cm²)	Sol. Substance (% Brix)
Average 1	**23.49**	**25.58**	**408.40**	**20.16**
Average 2	**24.42**	**25.01**	**231.96**	**16.26**
Average 3	**22.63**	**25.07**	**231.68**	**14.75**
Average 4	**20.79**	**23.63**	**448.64**	**14.93**
Average 5	**22.72**	**24.42**	**398.80**	**18.75**
Average 6	**22.84**	**25.91**	**289.60**	**11.28**
Average 7	**23.82**	**28.29**	**374.20**	**23.89**

Source: own measurements.

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THE IMPACT OF THE CULTIVATION SYSTEM ON THE YIELD AND QUALITY OF SWEET CHERRIES

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Abstract

The goal of this study, which was conducted between 2010 and 2022, was to evaluate the impact of the variety-rootstock association, the crown shape and the planting distance on the production and quality parameters of sweet cherries. Stationary experiments have been carried out using diverse sweet cherry varieties, such as Valerii Cikalov, Record, Ferrovia, Kordia, Regina, Stella, Skeena, Bigarreau Burlat, Lapins, grafted on Cerasus mahaleb, L. and Gisela-6 rootstocks, in various combinations, and which have been planted at diverse planting distances. The trees grafted on the Cerasus mahaleb, L., rootstock have shown rapid growth and an average fruit yield of 15.58-16.12 t/ha during the period of their full productivity. The harvest of the trees grafted on the Gisela 6 and MaxMa 14 rootstocks has been early and amounted to 12.50-14.58 t/ha. The varieties Bigarro Burlat, Ferrovia and Lapins, planted at a distance of 5 x 1.5 m, have given a yield of 17.98-20.07 t/ha, which is 12.2-24.9% higher than that of the trees which have been planted at a distance of 4 x 2.5 m.

Key words: *sweet cherry variety, cultivation system, rootstock, density, crown shape.*

INTRODUCTION

The assortment is the main factor in solving the requirements and difficulties of modern fruit growing at the disposal of the producer and consumer. The susceptibility to disease and pest attack of the existing varieties has negative effects on the environment and human health. Other major challenges facing fruit growers are the technology used and climate change.

The sweet cherry tree cultivation has developed a lot thanks to the assortment of vegetative rootstocks and high-quality self-fertile varieties. Due to the multiple variety-rootstock combinations, the numerous planting distances, as well as the ways of grouping the trees as a result of the diversity of the existing biological material, numerous researches regarding the tree crown shaping systems have been conducted (Long et al, 2014; Balan et al., 2022; 2023).

The research has shown that it is possible to grow orchards of high density, which, under favourable conditions of intensified technological processes, can produce the desired yields from a biological and technical point of view (Gjamovski et al., 2016; Long, 2003; Long et al., 2005; Sumedrea et al., 2014).

The range of moderate vigour and semi dwarfing vegetative rootstocks made it possible to plant sweet cherry orchards of high density, with trees with spindle-shaped crowns, as well as orchards with high yields per hectare and low production costs (Aglar et Yildiz, 2014; Aglar et al., 2016; Gyeviki et al., 2008; Long, 2003; Usenik et al., 2010; Vercammen, 2002; Balan et al., 2022).

The various vigour of the variety-rootstock combinations allows a good management of the orchard structure (Balan, 2009; 2015; Cimpoies, 2018; Mitre, 2020) and has a significant role in the vegetative growth, tree yield and fruit quality (Aglar et al., 2019; Aglar et al., 2016; Bujdosó et al., 2012). Thus, the variety-rootstock combination directly determines the cultivation system, the way of crown shaping and tree pruning, the orchard maintenance and work system (Calabro et al., 2009; Long et al., 2014; Pesteanu, 2022; Balan et al., 2023).

The issue related to the cultivation systems is quite controversial in specialized literature and in fruit growing practice. Currently, in the Republic of Moldova, the wild sweet cherry generative rootstocks (*Cerasus avium* L.), Mahaleb (*Cerasus mahaleb* L.) are still used in sweet cherry tree growing; the semi dwarfing vegetative rootstocks (Gisela 5), semi vigorous rootstocks (Gisela 6, PHL-C, Krymsk 6) and moderate vigour rootstocks (Maxima 14, Krymsk 5, Piku 1, Piku 4) are used less (Long et al., 2014; Balan et al., 2021; 2023).

That is why, the impact of the Gisela 6 and Mahaleb rootstocks on the growth and development of the Valerii Cikalov, Record, Ferrovia, Kordia, Regina, Stella, Skeena, Bigarreau Burlat and Lapins sweet cherry varieties, in different combinations and at different planting distances, has been studied. The aim of the research is to increase the productivity of sweet cherry orchards by identifying the highly productive variety-rootstock associations, which correspond to the climatic conditions, the biological production potential of the orchard, the technological procedures used to shape the tree crowns, the degree of mechanization, the amount of manual work and the management of pursued economic interests.

MATERIALS AND METHODS

Four stationary experiments have been performed to carry the studies. The Valerii Cikalov, Record, Ferrovia, Kordia, Regina, Stella, Skeena, Bigarreau Burlat and Lapins sweet cherry varieties, grafted on *Cerasus mahaleb* L. and Gisela-6 rootstocks in different combinations and at various planting distances, have been used. Four groups of eight representative trees each have been created to conduct the experiments. The scheme has been developed according to the polyfactorial principle with the placement of the groups through the randomized block system on two rows in the middle of the strip for each variety (Moiseicenco et al., 1994; Balan et al., 2001).

Experiment 1 was carried in the spring of 2010. The orchard was planted with Gisela 6 vegetative rootstocks, which had been bought in Greece, grafted with two buds of the Adriana, Ferrovia and Skeena sweet cherry varieties. The rows of trees were planted in a north-south direction at a distance of 4 x 2 m. The varieties were grouped in strips of 10 rows of each variety. Three shapes of crown were used: G1 – Small naturally improved crowns (the control group); G2 – Improved slender spindle crowns; G3 – Improved oblate vessel.

Experiment 2 was performed in the southern orchard area in the spring of 2010. The orchards were planted with Gisela 6 sweet cherry rootstocks, bought in Greece, which were grafted with two buds of the Bigarreau Burlat, Ferrovia and Lapins sweet cherry varieties. The rows of trees were planted in a north-south direction at a distance of 5 m between the rows and at a distance of 1.5 m, 2 m and 2.5 m on the row. The tree crowns were small: G1 – Small naturally improved crowns (the control group); G2 – Improved slender spindle crowns.

The interaction between the planting distance and the crown shape, as basic factors that determine the fruiting, the fruit yield and quality was studied.

Experiment 3 was conducted in the orchard which was planted in the autumn of 2003 in the village of Malaiesti. The Valerii Cikalov and Record sweet cherry tree varieties with big naturally improved crowns, grafted on the Mahaleb generative rootstocks and planted at a distance of 6 x 5 m were used.

Experiment 4 was conducted in 2011. The orchard was planted with sweet cherry trees of the Ferrovia, Kordia and Regina varieties which were grafted on Gisela 6 rootstock and planted at a distance of 4 x 2.5 m. Small crowns were studied: G1 – Small naturally improved crowns (the control group); G2 – Improved slender spindle crowns; G3 – Improved oblate vessel.

The interaction between the planting distance and the crown shape, as the basic factors that determine the fruiting, the fruit yield and quality was studied. Morphological descriptions, biometric measurements and statistical processing of the results were carried out. The methods of analysis, synthesis, tabulation, comparison and graphics were used to interpret the scientific results. The processed data are presented in average values for the 3-8 years of research. The difference between variants were compared with 5% probability (Dospehov, 1985).

Orchard management. In experiment 1, the soil was weeded naturally; in experiments 4 and 5, the soil in the orchards was weeded artificially. The strips of land 1.5-2 m wide were kept weed-free by weeding or superficial thinning to a depth of 5 cm with the feeler tiller. In experiment 2 and 3 the soil was maintained as a cultivated field. The trees in experiments 1, 4, 5 were drip irrigated, and the Watermark transducers installed at 20, 40 and 60 cm deep in each plot were used to monitor the soil moisture. The water was distributed by droppers fixed at a height of 40 cm from the ground in the direction of the row. Weather stations were installed in the orchards to determine the state of the environment and the plants.

RESULTS AND DISCUSSIONS

The sweet cherry trees, grafted on the Mahaleb rootstock and planted at a distance of 6 x 5 m, reached the optimal height and the diameter of the crown at the age of 11-14 (Table 1). Higher values of the tree height were recorded in the Valerii Cikalov variety (4.2 m), and of the crown diameter – in the Record variety (5.1 m). The varieties studied behaved similarly from the statistical point of view.

Table 1. The height and crown diameter of the sweet cherry trees, m (Mahaleb rootstock, the planting distance – 6 x 5 m)

Variety	The age of the trees			
	11 years old	12 years old	13 years old	14 years old
The height of the crown				
Valerii Cikalov	4.2	3.9	4.0	4.2
Record	3.9	3.7	3.5	4.0
LSD 5%	-	-	0.62	0.27
The diameter of the crown				
Valerii Cikalov	3.7	4.7	4.3	3.9
Record	4.3	4.7	5.1	4.2
LSD 5%	-	-	1.12	0.34

During the period of heavy bearing, the Valerii Cikalov and Record varieties, grafted on the Mahaleb rootstock, recorded a yield of 6.69 t/ha in 2012, and 28.2 t/ha in 2017 (Figure 1). The yield varied significantly from year to year and was not always statistically proved for each variety. Thus, in 2012, 2013, 2017 and 2018, the yield of the varieties was identical. In 2014, 2015, 2016 and 2017, a statistically proved yield of the varieties was recorded. During the eight experimental years, similar average yields of 15.71 t/ha were recorded for the Valerii Cikalov variety and 16.13 t/ha for the Record variety.

In the research years 2012-2014, the crown width of the Bigarreau Burlat variety varied in the control group from 120 cm to 262 cm (Table 2). For the trees with improved slender spindle-shaped crowns, the increase in the crown width was greater with the increase in the distance between the trees in a row. Thus, in the 2014 research year, the width of the crown of the trees, which had been planted at a distance of 5 x 1.5 m, was 245 cm, and of those which had been planted at a distance of 5 x 2.5 m – 260 cm.

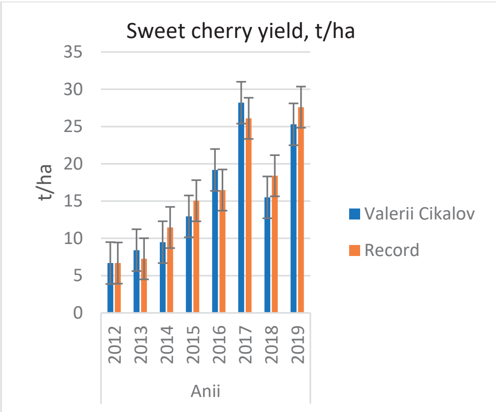


Figure 1. The yield of the sweet cherry trees, t/ha (Mahaleb rootstock, the planting distance – 6 x 5 m, the tree age – 10-17 years old)

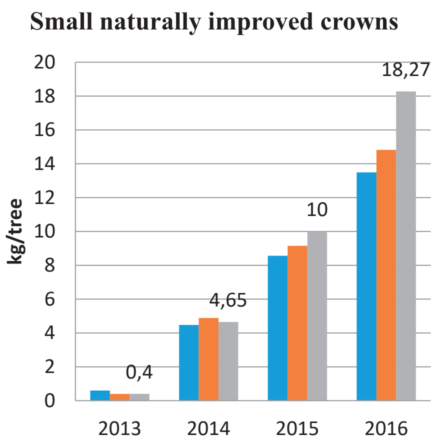
In the Ferrovia variety in the control group, the lowest indices were obtained in the first research years (2012-2013), and in the trees with improved slender spindle-shaped crowns, which were planted at a distance of 5 x 1.5 m, higher crown width values were recorded in 2014 (261 cm). In the Lapins variety in the control group, the crowns of which were small and naturally improved, the highest indices were recorded during the years 2013-2014 when the trees had been planted at the distance of 5 x 2 m, namely 172 cm and 262 cm, respectively. In the trees with improved slender spindle-shaped crowns, planted at a distance of 5 x 2 m, the highest indices were recorded in 2013-2014.

Table 2. The crown width of the sweet cherry trees depending on the planting distance and crown shape, cm (Gisela 6 rootstock, the tree age – 3-5 years old)

The shape of the crown	Planting distance, m	Bigarreau Burlat variety			Ferrovia variety			Lapins variety		
		2012	2013	2014	2012	2013	2014	2012	2013	2014
Small naturally improved crowns (control group)	5 x 1.5	120	147	258	132	150	254	130	165	254
	5 x 2	120	159	250	110	140	262	120	172	262
	5 x 2.5	120	150	249	135	180	248	130	152	254
Improved slender spindle crowns	5 x 1.5	116	140	245	128	162	261	140	169	260
	5 x 2	125	158	262	140	160	250	140	180	271
	5 x 2.5	110	165	260	128	175	250	140	180	260
LSD 5%	-	-	38.4	12.9	-	38.4	12.9	-	38.4	12.9

During the first 4 years, the yield of the Bigarreau Burlat, Ferrovia and Lapins varieties, grafted on Gisela 6, increased progressively from year to year. For example, in the Bigarreau Burlat variety, planted at a distance of 5 x 1.5 m, the fruit yield was 0.6; 4.47; 8.56 and 13.49 kg/tree, respectively (Figure 2). During the first two fruiting years, the yield did not depend on the distance between the trees in a row. Starting with the third year, the fruit yield per tree increased when the distance between the trees in a row was larger. Thus, in 2015, in the Bigarreau Burlat variety, which had been planted at the distance of 5 x 1.5 m, the fruit harvest was 8.56-6.93 kg/tree, and the trees, which had been planted at a distance of 5 x 2.5 m, yielded a better harvest which amounted to 10.0-12.75 kg/tree. In the seventh year of fruiting, the difference in yield between the groups was significantly greater than in previous years. Thus, in the trees of Bigarreau Burlat variety, which were planted at a distance of 5 x 1.5 m, the fruit yield was 13.49-14.21 kg/tree, while the trees, which were planted at the distance of 5 x 2.5 m, yielded a better harvest which amounted to 18.27-19.54 kg/tree i.e. it was better by 35.4-37.5% and was statistically proved. The Ferrovia and Lapins varieties yielded the same harvest; the harvest of the trees which were planted at smaller distances were worse and statistically proved.

In the first four years of fruiting, the positive impact of low densities on the yield per tree was evident; the increases were 7.8-34.3% in the Bigarreau Burlat variety, 19.2-41.1% in the Ferrovia variety and 14.4-35.9% in the Lapins variety. In the southern area, the orchards of 800 trees her hectare, in which the trees were planted at a distance of 5 x 2.5 m, produced a 34.3-35.9% better yield compared to the orchards in which the trees were planted at a distance of 5 x 1.5 m.



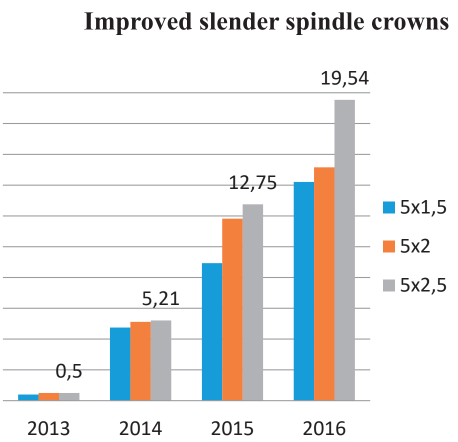


Figure 2. The yield of the sweet cherry trees of the Bigarreau Burlat variety depending on the planting distance and the crown shape, kg/tree (Gisela 6 rootstock, the age of the trees – 4-7 years old, *Terra-Vitis* Ltd)

It is worth mentioning that, during the period of tree growth and fruiting, the average fruit yield of the Bigarreau Burlat sweet cherry variety, related to the surface unit, varied from 6.66 to 9.51 t/ha, being higher by 13.3-24.9%, when the trees were planted at a distance of 5 x 1.5 m (Figure 3). The same trend was recorded with the Ferrovia and Lapins varieties – in high density orchards, the fruit yield was higher. In the Ferrovia variety planted at a distance of 5 x 1.5 m, the fruit harvest was better by 15.1-24.5%, and in the Lapins variety – by 12.2-18.9% compared to the varieties planted at a distance of 4 x 2.5 m.

In the fourth fruiting year, the Bigarreau Burlat, Ferrovia and Lapins varieties with improved slender spindle-shaped crowns, which were planted at a distance of 5 x 1.5 m, recorded productions of 17.98-20.07 t/ha. The studied varieties proved to be less valuable when they were planted at the distance of 5 x 2.5 m (14.61-17.54 t/ha) and in the trees with naturally improved crowns (14.61-16.15 t/ha), while the 5 x 2 m planting distance can be considered as medium valuable (15.16-17.24 t/ha) in terms of fruit production.

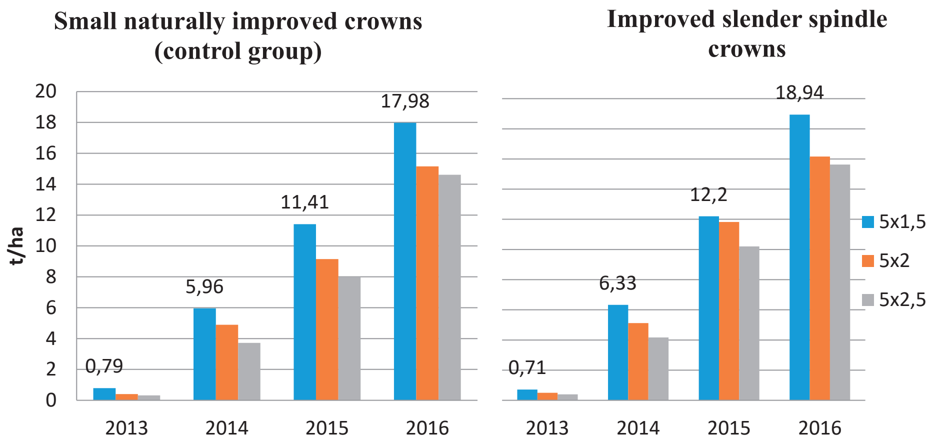


Figure 3. The yield of the Bigarreau Burlat sweet cherry variety depending on the planting distance and the crown shape, t/ha (the Gisela 6 rootstock, the tree age – 4-7 years old, *Terra-Vitis* Ltd)

The trees of the Ferrovia, Kordia and Regina varieties, grafted on Gisela 6 rootstock and planted at a distance of 4 x 2.5 m, began to bear fruit in the fourth year of vegetation, yielding 400-500 kg/ha. In the second fruiting year, a harvest of 4,600-5,000 kg/ha was recorded, which was not statistically proved (Figure 4). Starting with the third fruiting year, the yield increased significantly, being higher and statistically proved in the Ferrovia (12310-13290 kg/ha) and Kordia (11270-12830 kg/ha) varieties. In 2017 and 2020, the harvest decreased significantly due to unfavourable climatic conditions during the blossoming season – it was foggy, rainy and cold. Yields higher than 10000 kg/ha were recorded only in two out of seven years of tree fruiting, namely in 2016 and 2019. It should be mentioned that the crown shaping system did have a significant impact on the productivity of the studied varieties. The average yield during the first seven fruiting years recorded average indices for such orchards and was 8193-8308 kg/ha for the Ferrovia variety, 7650-8314 kg/ha for the Kordia variety and 7208-7877 kg/ha for the Regina variety.

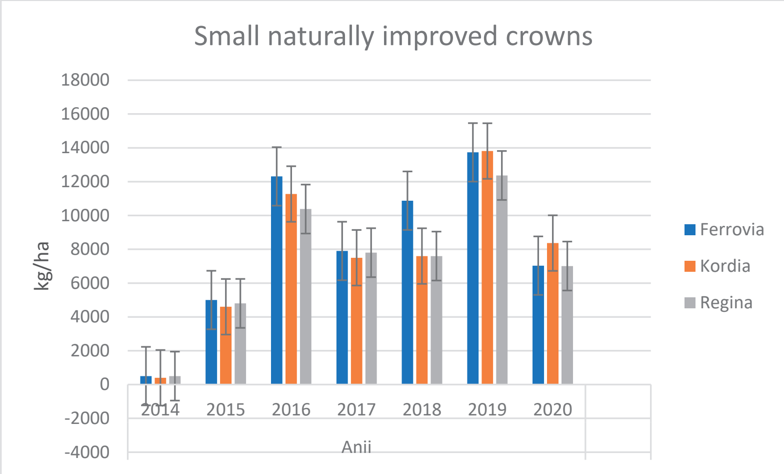


Figure 4. The yield of the sweet cherry trees, kg/ha (the Gisela 6 rootstock, the planting distance – 4 x 2.5 m, small naturally improved crowns, the age of trees – 4-10 years old)

The data regarding the crown parameters in the Adriana, Ferrovia and Skeena sweet cherry varieties, grafted on Gisela 6 and planted at a distance of 4 x 2 m, demonstrate that, during the growth and fruiting periods, the highest indices of the crown length were recorded in the Ferrovia variety (172 cm) in the trees with improved slender spindle-shaped crowns (Table 3). In the fifth year, the trees in the rows had intertwined, and the crown length was 195-220 cm. The width of the crowns also increased once the trees became older – from 110-190 cm in the third year to 195-245 cm in the fifth year of vegetation. Over the years, the Ferrovia variety proved to be more vigorous compared to the Adriana and Skeena varieties, but the growth indices were not distinctly significant.

Analysing the growth indices of the crowns of the sweet cherry trees, grafted on the Gisela 6 rootstock and planted at a distance of 4 x 2 m, it would be reasonable to say that they reached the optimal level once the trees entered the fruiting and growth periods.

In the fifth year of vegetation, the total length of the branches, including the annual branches, in the Adriana, Skeena and Ferrovia varieties was 50.7-56.2 m/tree, which was more than 80% (Table 4). In the first two years of vegetation, the number of annual branches increased moderately.

Table 3. The crown length and width of the sweet cherry trees depending on the variety and crown shape, cm (the Gisela 6 rootstock, the planting distance – 4 x 2 m, the tree age – 3-5 years old)

Variety	Crown length			Crown width		
	2014	2015	2016	2014	2015	2016
Small naturally improved crown						
Adriana	132	168	201	110	157	225
Ferrovia	150	173	215	160	173	224
Skeena	120	146	195	124	146	198
Improved slender spindle crown						
Adriana	120	168	195	124	157	214
Ferrovia	172	222	220	190	222	245
Skeena	135	165	200	142	164	195
LSD 5%	-	27	32	-	52	63

In the following two years it increased progressively geometrically; in the fifth year of vegetation, it was 64.3-72.3 pcs per tree. The average length of the annual branches is about 65 cm, which is a basic index that demonstrates the level of tree maintenance in the crown formation process. It is well known that the most suitable length of the annual branches, in order to accelerate the tree formation and fruiting, is 80-90 cm (Balan et al., 2001; Gradinariu, Istrate, 2009; Bucarciuc, 2022).

Table 4. The morphology of sweet cherry trees (the Gisela 6 rootstock, the planting distance – 4 x 2 m, the tree age – 5 years old, naturally improved crown)

Age of branch, years	The total length of the branches, m/tree			Number of branches, pcs/tree			The average length of the branches, cm		
	Adriana	Ferrovia	Skeena	Adriana	Ferrovia	Skeena	Adriana	Ferrovia	Skeena
1	43.3	42.2	46.3	64.3	65.3	72.3	67.2	64.1	64.1
2	5.8	5.9	5.8	10.3	12.0	10.3	57.4	49.0	56.8
3	2.4	2.5	3.0	5.6	6.0	6.0	41.2	42.4	50.9
4	1.2	1.2	1.1	3.3	3.3	6.0	36.9	36.9	31.3
Total	52.8	50.7	56.2	83.5	86.6	94.6	-	-	-

The Adriana, Ferrovia and Skeena sweet cherry varieties, grafted on Gisela 6 rootstock, began to bear fruit in the fourth year after planting – they yielded a harvest of 625-1562 kg/ha (Table 5). The Ferrovia variety proved to be more productive compared to the Adriana and Skeena varieties – it yielded a harvest of 1125-1562 kg/ha. In the second fruiting year, the mentioned varieties yielded a considerably better harvest which amounted to 4250-5000 kg/ha. As the trees grew, the fruit harvest tripled; in 2016, the Adriana variety yielded 11875-13000 kg/ha, the Ferrovia variety yielded 13250-14125 kg/ha and the Skeena variety yielded 16000 kg/ha. In the seventh year after planting, the sweet cherry yield doubled compared to the previous year and amounted to 21875-22500 kg/tree for the Adriana variety, and to 26250-28000 kg/ha for the Skeena variety.

The Ferrovia and Skeena varieties with both types of crown shapes gave better yields compared to the Adrian variety. In the following year (2017), the fruit yield decreased remarkably and was only 10750-10875 kg/ha in the Adriana variety, 12700-15750 kg/ha in the Ferrovia variety and 14000-16785 kg/ha in the Skeena variety. In the following years, the fruit yield remained at the level of 9316-18986 kg/ha, being higher in comparison with the Ferrovia and Skeena varieties.

Analysing the fruit yield indicators in the eighth to tenth years of tree growing season (2017-2020), and comparing them with the data presented by other authors (Long et al., 2014; Miter et al., 2012), it can be said that they are average for the sweet cherry orchards with trees grafted on the Gisela 6 moderate vigour rootstock.

As regards the impact of the variety and the crown shaping system, the Skeena variety with improved slender spindle-shaped crowns yielded the best harvest (14581 kg/ha), followed by the Ferrovia variety (12931 kg/ha). The lowest average yield was recorded for the Adriana variety (10659 kg/ha) in the group in which the trees had small naturally improved crowns.

Table 5. The yield of sweet cherry trees, kg/ha (the Gisela 6 rootstock, the planting distance – 4 x 2 m, the tree age – 4-12 years old)

Variety	Years								The average index (2013-2020)
	2013	2014	2015	2016	2017	2018	2019	2020	
Small naturally improved crowns									
Adriana	625	4375	11875	21875	10875	12958	13375	9319	10659
Ferrovia	1125	4875	13250	24750	15750	15222	10791	14277	12505
Skeena	625	4250	16000	26250	16875	17583	17042	16652	14409
Improved slender spindle crowns									
Adriana	875	4500	13000	22500	10750	14820	13125	10819	11298
Ferrovia	1562	5000	14125	24500	12700	15388	13541	16638	12931
Skeena	375	4375	16000	28000	14000	17500	17416	18986	14581
LSD 5%	-	435.2	971.8	1315.2	1429.1	1423.6	2305.7	1314.8	-

The total number of buds, including the flowering ones on annual branches, in sweet cherry trees depends on their length (Table 6). In the Ferrovia variety, as the length of the annual branch increased, the diameter of the branches increased from 4.7 to 11.7 mm, the total number of buds increased from 16.7 to 35.4 pcs, and the number of flower buds decreased from 9.0 to 3.8 pcs. The Skeena variety, being a self-fertile variety, constantly and moderately produces fruit on annual branches; flower buds on annual branches practically develop in the lower third of the branches, namely up to 1.9 pcs on long branches (20-100 cm) and up to 7 pcs on the short ones (20-40 cm).

Table 6. The distribution of buds on sweet cherry trees depending on variety and the length of annual branches (the Gisela 6 rootstock, the planting distance – 4 x 2 m, naturally improved crowns, the tree age – 6 years old)

The length of the annual branches	Diameter of annual branches, mm		Number of buds, pcs		Number of fruiting buds, pcs	
	Ferrovia	Skeena	Ferrovia	Skeena	Ferrovia	Skeena
20-40	4.7	4	16.7	14	9.0	7
20-60	6.7	8	19.7	18	6.4	5
20-80	8.2	8.2	27	23,8	5.2	5.4
20-100	11.7	10.8	35.4	33.4	3.8	1.9

When the trees are 5 years old, most of the short fruiting branches 2-5 cm long with a vegetative apical bud were found on 2-year-old branches (Table 7). Thus, in the Adriana variety, 139.1 short fruiting branches developed on 2-year-old branches, 46.3 pcs – on 3-year-old branches and 6.6 pcs on 4-year-old branches. The number of short fruiting branches decreased as the branches got older. The number of the short fruiting branches on most of the trees amounted to 192.0 pcs/tree. The number of short fruiting branches in the Ferrovia and Skeena varieties slightly differed from the Adriana variety, and was not statistically proved.

Table 7. The number of short fruiting branches in sweet cherry trees depending on variety and crown shape, pcs/tree (the Gisela 6 rootstock, the planting distance – 4x2m, the tree age – 5 years old, small naturally improved crown)

Variety	The number of the short fruiting branches			Total, pcs/tree
	Two-years-old branches	Three-years-old branches	Four-years-old branches	
Adriana	139.1	46.3	6.6	192.0
Ferrovia	119.9	54.6	6.3	180.8
Skeena	132.2	49.0	7.0	188.2
LSD 5%	37.45	17.42	4.33	-

During the growth and fruiting periods of the trees, the impact of the annual branches' length on the yield and quality of sweet cherries of the Kordia variety was also studied (Table 8). The sweet cherry tree is a species which produces many fruit. Under favourable climatic conditions, this fruit tree can produce 30-50% of the total number of flowers (Cimpoies, 2018). The research showed that, regardless of the length of the annual branches, the number of fruit was 34.0-44.4%. The diameter and weight of the sweet cherries, as well as the soluble dry matter, changed according to the length of the annual branches. As the branch length increased (40 cm), the diameter of the cherries (28.2 mm), their weight (10 g) and soluble dry substance (16.55%) increased compared to cherries that grew on shorter annual branches. Thus, the branches 40 cm long born higher quality fruit compared to shorter branches.

Table 8. The impact of the length of annual branches on the yield of the Kordia variety. (the Gisela 6 rootstock, the planting distance – 4 x 2.5 m, the tree age – 5 years old)

Length of the annual branches, cm	Number of sweet cherries, pcs	Fruit formation, %	Diameter of sweet cherries mm	Weight of sweet cherries g/fruit	Soluble dry matter Brix%
10 ± 2	99	38.0	22.7	7.4	14.02
20 ± 2	85	34.0	25.8	8.4	16.51
30 ± 2	131	44.4	26.0	8.7	16.46
40 ± 2	111	38.9	28.2	10.0	16.55

The data presented in Table 9 indicate the close interdependencies among the features considered as cherry quality elements, namely the fruit diameter (mm), the fruit weight (g), the fruit firmness (kg/cm^2), the soluble solids content (SSC, % Brix) and the titratable acidity (%). The variability of the indices of the sweet cherry diameter and weight was high and it depended on the variety, the shape of the crown, the fruit harvesting period and the climatic conditions. In the sixth year after planting, at harvest, the sweet cherry trees of the Skeena variety yielded fruit which had a diameter of 26.0-28.1 mm and a weight of 8.59-9.18 g/fruit. The diameter of the fruit produced by the trees with improved oblate vessel-shaped crowns was 28.1 mm, and the indices were statistically proved, compared to the fruit produced by the trees with small and naturally improved crowns and those with improved slender spindle-shaped crowns.

The SSC content of the sweet cherries varied between 18.0 and 18.6 % Brix. The trees with improved crowns of oblate vessel shape produced fruit with the highest concentration of soluble solids (18.6% Brix). The titratable acidity of the fruit was 0.85-0.89%, and the firmness was 2.87-2.98 kg/cm^2, which gave the sweet cherries a balanced acidity and firmness required by consumers.

Table 9. The impact of the crown formation system on the quality of fruit of the Skeena variety (the Gisela 6 rootstock, the planting distance – 4 x 2 m)

Crown shape	Fruit diameter, mm	Fruit weight, g	SSC, % Brix	Titratable acidity, %	Fruit firmness, kg/cm^2
Small naturally improved crowns	26.0	8.59	18.0	0.89	2.98
Improved slender spindle crowns	26.3	8.60	18.0	0.85	2.87
Improved oblate vessel	28.1	9.18	18,6	0.88	2.90
LSD 5%	1.13	0.42	0.85	0.33	0.21

The soluble solid content in sweet cherries was determined dynamically from the time of fruit reddening to the time of their harvesting (Table 10).

Table 10. The soluble solid content (SSC) in sweet cherries according to the colour, % (the Gisela 6 rootstock, the planting distance – 4 x 2.5 m, small naturally improved crown, the age of the tree – 6-7 years old)

Variety	Skin colour				
	Red	Bright ruddy	Dark red	Dark reddish brown	Dark brown
Year 2016					
Ferrovia	4.3	6.8	12.8	17.5	18.5
Regina	3.9	7.0	13.3	17.0	18.3
Year 2017					
Ferrovia	3.5	6.4	13.4	17.2	18.8
Regina	3.5	6.0	13.8	17.4	18.5

From the moment the fruit were red, the content of soluble solids grew much. In 2016, the soluble solid content (SSC) in the Ferrovia variety increased from 4.3%, in red fruit, to 18.5%, in dark brown fruit, and respectively from 3.9% to 18.3%, in the sweet cherries of the Regina variety. The same trend was observed in 2017, namely, starting from the phase when the fruit became red, the SSC increased until the sweet cherries became dark reddish-brown, then the rate of SSC storage decreased. The maximum SSC value was recorded during the phase of rapid skin colour change, which can be used to determine the optimal harvest time.

The size of fruit born by branches 20, 30 and 40 cm long recorded significantly higher indices compared to fruit born on branches 10 cm long. (Table 11). Thus, the diameter of the sweet cherries on 20-centimetres-long branches was larger by 14.2% (Adriana), by 23.6% (Ferrovia) and by 20.6% (Skeena) compared to the fruit born by 10-centimetres-long branches. The size of the sweet cherries on the 30- and 40-centimetres-long branches was larger compared to the sweet cherries formed on the 20-centimetres-long branches, but it was not significantly proved.

Table 11. The diameter of sweet cherries according to the length of the biennial branches, mm (the Gisela 6 rootstock, the planting distance – 4 x 2 m, small naturally improved crown, the age of trees – 6 years old)

Variety	The length of the branch								LSD 5%
	10 cm		20 cm		30 cm		40 cm		
	mm	%	mm	%	mm	%	mm	%	
Adriana	21.2	100	24.2	114.2	29.4	138.7	30.0	141.5	3.42
Ferrovia	21.2	100	26.2	123.6	27.4	129.3	29.0	136.8	4.36
Skeena	21.4	100	25.8	120.6	29.0	135.5	28.8	134.6	3.72

The position of branches in space plays a decisive role in growing quality sweet cherries. (Table 12). It is worth mentioning that the diameter and weight of the fruit decreased from branches oriented upward to branches oriented downward. Thus, the diameter of the fruit on the two-year-old branches with a spatial position upward increased in the Adriana variety by 23.1%, in the Ferrovia variety by 27.1%, and in the Skeena variety by 7.8% compared to the branches oriented downward; horizontal branches occupied an intermediate position. The results highlight the importance of controlling the direction of branch growth as needed.

Table 12. The diameter of the sweet cherries according to the position of annual branches in space, mm (the Gisela 6 rootstock, the planting distance – 4 x 2 m, small naturally improved crowns, the age of the trees – 6 years old)

Variety	Position of biennial branches						LSD 5%
	Upward		Horizontal		Downward		
	mm	%	mm	%	mm	%	
Adriana	29.3	123.1	29.0	121.8	23.8	100	4.95
Ferrovia	28.6	127.1	27.3	121.3	22.5	100	3.78
Skeena	29.0	107.8	29.0	107.8	26.9	100	2.36

The information analysis of the experimental data made it possible, through deduction and calculation, to establish the interdependence of the fruit diameter and weight according to the following formula: $\mathbf{Y = 1.39X + 13.72}$, where Y is the diameter of sweet cherries, mm; X is the weight of sweet cherries, g. (Balan et al, 2023).

CONCLUSIONS

The esearch was aimed at promoting sustainable integrated cultivation systems that would ensure early economic fruiting of sweet cherry trees and the production of quality, healthy fruit that is marketable. All these problems can be solved by determining the most suitable planting distance, crown formation and pruning systems, and by maintaining a balance between growth and fruiting. Currently, all sustainable integrated fruit growing systems are successfully used, which are based on geographical conditions, the degree of natural soil fertility, the relative strength of the variety-rootstock association, planting density, simple crown shape, and which are aimed at obtaining early high-quality yields.

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STRUCTURE OF THE VEGETATIVE ASSEMBLY OF APPLE TREES ACCORDING TO AGE AND THE BIOLOGICAL CHARACTERISTICS OF THE VARIETY

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Abstract

This work refers to the study of the structure of the vegetative ensemble of apple trees organized in the experimental orchards of SRL "Elit Fruct" and SRL "Prodcar". The apple varieties Granny Smith, Gala Delicious, Gala Buckeye Simmons, Golden Delicious, Golden Delicious Reinders, Red Velox and Fuji Kiku, grafted on M9, intended for the establishment of high density plantations, cultivated in the conditions of the central area of the Republic of Moldova. The vegetative growth of the trees in the apple varieties taken in the study is expressed, quantitatively, by the volume of vegetative growth accumulated annually by the size of the height and width of the crown of the trees, by the size of the surface and volume of the crown, as well as the level of soil coverage of the whole vegetative growth of trees.

*Key **words**: Apple variety, vegetative assembly, tree crown.*

INTRODUCTION

The apple is the priority fruit tree species in the country's fruit growing, which provides about 80% of the global fruit production. Obtaining large, constant and high-quality apple productions can only be achieved as a result of the implementation of modern technological links, and in addition to the technological processes applied in the orchard, the fruits must be exploited according to an appropriate technology, which allows maintaining the quality at the highest possible levels raised, from harvesting to delivery to the consumer [Bucarciuc, V. 2008].

The variety represents one of the basic links related to the production technology in apple culture and the exploitation of climatic conditions, being at the same time a mobile element, with continuous possibilities for improvement [Bucarciuc, V. 2008]. The assortment of varieties that are grown in a fruit-growing area is of particular importance, fruit growing being profitable only if the cultivated varieties are able to capitalize on local conditions, giving large, high-quality productions, competitive on the domestic and foreign markets [Babuc, V. 2012].

At the present time, the world assortment of the apple is very rich, representing about 10-12 thousand varieties obtained by man through the empirical selection of the most valuable forms existing in nature, as well as through the activity of research and scientific improvement [Cimpoieș Gh., 2000]. Thus, the large number of varieties is also explained by the fact that, in each apple-producing country, research institutions were opened to improve apple varieties and improve assortments [Bucarciuc, V., 2008].

The analysis of the evolution of the world range of apple culture demonstrates that, in the 21st century, there is a tendency to reduce the number of apple varieties cultivated in accordance with the conditions of environmental exploitation and the requirements of the international market. Today, in the large apple-producing countries, a small number of varieties are grown on huge areas, but with great production potential and quality fruit [Bucarciuc, V., 2008].

The most important cultivars cultivated in the world are those from the Red Delicious, Golden Delicious and Fuji groups [Babuc, V., 2012]. It is observed that, while the surfaces with red varieties remain stable, those with yellow varieties are decreasing, and the surfaces with bicolor varieties are increasing.

Vegetative growth and stem development in trees is determined by biological (variety, rootstock, resistance to diseases and pests) and technological factors (fruit load, provision of food and water), which condition the development of physiological processes [Babuc et al., 2008]. The influence of pedological factors, such as soil structure and fertility, restrictive soil factors, and climatic factors, such as solar energy, temperature, through its maximum and minimum values, but especially through the values recorded in the vegetation period (fruiting phenophases), the amount of precipitation, etc. [Babuc, V. et al., 2013]. From a genetic point of view, the variety is the basic factor in determining the culture system and technology [Bucarciuc, V., 2015].

MATERIALS AND METHODS

Experience 1. The research was carried out at SRL "Elit Fruct" in the village of Coșernița, Criuleni district, during the years 2015-2019. The plantation was founded in 2015, the varieties Red Velox and Golden Delicious Reinders grafted on the M9 rootstock were studied. The distance between rows was 3.2 m, and per row - 0.8 m, which is equivalent to 3900 trees/ha. When planting, the grafting site was placed 15-20 cm above ground level. Until the orchard was planted, the tree support system was installed - monoplane, simple, made of reinforced concrete poles, with a height of about 4.0 m above the ground, and a metal wire installed at a height of 50 cm from the ground level, which it is also used as a support for the irrigation system. In the first year of vegetation, 5 more metal wires were added. The first 2 wires were fixed at 80 cm from the ground and at 80 cm from each other, the following - at 160 cm, 240 cm and 320 cm from the ground, respectively. The experiment was set up in 4 randomized replicates of 8 trees each [Cimpoieș Gh., 2000].

Experience 2. The research was carried out at SRL "Prodcar" from Negureni village, Telenești district, during the years 2015-2019. The apple orchard was planted in 2014. In order to establish an assortment of apples intended for sustainable production in the ecopedo-climatic conditions of the central area of the Republic of Moldova, 4 apple varieties recently introduced into the intensive culture system were studied, namely Granny Smith, Gala Buckeye Simmons, Red Velox and Golden Delicious Reinders grafted onto M9 rootstock at 3.2 x 0.8 m spacing (3900 trees/ha). As a witness served the variety Granny Smith. The trees are driven in the form of a thin improved spindle and planted at a distance of 3.5 x 1 m (2857 trees/ha) in the north-south direction. When planting, the grafting site was placed 20 cm above ground level. Each variant included four repetitions of eight trees each. During the research, the maintenance and phytosanitary protection works of the trees, provided for in the technology of super-intensive apple culture, were applied in the orchard. The soil in the orchard is maintained by weeding and weeding the row of trees

RESULTS AND DISCUSSIONS

Vegetative growth and stem development in trees is determined by biological (variety, rootstock, resistance to diseases and pests) and technological factors (fruit load, provision of food and water), which condition the development of physiological processes [Babuc, V., 2012] The influence of pedological factors, such as soil structure and fertility, restrictive soil factors, and climatic factors, such as solar energy, temperature, through its maximum and minimum values, but especially through the values recorded in the vegetation period (fruiting phenophases), the amount of precipitation, etc. [Balan, V. et al., 2018]. From the genetic point of view, the variety is the basic factor in determining the culture system and technology.

The vegetative growth of the trees in the apple varieties taken in the study is expressed, quantitatively, by the volume of vegetative growth accumulated annually by the size of the height and width of the crown of the trees, by the size of the surface and volume of the crown, as well as the level of soil coverage of the whole vegetative growth of the trees (Table 1). The height of the crown, in the studied varieties, was 256-282 cm in the 3rd year after planting. The variety Red Velox, of weak vigor, recorded the lowest value (256 cm) of crown height.

The width of the crown at the base recorded maximum values (105-124 cm) admissible in relation to the distance of planting trees in a row (80 cm). The width of the crown at the top depends on the growth vigor of the variety and varied between 25 cm, in the variety Red Velox,

and 65 cm, in the variety Granny Smith (control) of high vigor.
The level of soil coverage with the vegetative assembly receiving solar energy has values of 32.8-38.8%. The difference in the use of the nutrition surface of the trees is insignificant, since the distances between the rows and within the row are optimal for the apple varieties grafted on the M9 rootstock in high density plantations.

Table 1 Structure of the vegetative ensemble of apple trees according to age and the biological characteristics of the variety

Variety	Crown height, cm	Crown width, cm		The level of ground cover with the projection of the crown, %	The lateral surface of the crown, thousand m^2/ha	Crown volume m^3	
		at the base	at the top			tree	ha
year 2017, age of the trees – 3 years							
Granny Smith (control)	282	124	65	38.8	24568	2.6	10155
Gala Buckeye Simmons	270	112	45	35.0	22850	2.1	8202
Red Velox	256	105	25	32.8	20975	1.6	6249
Golden Delicious Reinders	265	115	50	35.9	22654	2.2	8593
Fuji Kiku	265	116	45	36.3	22459	2.1	8202
year 2019, age of the trees – 5 years							
Granny Smith (control)	329	130	75	40.6	28630	3.4	13280
Gala Buckeye Simmons	335	130	65	40.6	28709	3.2	12499
Red Velox	285	130	45	40.6	24021	2.5	9765
Golden Delicious Reinders	333	130	70	40.6	28748	3.3	12889
Fuji Kiku	324	130	65	40.6	27849	3.2	12499

Based on the tree vigor data, the crown volume at the tree level was calculated and reported to the surface unit. The lateral crown surface of 3-year-old apple trees was 20975-24568 m^2/ha, and the crown volume was 1.6-2.6 m^3/tree and 6249-10155 m^3/ha, respectively.
Along with the growth and development of the trees, the parameters of the crown also increase. In 5-year-old trees, the height of the crown is 285-335 cm, depending on the variety, and the width of 130 cm remains constant in all the varieties studied (the distance between rows of 3.2 m does not allow wider crowns) [Balan, V. et al., 2001]. The width of the crown at the top varies from 45 cm, in the trees of the Red Velox variety, to 75 cm, in the Granny Smith variety (control). The soil coverage with the vegetative assembly reached the optimal level possible for the row spacing of 3.2 m and recorded values of 40.6% for all varieties.
The lateral surface of the crown in 2019 (24021-28709 m^2/ha) increased significantly compared to 2017 (20975-24568 m^2/ha) and achieved optimal values for such orchards. Crown volume, which depends on crown area, also increased significantly, constituting 2.5-3.4 m^3/tree and 9765-13280 m^3/ha, respectively. In 2019, crown area and volume were greater in Gala Buckeye Simmons, Granny Smith (control), Golden Delicious Reinders and Fuji Kiku varieties compared to Red Velox variety. Thus, in the 5th year of vegetation, the trees of the apple varieties studied achieved an optimal crown surface and volume, which characterizes the productive potential of the plantation. The data related to the structure of the vegetative ensemble of apple trees from SRL "Prodcar" are presented in Table 2. From the data obtained regarding the height of the trees, it follows that the trees of the Red Velox variety have the lowest height both in 2017 (272 cm) and in 2019 (271 cm). The medium to high vigor varieties (Gala Buckeye Simmons, Granny Smith (witness) and Golden Delicious Reinders) exceed the height of the less vigorous trees (Red Velox) by 50-100 cm.
The width of the crown in 4-year-old trees varies depending on the variety and is 118-132 cm at the base of the crown and 32-71 cm at its top. In the 6th year of vegetation, these indices increased insignificantly, and the crown of the trees did not exceed the space reserved by the planting distances between rows and per row.

Table 2. The structure of the vegetative ensemble of apple trees according to age and the biological particularities of the variety

Variety	Crown height, cm	Crown width, cm		The level of ground cover with the projection of the crown, %	The lateral surface of the crown, thousand m^2/ha	Crown volume m^3	
		at the base	at the top			Tree	Ha
year 2017, age of the trees – 4 years							
Granny Smith (control)	295	132	70	41.3	25779	2.97	11600
Gala Buckeye Simmons	295	127	60	39.7	25389	2.75	10741
Red Velox	272	118	32	36.9	22498	2.04	7968
Golden Delicious Reinders	285	126	71	39.4	25037	2.80	10936
year 2019, age of the trees – 6 years							
Granny Smith (control)	341	140	75	43.8	29568	3.66	14295
Gala Buckeye Simmons	355	140	75	43.8	30662	3.81	14881
Red Velox	271	140	42	43.8	22811	2.46	9608
Golden Delicious Reinders	338	140	70	43.8	29138	3.55	13866

The level of ground cover with crown projection in 4-year-old trees was 36.9-41.3%, varying slightly from one variety to another. The 6-year-old trees occupied the maximum planting area and reached values of 43.8% for this index.

The lateral area of the crown in the 4th year of vegetation varied from 22498 m^2/ha, for the Red Velox variety, to 25779 m^2/ha, for the Granny Smith variety (control), and at the age of 6 the trees had a maximum area of the crown of 30662 m^2/ha.

The volume of the crown differs both at the level of the tree and at the level of the surface unit. The crown of the trees provides the support structure for the branches, leaves and fruits, and through its structure it must ensure the penetration of solar energy to all skeletal, semi-skeletal and garnishing branches to maintain their coverage with fruit formations and to print a production volume as high as possible. The volume of the crown varies depending on the size of the trees, and these are influenced by the vigor of the variety. The studied varieties form a continuous crown in the direction of the row, which allows receiving no more than 43.8% of solar energy. In 4-year-old trees, the volume of the crown was 7968 m^3/ha in the Red Velox variety, increasing considerably in the high-vigor varieties, up to 10741-11600 m^3/ha. In 2019, the crown volume of 6-year-old trees reached optimal values and was 9608-14881 m^3/ha.

At the tree level, in the 6th year after planting, the recorded crown volume fluctuated quite a lot between varieties - from 2.46 m^3/tree, for the Red Velox variety, to 3.81 m^3/tree, for the variety Buckeye Simmons Gala. The crown volume data demonstrate that Gala Buckeye Simmons, Granny Smith (control), Golden Delicious Reinders and Fuji Kiku apple varieties, during the growth and fruiting period, formed an optimal vegetative ensemble for the rational use of solar energy and obtaining a yield enhanced by quality fruit. Similar results regarding the formation of the productive volume in modern orchards were obtained and argued by V. Balan (1997), Gh. Cimpoieș (2000), the data being a function of the size of the crown, the volumetric density coefficient of the lateral surface of the crown and the coefficient of effectiveness of the plantation.

Along with the growth and development of the trees, the parameters of the crown also increase. In 5-year-old trees, the height of the crown is 285-335 cm, depending on the variety, and the width of 130 cm remains constant in all varieties studied (the distance between rows of 3.2 m does not allow wider crowns).

The width of the crown in 4-year-old trees varies depending on the variety and is 118-132 cm at the base of the crown and 32-71 cm at its top. In the 6th year of vegetation, these indices increased insignificantly, and the crown of the trees did not exceed the space reserved by the planting distances between rows and per row.

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CONCLUSIONS

Vegetative growth and stem development in trees is determined by biological (variety, rootstock, resistance to diseases and pests) and technological factors (fruit load, provision of food and water), which condition the development of physiological processes [Babuc, V., 2012]. The influence of pedological factors, such as soil structure and fertility, restrictive soil factors, and climatic factors, such as solar energy, temperature, through its maximum and minimum values, but especially through the values recorded in the vegetation period (fruiting phenophases), the amount of precipitation, etc. [Balan, V. et al., 2018]. From the genetic point of view, the variety is the basic factor in determining the culture system and technology.

The volume of the crown differs both at the level of the tree and at the level of the surface unit. The crown of the trees provides the support structure for the branches, leaves and fruits, and through its structure it must ensure the penetration of solar energy to all skeletal, semi-skeletal and garnishing branches to maintain their coverage with fruit formations and to print a production volume as high as possible. The volume of the crown varies depending on the size of the trees, and these are influenced by the vigor of the variety. The studied varieties form a continuous crown in the direction of the row, which allows receiving no more than 43.8% of solar energy. In 4-year-old trees, the volume of the crown was 7968 m^3/ha in the Red Velox variety, increasing considerably in the high-vigor varieties, up to 10741-11600 m^3/ha. In 2019, the crown volume of 6-year-old trees reached optimal values and was 9608-14881 m^3/ha.

The level of soil coverage with the vegetative assembly receiving solar energy has values of 32.8-38.8%. The difference in the use of the nutrition surface of the trees is insignificant, since the distances between the rows and within the row are optimal for the apple varieties grafted on the M9 rootstock in high density plantations.

Along with the growth and development of the trees, the parameters of the crown also increase. In 5-year-old trees, the height of the crown is 285-335 cm, depending on the variety, and the width of 130 cm remains constant in all varieties studied (the distance between rows of 3.2 m does not allow wider crowns).

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WINFOLIA SYSTEM - INSTRUMENT FOR PEST AND DISEASE ATTACK EVALUATION IN PEACH AND NECTARINE ORCHARD

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Abstract

This study aims to screen new peach and nectarine cultivars for disease sensitivity. The peach orchard was planted in 2017 using 14 peach and 16 nectarines new cultivars, grafted on various rootstocks: Adesoto, Myrobalan 29C, Saint Julien A, and GF677 on two different planting systems: Trident and Vertical Axis. The planting distances varied from 4.0 x 1.5 m for Vertical Axis (1,666 trees/ha - 1,666 axis/ha) to 4.0 x 2.0 m for Trident (1,250 trees/ha - 3,750 axis/ha). The research was conducted over six years, and in the last year, 2023, leaves were analyzed in the growing season and at the end (late November). The leaves were harvested at three heights (bottom, half, and top of the tree canopy). Only ten leaves per cultivar were analyzed, and the incidence of the bacterial disease Xanthomonas arboricola pv. pruni (XANTPR) and Pseudomonas syringae pv. persicae (PSDMPE) was measured using a scanner and the WinFolia Software. Significant differences in bacterial disease incidence were registered in nectarine cultivars, according to the tree canopy, and the vertical axis showed more sensitivity.

***Key words**: Xanthomonas arboricola, Pseudomonas syringae, cultivars, sensitivity.*

INTRODUCTION

Unlike most other cultivated species, *Prunus persica* (L.) Batsch is a diploid species that self-pollinates naturally (Byrne et al., 2012). China is the world's largest producer of peaches and nectarines, followed by Spain, Italy, Greece, Turkey, and the U.S.A., with a global annual production of ~26 million tons in 2022 (FAOSTAT, 2024; EU Peach and Nectarine Consumption, 2022) In Europe and North America, the yearly consumption of peaches reaches 6.1 kg per person (compared to 15 kilograms of apples), a significant amount considering all fruits available during summer. (EU Peach and Nectarine Consumption, 2022).
Plant diseases still impact society and the economy today. According to the Food and Agriculture Organization (FAO), around 25% of crop loss is attributed to diseases, pests, and weeds. For example, each year, the rice blast disease destroys enough rice to equal Italy's rice production (Dean et al., 2005). The way that crop diseases spread is a crucial component. Viruses, bacteria, fungi, and other pests and pathogens are essential factors in the disease of leaves and plants, regardless of climatic changes (Wakelin et al., 2018). There are several reasons why measuring or estimating plant disease is necessary. Understanding the degree of attack is essential to quick management since disease and yield loss are strongly correlated.
In plant breeding, it is important to rate crop resistance and susceptibility to disease, but sometimes it may also involve the planting system (Stănică, 2019). Estimating plant resistance is a tool for crop protection so pesticides can be applied efficiently. Analysis of plant disease and symptom severity solves fundamental problems and concerns in plant stress biology (Martinelli et al., 2015).
Pseudomonas syringae pv. *persicae* (*P. s.* pv. *persicae*) was found for the first time in the Ardèche region in France and described under the name *Pseudomonas mors-pronotum* f. sp. *persicae*. The bacteria overwinter in cankers, dead and symptomless buds, and systemically infected branches (Vigouroux, 1970; Kennelly et al., 2007). During spring, bacteria that have

invaded leaf scars may become active, leading to bud blasts and die-back of shoots and branches (Young, 1987b). Symptom manifestation varies based on tree age, with trees under six years old being more vulnerable. It causes olive-green discoloration on shoots and branches during winter, quickly turning brown. A relationship exists between disease severity, plant dormancy, and early severe frost in autumn and winter (Young, 1987; 1988). The bacterium on young nectarine leaves initially creates angular, water-soaked patches that turn necrotic, measuring 1-2 mm in diameter and surrounded by a chlorotic halo. The necrotic tissue may vanish, causing a shot-hole effect. Young nectarine fruits may have superficial circular, dark olive-colored, greasy patches of 1-2 mm in size (Young, 1987b). *Xanthomonas arboricola* pv. *pruni* (Xap) is the source of bacterial spot, a dangerous peach disease that causes severe defoliation and black surface pitting, cracking, or blemishes on peach fruit. Identified for the first time in 1903 in the USA, the disease has spread worldwide and has been reported from all continents (Diagnostics, 2006; Socquet-Juglard et al., 2013). Eventually, severe leaf spot infections can cause early tree defoliation, reducing vigor and winter hardiness. Bacterial spots influence the worldwide economy. Under favorable conditions, bacterial spots can affect 100% of the fruit in peach orchards (Fleming et al., 2022). It is widespread in all fruit-growing areas on all stone fruits in Romania. Due to the attack, significant damage was recorded in plums, peaches, or apricots through strong defoliation and fruit fall in varieties susceptible to the attack of this bacterium (Florea et al., 2019). For the past 80 years, conventional disease measures have been frequently used to achieve acceptable accuracy and precision in naked-eye disease assessments. These techniques are overly subjective, however. There is now a chance to evaluate diseases more objectively thanks to new technology (reliability, precision, and accuracy) (West et al., 2003). The WinFolia software is one of these. The software's capabilities to accurately measure various leaf parameters, such as area, shape, perimeter, length, and width, allow for the early detection of pests and disease symptoms that may manifest on the leaves correlated to their color. Thus, this work aimed to screen new peach and nectarine cultivars for sensitivity to *Xanthomonas arboricola* pv. *pruni* (Smith) and *Pseudomonas syringae* pv. *persicae.*

MATERIALS AND METHODS

The peach orchard was planted in 2017 in the Experimental orchard of the Faculty of Horticulture of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, using 14 peach and 16 nectarines new cultivars, grafted on various rootstocks: Adesoto, Myrobalan 29C (M29C), Saint Julien A, and GF677 on two different planting systems: Trident and Vertical Axis.

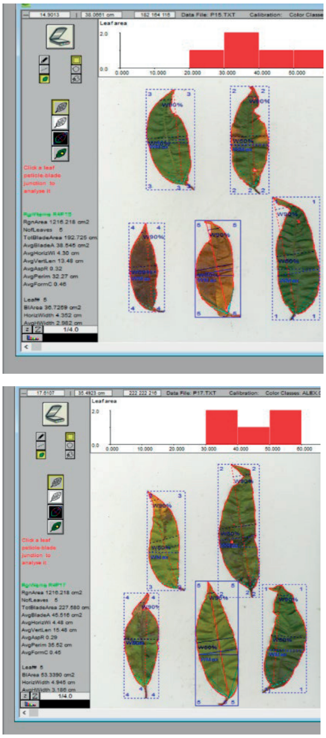
The planting distances varied between 4.0 x 1.5 m for Vertical Axis (1,666 trees/ha - 1,666 axis/ha) to 4.0 x 2.0 m for Trident (1,250 trees/ha - 3,750 axis/ha). The peach and nectarine cultivars are presented in Figure 1.

Peach		Nectarine	
Cultivar	**Rootstock**	**Cultivar**	**Rootstock**
Sugar Time	Adesoto	Honey Late	Saint Julien A
Springbelle	Mirobolan 29C	Nectaross	Saint Julien A
Cardinal	Mirobolan 29C	Stark Red Gold	Saint Julien A
Royal Majestic	Adesoto	Maria Anna	Saint Julien A
Royal Glory	Adesoto	Nectareine	Adesoto
Nabby	GF677	Guerriera	Saint Julien A
Royal Summer	GF677	Nectagrand 4	Saint Julien A
Royal Summer	SAINT JULIEN A	Caldessi 2000	Saint Julien A
Sweet Dream	GF677	Honey Royale	GF677
Royal Jim	Adesoto	Nectagrand 1	Saint Julien A
Sweet Henry	Adesoto	Big Top	GF677
Red Top	Mirobolan 29C	Big Fire	GF677
Sweet Ivan	GF677	Big Bang	GF677
Sweet Juana	GF677	Delta	Mirobolan 29 C
Gladys	GF677	Early Sun Grand	Saint Julien A
Lucius	GF677	Nectabelle	GF677

Figure 1. Peach and nectarine cultivars

The analyses were conducted over the 6 years (2018-2023). Thirty leaves per cultivar were harvested at three heights: base, middle, and top. The leaves were stored at 3°C and 90% humidity. Ten leaves were randomly selected from a total number of leaves and were analyzed using the Epson Expression 11000XL scanner, then interpreted by the WinFolia software. In 2023, leaves were evaluated twice during the vegetative period: first on July 11 and November 4, and then at the end of November on the 28th. Results and dendrograms depict this indication.

The WinFolia system, created by Regent Instruments Canada Inc., includes an Epson 11000XL scanner and image processing software (Figure 2).



a

b

Figure 2. Royal Summer/GF677 (a) and SJA (b)
(Trident system planting)

RESULTS AND DISCUSSIONS

Bacterial disease sensitivity determined with WinFolia system

None of the peach and nectarine cultivars were immune to the bacterial disease attack (Tables 1 and 2), confirming the results of Werner et al. (1986).

Comparing nectarine cultivars' sensitivity (Table 1), in the 2018-2023 period, Nectabelle/GF677 [39.68% on Vertical Axis (VA) and 37.66% on Trident (T)] presented the highest values on the attack. Big Bang/GF677 and Nectagrand1/SJA had similar values. The lowest values were at Big Fire/GF677 (26.25% - VA). Analyzing the dynamic in the 2023 months, Big Bang/GF677 recorded significantly higher values on 11.07.2023 - 55.7% (T) and 04.11.2023 - 58.75% (T).

The lower values at the end of November were primarily due to leaves drop. When comparing the influence of the system planting, Vertical Axis, and Trident, the Vertical Axis system generally showed higher values than the Trident towards the end of the analyzed period.

Table 1. Nectarine cultivars under Winfolia analysis: bacterial disease attack (%) in 2018-2023 on two different canopies, Vertical Axis and Trident

Genotype/Rootstock	2018-2023				11.07.2023				4.11.2023				28.11.2023			
	VA		T		VA		T		VA		T		VA		T	
Early Sun Grand/SJA	37.1259	abc	33.6418	ab	17.5260	bc	15.6800	cd	9.8280	def	19.5345	c	33.3010	abc	24.0530	bcd
Caldessi2000/SJA	33.4493	abcd	27.8447	bcd	4.6170	e	5.5910	de	5.0740	ef	17.4650	cd	33.4200	abc	46.2210	a
Nectagrand1/SJA	38.5968	ab	38.3987	ab	10.8940	bcde	9.4590	cde	46.1070	b	7.4730	de	44.6010	a	42.7780	ab
Nectagrand4/SJA	27.6083	cd	28.7337	bcd	10.1330	bcde	4.6130	e	7.5860	ef	6.5440	de	14.0150	d	32.4270	abcd
Guerriera/SJA	27.0685	cd	36.2822	ab	4.6750	e	5.8680	de	8.3350	ef	21.5280	c	17.4320	cd	12.6960	d
Nectabelle/GF677	39.6790	a	37.6634	ab	17.6260	bc	11.6290	cde	57.4750	a	5.8900	e	28.5930	abcd	20.4370	cd
Honey Late/SJA	32.5513	abcd	31.4535	abc	7.6420	cde	2.7890	e	7.7030	ef	3.6750	e	26.0850	bcd	14.5700	d
Nectaross/SJA	28.8390	bcd	27.6942	bcd	29.2710	a	17.0120	c	5.4910	ef	7.7740	de	17.5420	cd	14.6200	d
Nectareine/Adesoto	28.6438	bcd	22.1320	cd	6.6760	de	4.9720	e	4.5330	ef	4.1810	e	39.9690	ab	18.5810	d
Stark Red Gold/SJA	30.1023	abcd	31.4142	abc	16.0930	bcd	4.9720	e	2.9330	f	4.1810	e	20.8930	cd	18.5810	d
Big Bang/GF677	32.9617	abcd	41.5058	a	12.1230	bcde	55.8660	a	58.0150	a	58.7460	a	15.0660	cd	20.4380	cd
Big Fire/GF677	26.2520	d	34.1332	ab	16.4380	bcd	17.4320	c	15.4460	cde	43.5910	b	24.4960	bcd	16.6830	d

For the peach, in the 2018-2023 trends, the highest VA value was 48.43% (Sweet Ivan/GF677), while the lowest was 20.46% (Cardinal/M29C). For the Trident canopy, the highest value was 43.04% (Royal Summer/SJA), and the lowest was 14.30% (Sweet Henry/Adesoto). Vertical Axis and Trident systems showed an increasing trend in bacterial attack values over the year. Specific cultivars were more susceptible in the Trident canopy (ex., Royal Summer/GF677), while others presented uniform behavior on both systems (ex., Sweet Henry/Adesoto). Sweet Ivan/GF677 and Big Bang/GF677 cultivars generally showed higher values on the Vertical Axis than on the Trident canopy.

Research conducted by Adaskaveg & Förster (2023) focusing on the bacterial spot and other common bacterial diseases in stone fruits could share common observations regarding patterns (Adaskaveg & Förster, 2023).

Table 2. Peach cultivars under Winfolia analysis: bacterial disease attack (%) 2018-2023 on two different canopies, Vertical Axis and Trident

Genotype/Rootstock	2018-2023				11.07.2023				4.11.2023				28.11.2023			
	VA		T		VA		T		VA		T		VA		T	
Cardinal/M 29C			20.4553	ef			21.1610	bc			7.8460	de			20.0900	bc
Royal Summer/SJA	32.9998	de	43.0367	a	8.0700	ef	18.5280	c	4.6560	def	7.0240	de	36.1020	a	29.0440	abc
Sugar Time/Adesoto	29.5952	e	29.1993	bcde	22.9040	bcd	17.7600	c	15.3300	bc	24.4520	b	26.7920	a	30.4620	abc
Red Top/M29C	37.6228	bcde	34.3912	abcd	10.2880	def	34.4610	a	13.1990	bcd	15.4540	c	24.7040	a	28.7200	abc
Royal Glory/Adesoto	35.7567	cde	31.9617	abcde	30.6350	ab	31.2880	ab	17.4790	b	10.2070	cd	21.8710	a	33.8630	abc
Sweet Henry/Adesoto	36.9923	bcde	14.3003	f	4.0070	f	9.6920	c	1.6760	f	1.6390	e	30.6190	a	19.2920	bc
Sweet Juana/GF677	45.2302	abc	27.3847	cde	24.1360	bc	12.0590	c	1.0350	f	1.7800	e	22.2280	a	27.5040	bc
Royal Jim/Adesoto	39.6698	abcde	27.6295	cde	34.0170	ab	18.2600	c	44.1010	a	6.9820	de	40.2350	a	16.1030	c
Lucius/GF677	39.1632	abcde	34.1302	abcd	13.3500	cdef	17.3600	c	3.2790	ef	8.3110	de	30.0240	a	35.8220	abc
Royal Majestic/Adesoto	33.7687	de	39.1182	abc	31.2920	ab	36.1370	a	7.8440	cdef	12.7990	cd	25.2700	a	38.5220	abc
Sweet Ivan/GF677	48.4270	a	33.3300	abcd	20.7330	bcde	13.3150	c	8.1750	cdef	5.9320	de	36.3680	a	25.3010	bc
Sweet Dream/GF677	40.0648	abcde	36.7173	abc	22.6670	bcd	18.1350	c	8.0680	cdef	8.1030	de	30.7920	a	17.6710	bcbc
Royal Summer/GF677	36.2702	cde	31.1532	abcde	39.0120	a	17.2850	c	7.6850	cdef	6.8340	de	30.8440	a	50.1800	a
Nabby/GF677	47.2792	ab	40.6300	ab	33.8260	ab	21.7110	bc	7.7130	cdef	5.8130	de	39.8170	a	38.8740	ab

Analyzing the rootstock influence on the tree sensitivity to bacterial attack, Royal Summer/SJA presented lower values than Royal Summer/GF677 on the Vertical Axis and higher on the Trident system in 2018-2023. The trends were similar in the summer and beginning of fall in 2023. The trees on SJA are less vigorous than GF677, but the results were not in line for both canopies.

Morphological leaf parameters

WinFolia software analysis includes an accurately measured series of leaf morphological parameters, from which a leaf area comparison between cultivars and system planting is presented below.

The biological characteristics of the cultivar influence the trunk circumference and the constructive parameters of the canopies. Leaf area is correlated to tree vigor, canopy shape, and rootstocks (Peşteanu, 2021). At nectarine (Figure 3), comparing the cultivars grafted on similar rootstocks, there were differences in the leaf area on the SJA rootstock. Stark Red Gold/SJA had a leaf area of 6.95 cm^2 (VA), while Caldessi2000/SJA had a lower leaf area of 5.75 cm^2 (VA). On the GF677 rootstock, Nectabelle/GF677 had a higher leaf area (6.95 cm^2) than other cultivars on the same rootstock (ex., Big Fire/GF677 with 5.92 cm^2). On the Trident planting system, leaf area values were similar or slightly higher than on the Vertical Axis.

Genotype/ Rootstock	Leaf Area			
	VA		T	
Early Sun Grand/SJA	34.3089	ab	34.3012	b
Caldessi2000/SJA	28.7275	c	31.5578	b
Nectagrand1/SJA	33.4287	abc	33.7184	b
Nectagrand4/SJA	32.5815	abc	33.3962	b
Guerriera/SJA	30.8808	bc	32.5718	b
Nectabelle/GF677	34.7561	ab	35.3537	ab
Honey Late/SJA	31.5251	abc	33.6139	b
Nectaross/SJA	32.8342	abc	35.0963	ab
Nectareine/M29C	34.4914	ab	34.2653	b
Stark Red Gold/SJA	34.7576	ab	33.5407	b
Big Bang/GF677	33.4109	abc	31.9784	b
Big Fire/GF677	29.6077	bc	32.0596	b
Big Top/GF677	29.9222	bc	35.0355	ab

Figure 3. Nectarine cultivars under WinFolia analysis: leaf area (cm^2) 2018-2023 on two different canopies, Vertical Axis and Trident (values cumulated for five leaves)

For peach (Figure 4), the highest value on the Vertical Axis system was registered for Royal Summer/SJA (8.24 cm^2), even higher than on the vigorous rootstocks such as GF677 (Stănică et al., 2020) and M29C. As nectarine cultivars, the trees on the Trident system planting presented similar or slightly higher values than those on the Vertical Axis.

Genotype/ Rootstock	Leaf Area			
	VA		T	
Cardinal/M 29C			36.2005	cdefgh
Royal Summer/SJA	41.1986	a	40.8637	abc
Sugar Time/Adesoto	34.8799	bcd	37.5602	bcdefgh
Red Top/M29C	33.3087	bcd	36.1167	cdefgh
Royal Glory/Adesoto	31.2090	cd	38.4258	abcde
Sweet Henry/Adesoto	36.1509	bc	33.0709	gh
Sweet Juana/GF677	32.2844	cd	32.2208	h
Royal Jim/Adesoto	30.8315	d	34.4613	defgh
Lucius/GF677	33.0261	bcd	33.5553	efgh
Royal Majestic/Adesoto	33.3950	bcd	36.6003	bcdefg
Sweet Ivan/GF677	33.9028	bcd	34.6793	defgh
Sweet Dream/GF677	34.6267	bcd	34.5953	defgh
Royal Summer/GF677	37.7526	ab	38.8014	abcd
Nabby/GF677	34.7271	bcd	31.6928	h
Gladys/GF677	35.0761	bcd	33.0812	fgh
Springbelle/M29C			42.4258	a

Figure 4. Peach cultivars under WinFolia analysis: leaf area (cm^2) 2018-2023 on different canopies, Vertical Axis, and Trident (values cumulated for five leaves)

The dendrograms illustrate the similarities or differences between various peach and nectarine cultivars based on the degree of bacterial attack. The cultivars were analyzed across two canopies, Vertical Axis ("_axis") and Trident ("_t"), and different rootstocks. This analysis can support identifying patterns of resistance or susceptibility among the cultivars.

Both dendrograms (Figures 5 and 6) showed that the interaction between canopies, rootstock, and inherent cultivar traits shaped similarities or differences in bacterial disease levels among cultivars. Rootstocks like GF677 and Adesoto also contributed to cultivar clustering, highlighting bacterial resistance profiles.

Research by Tsogbadrakh et al. (2024) and Oliveira et al. (2018) they have identified comparable effects of canopy shape on disease susceptibility when canopies tend to have lower bacterial incidence than those with denser forms. These findings differed from our results for some cultivars, where the Vertical Axis system exhibited greater susceptibility.

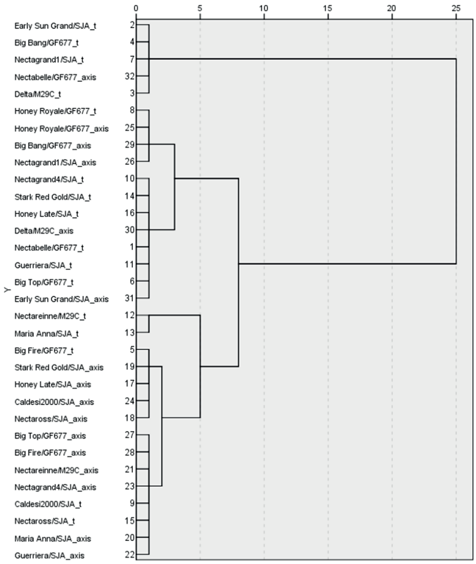


Figure 5. Nectarine cultivars dendrograms on bacterial disease sensitivity

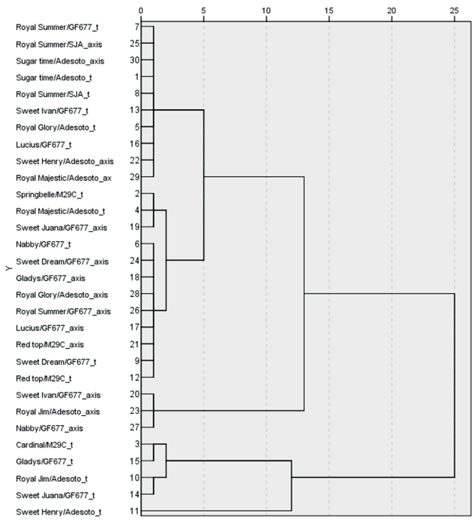


Figure 6. Peach cultivars dendrograms on bacterial disease sensitivity

CONCLUSIONS

The canopies and rootstock-cultivar combinations influenced leaf area, impacting plant health, disease resistance, and growth conditions. Trident canopy shape tended to exhibit higher leaf area values in some cultivars, suggesting favorable leaf development within this training system. Significant differences in vigor were observed among the cultivars studied, with Myrobalan 29C and GF677 generally presenting greater vigor. However, significant differences in bacterial disease incidence were registered in nectarine and peach cultivars, with the Vertical Axis showing more sensitivity for more cultivars.

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CORRELATION BETWEEN SWEET CHERRY QUALITY TRAITS AND FRUIT CRACKING INDEX AT THREE ROMANIAN VARIETIES

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Abstract

Cracking in sweet cherry is often mentioned as a serious problem in many commercial orchards. Research was conducted on reducing the phenomenon of cracking and keeping in mind that cheap, easily accessible, effective solutions for farmers in the specific climatic conditions of our country, is of particular importance. Fruit quality is the target of every farmer. In an attempt to obtain higher quality fruit, it is possible that sometimes, especially if significant rainfall occurs during the ripening period, producers neglect the cracking susceptibility of their varieties. In 2023 we evaluated three Romanian sweet cherry cultivars from the perspective of fruits quality parameters as follows: weight, firmness, pH, total soluble solids, colour. `Special`, `Tentant` and `Severin` cvs. grafted on `IP-C8` rootstock have been quantitatively assessed by the cracking index. `Severin` recorded the largest fruits while `Special` registered the highest content of total soluble solids and these traits represent ones of the most important quality attributes in relation with the intensity of sweet cherry fruit cracking.

Key words: *sweet cherry, cracking index, fruit quality, spray treatments.*

INTRODUCTION

In recent years, Romania has consistently maintained in the first top 10 countries ranked in terms of sweet cherries production in Europe (Faostat, 2022). Due to an extensive favourable geographical conditions for growing (many hilly areas with altitudes of 600-700 m) and a long traditions of sweet cherry cultivation, considering also the economic efficiency of the crop that can generate important incomes for fruit growers, the cherries are in the main attention for farmers and investors too.

Nevertheless sweet cherry production faces a lot of challenges. Certainly the most important ones are spring frosts and fruit cracking. Spring frosts is a threat of a high risk for the production of sweet cherries in temperate climate regions through cold climate regions, especially when the bacterial canker infection follows this low temperature wave (Demirsoy et al., 2022), and its severity is determined by many factors, such as the cultivar, the pruning intensity, the duration of the freeze and the stage of bud dormancy (Webster and Looney, 1996). Such accidents are quite often and become more often due to the climate change in all areas where sweet cherry is cultivated in Romania. If occurs during the blooming period, between 10 to 90% of the flowers can be affected (Chitu et al., 2020).

On the other hand, the unpredictable weather specific to the sweet cherries ripening period (May-June) causes big problems for farmers in many years. In a multi-year characterization (1961-2023) of Romania's climate, the National Meteorological Administration indicates that May and June are the months characterized by periods of sunny and warm days (sometimes too hot) that alternate with periods of cold and rainy days. The rains have many times torrential feature. In addition, June is the richest month in precipitation among all the months of the year.

In terms of cracking susceptibility, fruit of all cultivars cracks, but some differences between the sweet cherry cultivars exist (Knoche and Winkler, 2019). Research should be conducted towards the use of less susceptible cultivars to cracking and their selection for the establishment of new orchards, especially in areas where the rain risk is present during the picking time. These sweet cherry varieties that prove to be tolerant to cracking, can be a valuable source of genes for the sweet cherry breeding programs.

MATERIALS AND METHODS

The biological material used in the present research was represented by three Romanian sweet cherry varieties grafted on IP-C8 vegetative rootstock and established in a non-irrigated eight years old orchard of the Research Institute for Fruit Growing Pitesti - Maracineni (Figure 1).

Figure 1. Eight years old orchard at RIFG Pitești - Mărăcineni

The main traits of the sweet cherry cultivars are as follows:

Special - is a bitter-sweet taste variety, specially bred for processing as jam and for alcoholic beverages, a better alternative to the varieties used for 'kirschwasser' or older similar varieties present in the official ISTIS assortment list.

Figure 2. 'Special' fruit

The fruit is medium-sized (6 g), with a deep stylar point, dark red to black skin, good firmness of the flesh, dark red flesh and juice (Figure 2).

Tentant – is a variety for fresh consumption, with a very firm purple-red pulp and sweet taste. Flowering is starting late and the tree is medium as vigour, with upright or semi-upright habit and high productivity. It is tolerant to *Monilia* sp. and *Blumeriella jaapii* (Rehm Arx.) and can be a good substitute for 'Kordia' in a high density orchard (Figure 3).

Figure 3. 'Tentant' sweet cherry variety fruits

Severin – variety bred for fresh consumption, with large fruits (9g), dark red skin, firm pulp and sweet taste (Figure 4). The productivity is medium and the tree is tolerant to *Monilia* sp. and *Blumeriella jaapii* (Rehm Arx.).

Figure 4. 'Severin' sweet cherry variety fruits

The cracking susceptibility of the varieties was assessed by calculating the cracking index, using the Verner (1957) method revised later by Christensen (1972).

For each sweet cherry variety, 50 fruits at full maturity were picked, without any disorders, cracks or bumps and were placed in distilled water at 20°C for 6 hours. Every 2 hours the fruits have been checked, and those that were found cracked have been removed from the water and counted (Figure 5).

Figure 5. Immersing sweet cherry fruits in distilled water for 6 hours

The cracking index (CI) was calculated according to the formula below:

$$CI= (5a + 3b + c) / 250) \times 100$$

where a, b and c represent the number of fruits cracked after 2, 4 and 6 hours of immersion, total number of fruits immersed = 50, maximum cracking 50 × 5 = 250

The quality traits of the sweet cherry fruits have been evaluated considering the following items (Figure 6):

- weight – measured with a high precision electronic scale;
- fruit firmness – using a non-destructive penetrometer, Bareiss Qualitest HPE, with 0.25 cm^2 plate and cylindrical tip;
- the skin color - was assessed using the Konica Minolta CR 400 Chroma Meter;
- total soluble solids (TSS) – was measured by refractometric method using a Hanna HI 96801 portable refractometer;
- pH of the fruit juice - was determined using the Mini Lab pH meter.

For the evaluation of the fruit's skin colour, we used the CIELAB system. The colour is represented using coordinates in a uniform colour space consisting of the brightness variable L* and the chromaticity indices a* and b*. Thus, if the L* coordinate provides information regarding the brightness, the positive values of a* are located on the red axis and the negative ones on the green axis, and the positive values of b* are located on the yellow axis and the negative ones on the blue axis.

Figure 6. Sweet cherry fruit measurements

Statistical analysis of data was done using analysis of variance (one way-ANOVA), followed by post hoc Duncan's Multiple Range Test (DMRT) to measure specific differences between samples means.

The relationships between parameters were also performed in order to determine, above all, the relationships between fruit quality parameters and CI, by Pearson's correlation.

The aim of the researchwas to emphasize the relationship between some quality traits of three sweet cherry Romanian varieties and their susceptibility to cracking.

RESULTS AND DISCUSSIONS

Sweet cherry fruit quality is closely related with the cultivar, but also with the specific environmental conditions of the current year, and is decisively affected when abundant precipitation is recorded before or during harvesting. In 2023, 12th of June was considered the harvesting day for full ripen stage of 'Tentant' and 'Special' varieties and two days later, on June 14, for 'Severin'. In setting up the experiment, we considered very relevant for an objective evaluation, to choose sweet cherry varieties that have the same or very close harvesting time. One week behind the harvest date, no precipitation occurred (Figure 7), and even that 7.2 mm of precipitation fell on the eve of the harvest, it wasn't affect the fruits. As a result of this environmental condition, in the field we counted only 2.5% of cracked fruits.

According to the data registered for each variety (Table 1) in terms of **fruit weight**, 'Severin' presented the largest fruits (7.67 g), very closed followed by 'Tentant' (7.56 g) with no statistic differences.

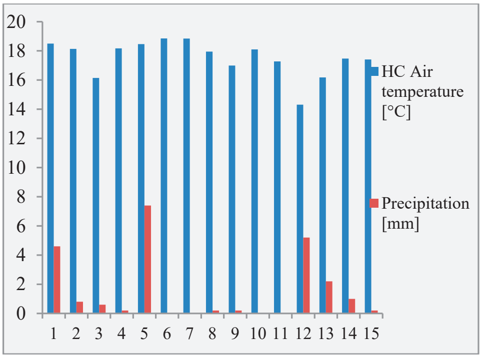

Figure 7. Environmental conditions during the harvest time (1-15 June, 2023)

Table 1. Analyzed quality parameters of the sweet cherry varieties

Cultivar	Weight (g)	Firmness (HPE)	pH	TSS (°Brix)
Special	5.57^{b}	32.1^{b}	3.53^{b}	21.70^{a}
Tentant	7.56^{a}	51.2^{a}	3.62^{a}	16.21^{b}
Severin	7.67^{a}	34.1^{b}	3.44^{c}	11.78^{c}

*Values followed by the same letter are not statistically different according to DMRT (P<0.05).

The lowest cracking index (Table 2) of ‘Severin’ variety (6.4) did not support the results obtained by Perreira et al. (2020) in case of cultivar ‘Lapins’ and ‘Early Bigi’ that showed a higher CI correlated with the bigger size of the fruits. In other particular cases and varieties, as results obtained by Stojanović et. al. (2013) the late ripening varieties are less susceptible to cracking and not the heaviest ones.

In our research, we noticed a significant negative correlation between weight and the cracking index (Table 3). The same conclusion was indicated in the experiments initiated by Demirtaş and Aydinli (2020) who could not demonstrated a strong connection between the fruit size and the cracking index in 16 sweet cherry early ripening genotypes.

Table 2. Colour and Cracking Index of the three sweet cherry varieties

Cultivar	L*	a*	b*	CI
Special	23.25^{c}	6.15^{c}	1.16^{c}	38.4^{a}
Tentant	24.72^{b}	13.00^{b}	2.78^{b}	35.6^{b}
Severin	25.07^{a}	15.86^{a}	4.04^{a}	6.4^{c}

*Values followed by the same letter are not statistically different according to DMRT (P<0.05).

Although 'Special' and 'Severin' varieties showed similar **firmness** values (Table 1), their behaviour was totally differently once the fruits were introduced to water for 6 hours. These data are in line with other experiments that did not reveal a very strong correlation between firmness and the cracking index (Perreira et al., 2020). In our case, the correlation is significant at the 0.05 level and highlight big differences between the CI values of ‘Special’ and ‘Tentant’ varieties (Table 3).

Variations of **pH** can influence taste, flavour, consistency, fruit shelf life and depreciation of juice. Comparing to the finding of Kappel et al. (1996) where the pH was around 3,8 and those reported by Skrzyński et al. (2016), where 10 of the 14 sweet cherry genotypes had pH below 3.5 and only four cultivars had higher pH value than 3.8 our sweet cherry varieties significantly varied between 3.44 at ‘Severin’ to 3.62 at ‘Tentant’.

Table 3. Correlations between cracking index and the biometric attributes of the sweet cherry varieties

	Cultivar	Weight (g)	Firmness (HPE)	pH	TSS (°Brix)	L*	a*	b*	CI
Cultivar	1	.821(**)	.070	-.262(*)	-.958(**)	.448(**)	.693(**)	.634(**)	-.905(**)
Weight (g)		1	.415(**)	-.027	-.817(**)	.452(**)	.651(**)	.565(**)	-.563(**)
Firmness (HPE)			1	.425(**)	-.097	.170	.169	.083	.250(*)
pH				1	.199	-.050	-.132	-.180	.440(**)
TSS (°Brix)					1	-.417(**)	-.661(**)	-.595(**)	.842(**)
L*						1	.885(**)	.921(**)	-.338(**)
a*							1	.972(**)	-.558(**)
b*								1	-.554(**)
CI									1

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

Under our specific experimental conditions, the increase in pH was positively correlated with the cracking index (Table 3).

Concerning the **total soluble solids**, the spectrum of values ranged from 11.78 °Brix at 'Severin' to 21.7 °Brix at 'Special' (Table 1). Even that Kappel et al. (1996) considered optimal a value of TSS = 15 for the harvest time, the specific color and the very balanced taste of the fruits assured us that the moment of harvesting for 'Severin' was the right decission. Similar values were also reported by Ruiz-Aracil et al. (2023), in the case of 'Early Lory' cultivar and after foliar applications of 0.5 mM MeJA.

Zheng et al. (2016) have shown that flavour and sweetness are main priorities in consumer acceptance, followed closely by colour, size and firmness of sweet cherry fruits. Also important we consider to research if there is any connection between the intensity of these attributes and the susceptibility to fruit cracking.

In our research, the correlation between TSS and CI is consistent and positive (Table 3) even that the differences between the varieties were statistically assured for both parameters.

Analyzing the 3 coordinates for **skin colour** of the fruits, we remarked that the bright red skin color of 'Severin' was the lightest one in contrast with 'Special' variety where the skin was darker (Table 2). Overall, 'Severin' cherries had recorded higher values than 'Special' and 'Tentant' cultivars in all parameters, and significant differences ($p < 0.05$) were found for all L*, a* and b* coordinates. We found significant negative correlation between skin colour and CI. Chełpiński et al. (2019) considered that the color measured with a spectrophotometer is a sufficient tool for appreciating the degree of ripening of the fruits. Low values of the coordinates L*, a* and b* generally assume darker and therefore over ripen fruits, correlated with higher CI values.

The lowest average value of **fruit cracking index** was recorded in the cultivar 'Severin' (6.4), while the highest value was fund for the cultivars 'Special' (38.4) and 'Tentant' (35.6). These values split the varieties in two out of the four groups established by Christensen (1972): 'Severin' in the group of low susceptible (CI < 10.0) and 'Special' and 'Tentant' in cracking susceptible group, were the cracking index values range between 30.1 and 50.0. We expect some differences in rankings and cracking index values depending on the year. For instance, Demirtaş and Aydinli (2020) found for one of the studied genotypes value of 5.7 for cracking index in the first year, and 43 for the 2nd year of experimentation.

CONCLUSIONS

Not all quality traits of the 'Severin', 'Special' and 'Tentant' Romanian sweet cherry varieties proved a strong correlation with the cracking index.

'Special' had the best coloured fruits, the highest TSS content and the lowest fruit firmness. The highest cracking index at the moment of the variety ripening time has to be connected to the rain fell on the eve of the harvest.

Some parameters such as the TSS content, better coloration of the fruit skin indicated a good correlation with the cracking index.

For the tested sweet cherry varieties, neither the firmness, nor the fruit weight did not confirm the expectation of a higher cracking index.

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THE EFFECT OF THE CULTURE SUBSTRATE ON THE CONTENT OF BIOACTIVE COMPOUNDS IN SOME RASPBERRY GENOTYPES

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Abstract

Raspberries are shrubs that belong to the genus Rubus idaeus L., Rosaceae's family. The raspberry culture is one of the most widespread among fruit bushes. Raspberries include a large number of varieties with different ripening periods, with summer and autumn fruiting of the remontant ones. The fruits of the genus Rubus are among those rich in bioactive compounds (anthocyanins, dietary fiber, vitamins, minerals and carbohydrates), so beneficial for human and animal health (Vega et al., 2021) The objective of the paper was to evaluate the bioactive compounds with antioxidant properties from raspberry fruits obtained from plants grown on different culture substrates, such as: manure, garden soil, forest compost, semi-fermented compost and spent mushroom substrate (SMS), applied to the soil. The studied plantation was established in the spring of 2020, and the presented results refer to the fruits harvested in 2022. The experiments were set up in the field within SCDP Băneasa - the Moara Domnească Afumati Experimental Farm.

***Key words**: raspberry cultivation, nutritive substrates, bioactive compounds.*

INTRODUCTION

Red raspberry (*Rubus idaeus* L.), a herbaceous plant in the Rosaceae family, is a shrub described as a nanophanerophyte. Raspberry, is an important commercial product in fresh or processed form due to its nutritional, medicinal and cosmetic uses (Gülcin et al., 2011; Papaioanou et al., 2018).

Raspberries contain high concentrations of important nutrients, bioactive compounds and phytochemicals. Raspberries are also an excellent source of vitamin C. It is well known that vitamin C has health and wellness attributes that make the fruit very popular among consumers (Papaioanou et al., 2018). Raspberries contribute to the nutritional value of a diet. They contain phytochemical components with documented biological activity, many of which were originally investigated for their *in vitro antioxidant properties* .

The chemical constitution of raspberry leaves, which are a by-product of raspberry processing, has not been studied as thoroughly as its fruit. In particular, the healing properties of raspberry leaves, known since ancient times, are prescribed for the treatment of a wide variety of diseases, for example, by including them in herbal preparations used to relax the uterus during childbirth. Other applications of raspberry leaves include their use as an additive to beverages, nutritional supplements, functional herbal preparations, teas and chocolate to enhance their nutritional and flavor-forming properties (De Santis et al., 2022; Wu et al., 2022). *Rubus* fruits have been shown to be an increasingly important source of bioactive substances due to their antioxidant, anti-inflammatory, chemopreventive and antibacterial properties, positive effects on blood lipids and atherosclerosis mentioned above, as well as their advantageous composition. Therefore, due to their biological effect, they can potentially be applied as a health promoting factor (Schulz and Chim, 2019).

The objective of the work was the evaluation of the bioactive compounds with antioxidant properties from raspberry fruits (*Rubus idaeus* L.) in correlation with their culture substrate.

MATERIALS AND METHODS

The study was carried out at the Moara Domnească Experimental Base, located NE of Bucharest (in Câmpia Vlăsiei, a subunit of the Romanian Plain), in Ilfov County, just about 17 km from Bucharest. The farm belongs to the Research and Development Station for Pomiculture (RDSFG) Băneasa.

The experimental plot was established in early spring 2020 by planting three varieties of raspberry (*Rubus idaeus* L.): "Citria", "Przehyba" and "Tulameen", on different nutrient substrates (peat moss, semi-fermented compost, forest compost, and mixture of the 4 substrates in equal amounts), at distances of 3.0 m between rows and 0.5 m apart in each row.

The environment in which the plant grows and develops is one of the most important factors in agriculture. Substrates must be able to provide adequate water, nutrients and oxygen for the plant, as well as support for the whole plant. (Kang et al., 2004; Miller and Jones, 1995)

The better the substrate, the healthier and more vigorous the plant

Biochemical investigations were carried out at the Faculty of Biotechnologies of USAMV-Bucharest, using spectrophotometric determination methods.

Determination of total ascorbic acid content in fruit juices was performed by spectrophotometric method using potassium permanganate ($KMnO_4$) as chromogenic reagent (Zanini et al., 2018; Elgailani et al., 2017). The concentration of ascorbic acid in the samples was expressed in mg/L.

Determination of total anthocyanins content

Total anthocyanins content (TA) was carried out using the pH differential spectrophotometric method (Giusti & Wrolstad, 2000).

Determination of total phenolics

For determination of TPC, a method with Folin - Ciocalteu reagent (Sigma-Aldrich) (Singleton, 1999)

Determination of antioxidant activity

It was determined by two methods: the DPPH (2,2-diphenyl-1-picrylhydrazyl) method and the phosphomolybdate method for total antioxidant capacity.

Statistical analysis

All measurements were carried out in three replicates (n = 3) and results were presented as means ± standard deviations (SD). Anova test and Duncan test were performed with SPSS software.

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RESULTS AND DISCUSSIONS

The biological material consisted of three varieties of raspberry (*Rubus idaeus* L.): "Citria", "Przehyba" and "Tulameen", from the Băneasa Pomiculture Research and Development Station - Bucharest. A brief characterization of the three raspberry cultivars and the general appearance of the fruit are presented in Table 1.

Table 1. General description of the raspberry genotypes studied

Genotype	General description	The appearance of the fruit
"Citria"	**The "Citria" raspberry (origin Romania)** is a variety with yellow fruits. It forms a bush with a tall stem, with few branches and medium sprouting capacity. The fruits are medium-sized, short-shaped, conical, with small seeds, yellow in color and excellent taste. The planting period is spring and autumn, and the harvesting period in June. The fruits can be consumed fresh or processed. It is an extremely early variety, adaptable to different environmental conditions, resistant to frost and raspberry diseases and is very productive.	
"Przehyba"	**"Przehyba" (origin Poland)** is an early variety, created in recent years and appeared on the market in 2016. It is available from autumn 2017 for testing, and from 2018 for professional crops. It keeps very well, has good firmness and withstands transport in suitable packaging. The Przehyba raspberry belongs to seasonal varieties with an early ripening period. The fruits are elongated, cylindrical, of a bright red, of sizes that come out of any pattern, up to 5 cm in length, very sweet and tasty. It is a dessert variety.	
"Tulameen"	**The "Tulameen" raspberry variety (origin Canada**), is a variety that easily adapts to environmental conditions, being widespread in many climate zones sweet noted for its excellent flavor. For optimal development, it needs a rich, deep soil that retains water quite well but not with excess moisture and sunny exposure. The fruit is very large, weighing between 5-7 g, intense red, shiny, conical, elongated. Fruit characters of Tulamen, apparent derived from black raspberries,include late ripenng and relatively firm textured fruit.	

The soil at Moara Domnească is a reddish prevosol. Several soil profile analyses were carried out in the in-house agrochemical laboratory to determine the soil's physico-chemical properties.

The following soil characteristics were determined (by particle size analysis to determine the clay, dust and sand content of the soil): a high percentage of clay ranging from 40.55% in the upper horizon 0-40 cm to 41.63% at depths of 41-53 cm and 47.39% at depths greater than 54 cm (Table 2).

Table 2. The granulometric composition of the soil (Experimental Base Moara Domnească, 2019)

Horizon	Depth (cm)	Clay (%)	Coarse sand (%)	Fine sand (%)	Dust (%)	Texture
Ao	0-40	40.55	0.36	34.33	24.75	Clay loam
Ao/Bt	41-53	41.63	0.52	21.54	56.28	Clay loam
Bt	54-200	47.39	0.37	27.59	30.34	Clay loam
C	Over 200	36.18	0.42	32.04	32.04	Clay loam

The clay texture results in low nutrient mobility and poor soil water permeability.
Soil humus content is good in the first 40 cm of the profile, where most of the roots of young trees, reaching a value of 3.26%, then drops sharply to 1% in the Bt horizon profile (Table 3).

Table 3. Physical and chemical properties of the profile soil (Experimental Base Moara Domnească, 2019)

Properties \ **Horizons**	Ao	Ao/Bt	Bt	C
Humus (%)	3.26	1.87	1.0	1.0
Soluble Ca (mg/100 g soil)	55	32	32	30
Hydrolitic acidity (meq)	2.8	2.04	1.72	0.18
Exchangeable Bases (meq)	22.6	23.62	26.28	-
Total cation exchange capacity (meq)	28.65	28.04	30.01	-
Degree of saturation in bases (%)	78.94	84.28	87.53	-
pH	6.4	6.6	6.8	8.3
Total N (%)	0.144	0.102	0.075	0.07
Soluble P (mg / 100 g soil)	50	40	40	30

The pH is slightly acidic at the soil surface (6.4), reaching alkaline in the C horizon (8.3). Other indicators such as nitrogen index (NI), hydrolytic acidity, humus, organic carbon were determined during 2020
The climate at Moara Domnească is temperate continental
The plants were grown on different nutrient substrates (peat moss, semi-fermented compost, forest compost, and a mixture of the 4 substrates in equal amounts) (Table 4).

Table 4. Variants of nutrient substrates in which blackberry varieties are planted

Nr. crt.	Variants of nutrient substrates
1	Control
2	Bramble
3	Mushroom Compost
4	Forest Compost
5	Semifermented Compost
6	Mixture Compost 25 % of the 4 substrate variants

Biochemical determinations were performed by analyzing fresh raspberry fruit from "Citria", "Przehyba" and "Tulameen", Fruits were obtained from plants grown on different culture substrates: peat moss, compost from mushroom growing substrates, forest compost, semi-fermented compost and compost substrates, forest compost and semi-fermented compost). The plants produced fruit on all substrates used for their culture.

Determination of total ascorbic acid content
The determination of the total content of ascorbic acid in fruit juices was carried out by the spectrophotometric method using potassium permanganate ($KMnO_4$) as a chromogenic reagent (Zanini et al., 2018; Elgailani et al., 2017). The decrease in absorbance was measured when a potassium permanganate solution was reacted with ascorbic acid solution in acid medium.
The results for the total ascorbic acid content (g/L) of blackberry fruit are summarised in Table 5 and Figure 1.

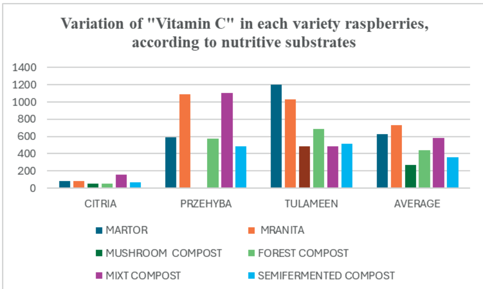

Figure 1. Variation in total ascorbic acid content (g/L) of three varieties of raspberry as a function of nutrient substrates

To raspberry fruits from the "Citria" variety obtained from the plants grown on different substrates, it was found that, compared to the control substrate (84.90 ± 1.02 mg/L), the highest amounts of vitamin C were recorded in the fruits of the plants grown on the substrate

(157.35 ± 1.20 mg/L) represented by the compost obtained from the mixture of 25% each of the other substrates taken into the study (Mraniță; Mushrooms Compost; Forest compost; Semi-fermented compost).In the case of fruits from plants grown on the other substrates, the vitamin C content varied between 50.28 ± 0.47 mg/L and 79.40 ± 0.47 mg/L (Figure 1).

Table 5. Total ascorbic acid content (mg/L) of raspberry fruit

Descriptive Statistics

Dependent Variable: Total Ascorbic Acid (mg/l)

Substrat	Variety	Mean	Std. Deviation	N
CONTROL	CITRIA	84.90	1.02	3
	PRZEHYBA	592.13	0,61	3
	TULAMEEN	1204.29	1.72	3
	AVERAGE	627.11	1.12	9
BRAMBLE	CITRIA	79.4	0,47	3
	PRZEHYBA	1087	1.00	3
	TULAMEEN	1027.86	1.77	3
	AVERAGE	731.42	1.08	9
MUSHROOM COMPOST	CITRIA	50.28	0,47	3
	PRZEHYBA	-	-	3
	TULAMEEN	484.67	1.01	3
	AVERAGE	267.48	0.74	9
FOREST COMPOST	CITRIA	56.1	0,48	3
	PRZEHYBA	574.00	1.0	3
	TULAMEEN	688.33	1.04	3
	AVERAGE	439.48	0.84	9
MIXTURE COMPOST	CITRIA	157.35	1.20	3
	PRZEHYBA	1106	1.00	3
	TULAMEEN	485.17	1.04	3
	AVERAGE	582.84	1.08	9
SEMIFERMENTED COMPOST	CITRIA	67.72	0.50	3
	PRZEHYBA	485.67	0.76	3
	TULAMEEN	514.33	0.76	3
	AVERAGE	355.91	1.01	9

In the raspberry fruits of the "Przehyba" variety, the highest values of vitamin C compared to the control (592.13 ± 0.61 mg/L) were obtained in the fruits from the plants grown on the substrates (mixed compost) (1106 ± 1.0 mg/L) and (bramble) (1087 ± 1.0 mg/L) (Figure 1).

On the substrate variant (compost from mushroom culture) no fruit was obtained until the time of harvest, as a result the experiments could not be performed. On the substrate variant forest compost, the ascorbic acid content (574.00 ± 1.0 mg/L) was close to that obtained from the fruits of plants grown on the control variant (592.13 ± 0.61 mg/L). The lowest values of vitamin C, 485.67 ± 0.76 mg/L, were recorded in the fruits of plants grown on semi-fermented compost substrate (Table 5, Figure 1).

In the raspberry fruits from the "Tulameen" variety, the highest values regarding the total content of ascorbic acid (vitamin C) were obtained in the case of the fruits from plants grown on bramble substrate (1027.86 ± 1.77 mg/L) and from the control variant (1204.29 ± 1.72 mg/L), followed by the variant (forest compost substrate) with 688.33 ± 1.04. In the fruits obtained from the variants (484.67 ± 1.01 mg/L) (514.33 ± 0.76 mg/L) and (485.17 ± 1.04 mg/L), the values were relatively close (Table 5, Figure 1).

From the data regarding the total content of ascorbic acid (vitamin C) in the raspberry fruits obtained from the plants grown on the different culture substrates, it can be noted that the mixed compost substrate (V5) was conducive to the accumulation of vitamin C in large quantities in the case fruits from the "Citria" and "Przehyba" varieties. In the "Tulameen" raspberry variety, vitamin C recorded significant levels in the control and substrate variant V1 (bramble). The V1 variant was conducive to the accumulation of vitamin C also in the case of the "Przehyba" variety.

Among the 3 analyzed varieties, the highest levels of vitamin C are found in the "Tulameen" and "Przehyba" varieties.

Determination of total monomeric anthocyan

The intense red color of the raspberry itself is related to its composition, including the content of anthocyanins, the concentration of which is influenced by several factors, such as the variety, the stage of ripening and the climatic and soil characteristics of the cultivation areas, among others Anthocyanins, and by extension raspberry color, are important elements for growers because they improve consumers' perception of quality.

The results regarding the total content of anthocyanins (mg equivalent of cyanidin-3-glucoside/L) from raspberry fruits resulting from plants grown on different substrates are summarized in Table 6 and Figure 2.

Table 6. Total monomeric antocyan content (mg/L) of raspberry fruit

Descriptive Statistics				
Dependent Variable: Total Ascorbic Acid (mg/l)				
Substrat	Soi	Mean	Std. Deviation	N
CONTROL	CITRIA	1.4	0.1	3
	PRZEHYBA	50.6	0.65	3
	TULAMEEN	62.05	0.21	3
	AVERAGE	38.02	0.32	9
BRAMBLE	CITRIA	5.49	0.02	3
	PRZEHYBA	20.19	0.3	3
	TULAMEEN	76.33	0.6	3
	AVERAGE	34.00	0.31	9
MUSHROOM COMPOST	CITRIA	1.82	0.02	3
	PRZEHYBA			3
	TULAMEEN	66.5	0.49	3
	AVERAGE	34.16	0.26	9
FOREST COMPOST	CITRIA	6.33	0.24	3
	PRZEHYBA	6.7	0.99	3
	TULAMEEN	99.08	0.26	3
	AVERAGE	58.37	0.50	9
MIXTURE COMPOST	CITRIA	5.99	0.01	3
	PRZEHYBA	37	0.23	3
	TULAMEEN	82.92	0.21	3
	AVERAGE	41.97	0.15	9
SEMIFERMENTED COMPOST	CITRIA	4.99	0.01	3
	PRZEHYBA	40.96	0.11	3
	TULAMEEN	59.62	0.52	3
	AVERAGE	35.19	0.21	9

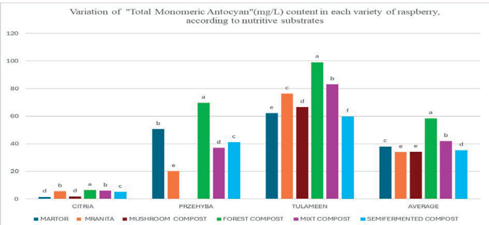

Figure 2. Variation of Total monomeric antocyan (mg/L) content of three varieties of raspberry as a function of nutrient substrates

The raspberry variety "Citria" is a variety with yellow fruits, as a result the total content of anthocyanins in mg cyanidin-3-glucoside equivalents / L was lower than in the raspberry varieties with red fruits.

In the results of this work, in the fruits of "Citria" obtained from plants grown on different substrates, high values of anthocyanins were noted, relatively close, in the case of the fruits obtained from the substrate variants of: forest compost (6.33 ± 0.24 mg/L), semi-fermented compost (5.99 ± 0.01 mg/L), bramble substrate (5.49 ± 0.02 mg/L) and respectively on the mixed compost substrate (4.99 ± 0.01 mg /IT). The lowest anthocyanin values were recorded in the control (1.40 ± 0.1 mg/L) and the substrate variant represented by compost from mushroom culture (1.82 ± 0.02 mg/L) (Table 6 and Figure 2).

In the raspberry variety "Przehyba" with bright red fruits, the highest amount of anthocyanins, 69.7 ± 0.99 mg ECy 3-Glu/L, was recorded in the case of the fruits of the plants grown on the forest compost variant (V3), followed by the control variant with 50.6 ± 0.65 mg ECy 3-Glu/L and respectively by V5 (mixed compost) with 40.96 ± 0.11 mg ECy 3-Glu/L and V4 (semi-fermented compost) with 37.0 ± 0.23 mg ECy 3-Glu/L. The lowest values of anthocyanins were recorded in the fruits of the plants grown on the V1 variant of the bramble substrate (20.19 ± 0.30 mg ECy 3-Glu/L) (Table 6 and Figure 2).

In the **raspberry variety "Przehyba"** with bright red fruits, the highest amount of anthocyanins, 69.7 ± 0.99 mg ECy 3-Glu/L, was recorded in the case of the fruits of the plants grown on the forest compost variant (V3), followed by the control variant with 50.6 ± 0.65 mg ECy 3-Glu/L and respectively by V5 (mixed compost) with 40.96 ± 0.11 mg ECy 3-Glu/L and V4 (semi-fermented compost) with 37.0 ± 0.23 mg ECy 3-Glu/L. The lowest values of anthocyanins were recorded in the fruits of the plants grown on the V1 variant of the bramble substrate (20.19 ± 0.30 mg ECy 3-Glu/L) (Table 6 and Figure 2).

In the **"Tulameen" raspberry variety** with intense red fruits, the results revealed that the highest content of anthocyanins was in the fruits from plants grown on the forest compost substrate (V3), with 99.08 ± 0.26 mg ECy 3-Glu/L, followed by the data obtained from the fruits of plants grown on V4 - semi-fermented compost - with 82.92 ± 0.21 mg ECy 3-Glu/L and V1 - blackberry with 76.33 ± 0.60 mg ECy 3-Glu/L. On the variants of substrates V2 (compost from mushrooms), control substrate (C) and V5 (mixed substrate) the results were relatively close. The lowest values of the anthocyanin content were recorded for the substrate variant V5 - compost mixture (Table 6 and Figure 2).

Following the results obtained regarding the content of anthocyanins in the fruits of plants grown on different substrates, it is noted that the most favorable substrate for the accumulation of anthocyanins in the fruits was represented, for all three raspberry varieties, by the compost substrate of forest (V3). Among the 3 raspberry varieties analyzed, the "Tulameen" and "Przehyba" varieties have the highest levels of anthocyanins.

Anthocyanins are a group of natural phenolic compounds responsible for the color of many plants, flowers and fruits. These compounds are of great importance due to their proven pharmacological activities. Forest fruit extracts (*Rubus* spp.) contain large amounts of anthocyanins, and their consumption either in food or in some therapeutic applications is common. The anthocyanin composition of the fruits of different species of Rubus species are quite distinct. Raspberry (*R. idaeus*) contains a distinct spectrum of anthocyanins (Dugo et al., 2001). The major components are cyanidin and cyanidin-3-sophoroside, with smaller amounts of other anthocyanins, including cyanidin-3-(2G-glucosylrutinoside), cyanidin-3-glucoside, cyanidin-3-rutinoside, pelargonidin and its glycosides. More recently, Chen et al. (2007) established an ultrasound-assisted extraction of anthocyanins from *R. idaeus*. The anthocyanin composition of the extracts was identified by high-performance liquid chromatography-mass spectrometry. The content of anthocyanins, found in large quantities in red raspberries, is influenced by several factors, such as: the variety, the ripening stage and the climatic and pedological characteristics of the cultivation areas, among others (Stamenkovic et al., 2019).

Determination of total phenol content

The total phenolic content, expressed as gallic acid equivalent, **in "Citria" with yellow fruits,** originating from plants grown on different substrates, varied from 355.86 ± 1.78 mg EAG/L, with the highest value (recorded in the sample control), at 175.38 ± 1.26 mg EAG/L (value recorded in samples V5- compost mixture). Among the samples from plants grown on different substrates, the highest values were recorded in sample V4 – semi-fermented compost (197.32 ± 1.13 mg EAG/L). The other samples had close values (Table 5 and Figure 2). **In the red fruits of raspberry cv. "Przehyba"**, the highest values (125.67 ± 1.13 mg EAG/L) were recorded in sample V3 (forest compost), and the lowest (85.78 ± 0.80 mg EAG/L) in sample V1 (bramble). With the exception of the compost substrate variant from mushroom culture for which no fruits were obtained until the analysis was carried out, the control sample (M) and the other samples (V4 and V5) recorded close values (Table 7 and Figure 3 . **In the case of red raspberry fruits cv. "Tulameen"**, the most significant values were noted in samples V4 (256.43 ± 0.89 mg EAG/L) and V3 (220.41 ± 1.13 mg EAG/L), and the lowest in the control sample (137.41 ± 0.88 mg EAG/L) (Table 7 and Figure 3).

As noted from the obtained results, the concentration of polyphenols can vary depending on the variety and the specific nutritional needs of each variety, provided by the culture substrate. Probably, the raspberry studied had different climatic and agrotechnical conditions during growth, which it could have a significant impact on the amount of analyzed compounds. Contaminants from fertilizer use and the lower nutritional quality of the fruit could affect the low concentration of these compounds.

Table 7. Total Poliphenoli content (mg EAG/L) of raspberry fruit

Descriptive Statistics

Dependent Variable: Total Ascorbic Acid (mg/l)

Substrat	Variety	Mean	Std. Deviation	N
CONTROL	CITRIA	355.86	1.78	3
	PRZEHYBA	110.51	1.02	3
	TULAMEEN	137.41	0.88	3
	AVERAGE	201.26	1.23	9
BRAMBLE	CITRIA	188.08	0.47	3
	PRZEHYBA	85.78	0.80	3
	TULAMEEN	176.74	1.23	3
	AVERAGE	150.20	0.83	9
MUSHROOM COMPOST	CITRIA	187.72	1.48	3
	PRZEHYBA			3
	TULAMEEN	169.33	1.41	3
	AVERAGE	178.53	1.45	9
FOREST COMPOST	CITRIA	181.22	0.83	3
	PRZEHYBA	125.67	1.13	3
	TULAMEEN	220.41	0.89	3
	AVERAGE	175.77	0.95	9
MIXTURE COMPOST	CITRIA	197.32	1.13	3
	PRZEHYBA	112.23	1.24	3
	TULAMEEN	256.43	0.89	3
	AVERAGE	188.66	1.09	9
SEMIFERMENTED COMPOST	CITRIA	175.38	1.26	3
	PRZEHYBA	115.74	0.81	3
	TULAMEEN	166.49	1.24	3
	AVERAGE	152.54	1.10	9

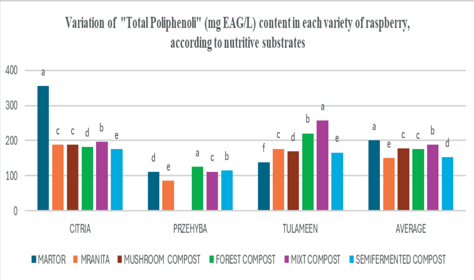

Figure 3. Variation of total poliphenoli content (mg EAG/L) of three varieties of raspberry as a function of nutrient substrates

Polyphenols can act in several ways: as reducing agents, as compound that blocks free radicals, chelates metal ions that catalyze oxidation reactions (thus preventing reactions caused by a single active oxygen atom) and inhibits the activity of oxidative enzymes such as lipoxygenases. Polyphenols have an ideal chemical structure to scavenge free radicals and form chelates with metals, making them more effective than antioxidant vitamins (Ruskovska et al., 2020; Kruk and et al., 2022).

Determination of Total oxidant capacity (µg/ml)

The total antioxidant capacity of the samples was evaluated by the phosphomolybdate method, according to the procedure described by Prieto (Prieto et al., 1999). The results were expressed in µg equivalent of ascorbic acid/ml.

In the phosphomolybdenum test, which is a quantitative method for evaluating antioxidant capacity, all analyzed samples presented different degrees of activity, as shown in Table 7 and Figure 2. **In the "Citria" cultivar**, all the samples obtained from different substrates showed an antioxidant activity ranging between 306.92 ± 1.77 µg/ml of ascorbic acid (for the V1 variant - bramble substrate) and 202.79 ± 0.78 µg/ml of ascorbic acid (at V2 – mushroom compost). A significant total antioxidant activity of 284.01 ± 1.54 µg/ml of ascorbic acid was also recorded in fruit samples from plants grown on semi-fermented compost (V4).

In the "Przehyba" cultivar, the highest antioxidant activity was recorded in fruit samples from plants grown on forest compost substrate (V3) (206.76 ± 0.63 µg/ml of ascorbic acid), on semi-fermented compost substrate (V4) (143.47 ± 0.17 µg/ml of ascorbic acid) and on mixed compost substrate (V5) (138.53 ± 0.42 µg/ml). The lowest antioxidant activity was presented by the fruit samples from the plants grown on bramble substrate (V1) (108.77 ± 0.16 µg/ml). On the substrate variant V2 - the fruits did not develop.

In the "Tulameen " variety, in the raspberry samples from different substrates, a significant total antioxidant capacity is possessed by the fruit samples from the forest compost substrate (V3) (211.35 ± 0.63 µg/ml) and respectively those from the substrate of sea buckthorn (V1) (201.48 ± 1.49 µg/ml). The sample from the mushroom compost substrate (V2) presented the lowest antioxidant activity, equivalent to 143.16±0.77 µg/ml of ascorbic acid (Table 8, Figure 4).

Table 8. Total oxidant capacity(µg/ml) content of raspberry fruit

Descriptive Statistics				
Dependent Variable: Total Ascorbic Acid (mg/l)				
Substrat	Variety	Mean	Std. Deviation	N
CONTROL	CITRIA	275.92	1.05	3
	PRZEHYBA	141.23	0.25	3
	TULAMEEN	232.37	1.21	3
	AVERAGE	216.51	0.84	9
BRAMBLE	CITRIA	306.92	1.77	3
	PRZEHYBA	108.77	0.16	3
	TULAMEEN	201.48	1.49	3
	AVERAGE	205.72	1.14	9
MUSHROOM COMPOST	CITRIA	202.79	0.78	3
	PRZEHYBA			3
	TULAMEEN	143.16	0.77	3
	AVERAGE	172.98	0.78	9
FOREST COMPOST	CITRIA	227.31	1.18	3
	PRZEHYBA	206.76	0.63	3
	TULAMEEN	211.35	0.63	3
	AVERAGE	215.14	0.81	9
MIXTURE COMPOST	CITRIA	284.01	1.54	3
	PRZEHYBA	143.47	0.17	3
	TULAMEEN	170.51	0.13	3
	AVERAGE	199.33	0.61	9
SEMIFERMENTED COMPOST	CITRIA	230.49	1.61	3
	PRZEHYBA	138.53	0.42	3
	TULAMEEN	161.19	1.06	3
	AVERAGE	176.74	1.03	9

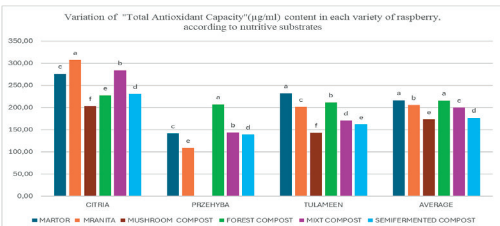

Figure 4. Variation of Total oxidant capacity (µg/ml) content of three varieties of raspberry as a function of nutrient substrates

Determination of antioxidant activity using DPPH radical scavenging activity . Fruit samples were evaluated for antioxidant activity using the DPPH method and were expressed as percentage DPPH · inhibition (Table 9)

Table 9. Total Antioxidant activity of the samples expressed as DPPH (RSA %) content of raspberry fruit

variants	Substrate type/variety	DPPH RSA%*		
		Citria	Przehyba	Tulameen
m	Blank substrate	**87.14±0.38**	**94.37±0.24**	**88.22±0.48**
V1	garden soil	61.38±0.48	59.92 ±0.79	82.01±0.33
V2	Compost from mushrooms	78.58±0.09	when	**90.79±0.45**
V3	Forest compost	77.26±0.98	59.93±0.79	80.61±0.66
V4	Semi-fermented compost	**88.30±0.25**	**79.93±0.73**	83.50±0.86
V5	Compost mix	**88.40±0.09**	**77.48±0.20**	**88.11±0.57**

Regarding the antioxidant activity of the plant raspberry fruits, significant differences were found between the control samples of the three cultivations and the tested samples. **In "Citria",** a strong antioxidant activity - with approximately the same values, was found both in samples V4 - semi-fermented compost (88.30 %) and V5 - compost mixture (88.40%), as well as in the control sample (87.14%) (Table 9). **In "Przehyba "**, a high antioxidant activity was registered especially in the control sample (94.37%) and similar to "Citria", in samples V4 - semi-fermented compost (79.93%) and V5 - mixed compost (77.48%). **The fruits of the "Tulameen" variety** also recorded antioxidant activities of over 80%, among which the sample grown on mushroom compost substrate (V2) (90.79%) stands out.

The strong antioxidant activities of these raspberry samples may be a possible result of the high level of phenolic compounds and flavonoids that have been shown to have antioxidant properties (Zheng et al., 2009). These results were consistent with previous studies that reported that phenolic compounds in various plant extracts are the major constituents with the free radical scavenging property of donating a hydrogen atom from their phenolic phenolic hydroxyl (Wang et al., 2011).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical that is frequently used in the measurement of antioxidant activities due to the following strengths: direct inhibition measurement, simplicity, and rapid analysis (Mammadov et al., 2011).

The results of the antioxidant evaluation based on the two models (DPPH and Total Antioxidant Capacity) used in this study , showed that raspberry samples from plants grown on different substrates possess an interesting antioxidant activity that varies depending on the culture substrate and the variety of raspberries that benefited from these substrates.

Table 10 includes the data obtained regarding the biochemical compounds with antioxidant activity in raspberry fruits in correlation with their culture substrates.

Table 10. Biochemical compounds with antioxidant activity in raspberry fruits harvested from plants grown on different substrates

	The variety	VIT. C (mg/L)	ANTHOCYANS (mg/L)	Phenols mg GAE/L	DPPH RSA%	CAT µg/ml Total antioxidant capacity
1	**Citria -witness**	**84.90±1.02**	**1.40±0.1**	**355.86±1.78**	**87.14±0.38**	**275.92±1.05**
2	**V1**	79.40±0.47	5.49±0.02	188.08±0.47	61.38±0.48	306.92±1.77
3	**V2**	50.28±0.47	1.82±0.02	187.72±1.48	78.58±0.09	202.79±0.78
4	**V3**	56.10 ± 0.48	6.33±0.24	181.22±0.83	77.26±0.98	227.31±1.18
5	**V4**	67.72 ± 0.50	5.99 ± 0.01	197.32±1.13	88.30±0.25	284.01±1.54
6	**V5**	157.35 ± 1.20	4.99 ± 0.01	175.38±1.26	88.40±0.09	230.49±1.61
7	**Przehyba - witness**	**592.13±0.61**	**50.6 ± 0.65**	**110.51±1.02**	**84.37±0.76**	**141.23±0.25**
8	**V1**	**1087 ± 1.0**	20.19±0.30	85.78±0.80	59.92 ±0.79	108.77±0.16
9	**V2**					
10	**V3**	574.00 ± 1.0	69.69 ± 0.99	125.67±1.13	59.93±0.79	206.76±0.63
11	**V4**	485.67 ± 0.76	37.00±0.23	112.23±1.24	79.93±0.73	143.47±0.17
12	**V5**	1106 ± 1.0	40.96±0.11	115.74±0.81	77.48±0.20	138.53±0.42
13	**Tulameen - witness**	**1204.29 ± 1.72**	**62.05 ± 0.21**	**137.41±0.88**	**88.22±0.48**	**232.37±1.21**
14	**V1**	1027.86 ± 1.77	76.33±0.60	176.74±1.23	82.01±0.33	201.48±1.49
15	**V2**	484.67 ± 1.01	66.5±0.49	169.33±1.41	90.79±0.45	143.16±0.77
16	**V3**	688.33 ± 1.04	99.08±0.26	220.41±.1.13	80.61±0.66	211.35±0.63
17	**V4**	514.33 ± 0.76	82.92±0.21	256.43±0.89	83.50±0.86	170.51±1.03
18	**V5**	485.17 ± 1.04	59.62±0.52	166.49±1.24	88.11±0.57	161.19±1.06

M: Witness; V1: Bramble; V2: Compost from mushrooms; V3: Forest compost; V4: Semi-fermented compost; V5: Compost mix.
* Data are expressed as mean and standard deviation (±SD) of three replicates (n= 3).

CONCLUSIONS

The results of the study led to the following conclusions:

- From the data regarding **the total content of ascorbic acid (vitamin C)** in the raspberry fruits obtained from the plants grown on the different culture substrates, it was noted that the **mixed compost substrate (V5)** was conducive to the accumulation of vitamin C in a significant amount in the case of the fruits from the "**Citria" and "Przehyba**" varieties.
- **"Tulameen"** raspberry variety , vitamin C recorded significant levels both in **the control variant and in the substrate variant V1 (mraniță**).
- The bramble substrate was conducive to the accumulation of vitamin C also in the case of the "Przehyba" variety.
- Among the 3 raspberry varieties analyzed, the highest levels of vitamin C were found in the "Tulameen" and "Przehyba" varieties (the last one without the V2 substrate variant);
- Following the results obtained regarding the content of anthocyanins in the fruits of plants grown on different substrates, it was noted that the **most favorable substrate for the accumulation of anthocyanins in the fruits was represented, in all three raspberry varieties, by the compost substrate of forest (V3).**
- Among the 3 raspberry varieties analyzed, the "Tulameen" and "Przehyba" varieties had the highest levels of anthocyanins.
- **For cv. "Citria"** with yellow fruits, the most favorable substrate both for the accumulation of phenolic compounds and for the two antioxidant activity evaluation models was the **semi-fermented compost substrate (V4)**; A significant accumulation of phenols, with a high total antioxidant activity, was also recorded in the case of "Citria" raspberry fruits grown on bramble substrate (V1);
- **For cv. "Przehyba"** with red fruits, the most favorable substrate for the three parameters analyzed was also **semi-fermented compost (V4);** Significant results were also obtained in the case of fruits from the substrate represented by forest compost, but only for the supply of phenols and for an increased total antioxidant capacity;
- **For cv. "Tulameen"** with red fruits, for the accumulation of phenolic compounds in high quantities, the favorable substrates were **forest compost (V3)** and semi-fermented compost **(V4)**; For the accumulation of compounds with DPPH antioxidant activity, **the growth substrates V2 (compost from mushrooms)** and **V5 (compost mixture) stood out** , and for the total antioxidant capacity and viability , the best results were obtained in the case of the samples from substrates V1 (bramble) and V3 (forest compost).

The results obtained may vary depending on the variety and the specific nutritional needs of each variety, provided by the culture substrate. Probably, the raspberry studied had different climatic and agrotechnical conditions during growth, which it could have a significant impact on the amount of analyzed compounds. Contaminants from fertilizer use and the lower nutritional quality of the fruit could affect the low concentration of these compounds.

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ASSESSMENT OF THE BIOCHEMICAL QUALITIES AND COLOUR PARAMETERS OF FRESH AND FROZEN FRUITS OF HIGHBUSH BLUEBERRIES GROWN IN BULGARIA

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Abstract

In recent decades, the attention of scientists has been focused on the fruits of Vaccinium L. due to their significant potential to be used in the food, pharmaceutical, and cosmetic industries. In the present experiment, the biochemical composition of fruits of four introduced highbush blueberry varieties (Bluecrop, Bluegold, Spartan, Toro) grown in a demonstration plantation of RIMSA-Troyan was analyzed. The changes in the biochemical composition of fresh and frozen fruits after 1-year of storage at - 18°C (in refrigerated conditions) were compared. The fresh fruits of Bluegold are distinguished by a higher content of total and inverted sugar, ascorbic acid, tannins, anthocyanins, and pectin than the other studied varieties. In Bluecrop fruits, after one year of storage under refrigerated conditions, higher content of dry matter weight, ascorbic acid, tannins, and pectin were reported. The best results in terms of colour brightness were reported for fruits of the Spartan variety (22.68).

Key words: *blueberry, chemical composition, colour parameters, storage, Vaccinium corymbosum L.*

INTRODUCTION

In conditions of a quite polluted environment and irrational nutrition, humanity pays attention to the connection between the fruit diet and the preservation of human health. Fresh fruits have always had an essential role in the traditional diet and healthy menu of people. They are used for fresh consumption and processing, such as freezing, production of fruit juices and preserves, drying, and as an additive in sugar products. Blueberry fruits consist of balanced amount of sugars (invert and sucrose), organic acids, vitamins, tannins, pectin, anthocyanins, polyphenols, etc., which are used in the food, pharmaceutical and medical industries (Hung et al., 2004; Puupponen-Pimiä et al., 2005; Kalt et al., 2007; Szajdek & Borowska, 2008; Petronelli et al., 2009; Gilbert et al., 2014; Lin et al., 2016; Correia et al., 2017; Shi et al., 2017; Dare et al., 2022; Ferrão et al. 2022; Felipe et al., 2022, Bai et al., 2023; Antal, 2024). Their biochemical composition and sensory evaluation are not constant, but they change depending on the variety, ripeness stage, abundance of the harvest, applied agrotechnical events, the influence of soil and climatic conditions in the growing region, altitude, etc. (Skupień, 2006; Bryla & Strik, 2007; Leposavić, 2014; Gilbert et al., 2015; Solovchenko et al., 2019; Spinardi, 2019; Niedbała et al., 2022).

The present study aims to evaluate the biochemical composition (qualitative characteristics) and colour parameters in fresh and frozen (-18°C) fruits of the genus *Vaccinium*.

MATERIALS AND METHODS

Fruits of four highbush blueberry varieties (Bluecrop - standard, Bluegold, Spartan, and Toro) were gathered from a demonstration plantation of RIMSA over three years.

The planting scheme is 3.00 m x 1.50 m, as inter and intra-row spacings are with natural grass cover. The area around the stems is maintained in black fallow using tillage. The collection plantation is located 474 m above sea level, on an eastern exposure slope. Drip irrigation was provided during the vegetation.

The heaviest rainfall in the period from March to July in the region was registered in the 2020 vegetation season (346 l/m^2), whereas the lowest amount was in 2021 (232.1 l/m^2). In

2022, the vegetation sum of average monthly rainfall (from March to July) was 253.3 l/m^2. In the last two years of the experimental period, the lowest reported vegetation precipitation amount for the last five years was reported (Atanasova, 2021).

The average monthly temperature sum from March to July does not differ significantly in the research period and is from 13.9°C (2020) to 14.3°C (2022).

The relative humidity for vegetation season from March to July within the three years is in the range of 69-81%, as the lowest was in 2022.

The varieties included in the study are distinguished by growth habitus, fruit ripening period, and resistance to abiotic and biotic stress factors.

Ripe fruits were picked by hand and put in polythene boxes. The following biochemical indicators of fresh and frozen fruits were observed (after storage one year of storage at -18°C in laboratory freezer, model Indesit):

- dry matter (DM) (%), total soluble solids (TSS) and sugars (total, invert and sucrose %) had been made, according to the methods described by Donchev et al., 2001;
- titratable acidity (malic acid %), ascorbic acid (mg %) and tannins substances (%) had been made according to the method of Donchev et al., 2000, using 1: 4 H2SO4, 0.1 N KM4O4 and indigo carmine as indicator for tannins quantification;
- total anthocyanins (mg %) was made using the method of Fuleki & Francis (1968);
- pectin according to the method of Melitz (Donchev et al., 2000) has been performed, using 0.1 N NaOH, CH3 COOH, CaCl 2, AgNO3;
- total polyphenols (mg/100 g) has been made using the method of Folin-Ciocalteu, (Singleton & Rossi, 1965).

Colour parmeteres

The indicators were reported according to the CIE Lab system. The colour coordinates L, a, and b were measured: (L) – colour brightness; (+a) - red; (-a) – green; (+ b) – yellow; (- b) – blue. The colour analysis has been made with Color meter CM-200S.

The colour tone value or dominant wavelength is represented by the ratio a/b.

The data were statistically processed by the software product MS Excel – 2010.

RESULTS AND DISCUSSIONS

Tables 1, 2, and 3 present the results for the main biochemical composition of four blueberry varieties, including dry weight matter, total soluble solids, total sugars, inverted sugar, sucrose, malic acid, ascorbic acid, tannins, anthocyanins, pectin and total polyphenols. Our study shows differences in some quality indicators among varieties.

Table 1. Biochemical composition of fruits of some highbush blueberry varieties, 2022 harvest

Indicators / Varieties	D.M (%)	TSS (%)	Total sugars (%)	Inverted sugar (%)	Sucrose (%)	Acids (as malic) (%)	Ascorbic acid (mg %)	Tannins (%)	Total anthocyanins (mg/%)	Pectin (%)	Total polyphenols mg/100 g
Bluecrop	18.74	14.00	8.90	8.90	0	0.20	17.60	0.219	20.32	1.02	229.73
Bluegold	17.38	12.50	16.70	14.30	2.28	0.27	21.12	0.263	49.03	1.51	197.31
Spartan	14.63	10.00	12.10	11.45	0.62	0.27	17.60	0.197	42.26	0.39	149.19
Toro	16.10	9.50	8.35	5.70	2.52	0.27	12.32	0.197	27.42	0.72	166.88
Mean	16.71	11.50	11.51	10.09	1.36	0.25	17.16	0.22	34.76	0.91	185.78
SE	0.88	1.06	1.92	1.83	0.62	0.02	1.81	0.02	6.60	0.24	17.70
St Dev	1.76	2.12	3.83	3.66	1.24	0.04	3.63	0.03	13.19	0.48	35.41
CV	10.52	18.45	33.30	36.32	91.28	13.86	21.14	14.21	37.96	52.26	19.06
Amplitude min - max	14.63-18.74	9.5-14	8.35-16.7	5.7-14.3	0-2.52	0.2-0.27	12.32-21.12	0.197-0.263	20.32-49.03	0.39-1.51	149.19-229.73

The comparative analysis shows that the dry matter weight in the fresh fruits is from 14.63% (Spartan) to 18.74% (for Bluecrop), with a low coefficient of variation of 10.52%.

The highest content of TSS was reported for Bluecrop with 14.00% and Bluegold with 12.50%. The average value is 11.50%, with an average coefficient of variation of 18.45%.

The content of carbohydrates in blueberry fruits includes various types of sugars (total, inverted sugar, and sucrose), which are quickly absorbed by the human body and are an indispensable energy source (Mondeshka, 2005).

The variation in the content of total sugars is significant. The highest content is reported for Bluegold with 16.70%, and Toro with 8.35%, whereas the lowest content is observed in Bluecrop with 8.90%. The average value of the indicator for varieties is 11.50%, with an average coefficient of variation of 33.30%. A similar coefficient of variation (36.32%) was also reported for inverted sugar. Bluegold has the highest content with 14.30%, whereas Toro shows the lowest with 5.70%. The average indicator among varieties is 10.09%. No sucrose was reported in Bluecrop, as it is from 0.62% (Spartan) to 2.52% (Toro), with a very high coefficient of variation.

In agreement with the present results, Leposavić, (2014) analyzed the main biochemical composition of the fruit of five blueberry genotypes (Bluecrop (control), Duke, Reka, Nui, and Ozarkblue) and found the highest amount of soluble dry matter, total and reducing sugars in the fruit of Ozarkblue, least of all in those of Nui.

It is a well-known fact that fruit acids are associated with accelerating the metabolism and increasing the defenses of the human body. In the present paper, they are represented by malic and ascorbic acid (Mondeshka, 2005). The established organic acids were in close values among the varieties in the range of 0.2-0.27%. The highest ascorbic acid content was found in Bluegold (21.12 mg/%), whereas the lowest was in Toro (12.32 mg/%). The average value of the indicator is 17.16%, with an average coefficient of variation of 21.14%.

The astringent taste of blueberry fruits is due to the content of tannins, which have anti-inflammatory properties for colds (Mondeshka, 2005). The largest amount was registered in Bluegold (0.263%) and Bluecrop (0.219%) varieties (VC-14.21%).

The highest anthocyanin values were observed in Bluegold (49.03 mg/%) and Spartan (42.26 mg/%), whereas the lowest were in Bluecrop (20.32 mg/%). The average content of anthocyanins is 34.76 mg/%, with a very high coefficient of variation (37.96%).

The highest pectin level was reported in Bluegold (1.51%), followed by Bluecrop (1.02%), and the lowest was in Spartan (0.39%).

Total polyphenol values are from 149.19 mg/100 g (Spartan) to 229.73 mg/100 g (Bluecrop). The average value of the indicator is 185.78%, with an average coefficient of variation of 19.06%.

Similar to our results were obtained by Skupień (2006), who compared the main biochemical composition of four highbush blueberry cultivars (Spartan, Bluecrop, Jersey, and Blueray) grown in an orchard in the Szczecin region. The author found that the fruits of Bluecrop had the highest total content of polyphenols, and those of Blueray were distinguished by the highest amount of total acids. The lowest total content of total polyphenols was analyzed in Jersey berries.

Frozen Fruits

Freezing fruits is one of the most practical methods of preservation and storage of their valuable biochemical components. The species and varietal specificity, agrotechnical practices, duration, and storage conditions are of primary importance for their preservation (Lohachoompol et al., 2004; Poiana et al., 2010; Celli et al., 2015; Stamenković et al., 2019; Žlabur et al., 2021; Wang et al., 2024;)

The changes that occurred in the biochemical composition of blueberry fruits after one year of storage at -18°C are presented in Table 2

The dry matter weight of the frozen fruits is from 15.84% (Toro) to 19.39% (Bluecrop), with a low coefficient of variation (CV-8.91%). More significant differences were observed compared to fresh fruit in the Spartan variety with approximately four units more in frozen fruit.

Table 2. Biochemical composition of frozen fruits of some highbush blueberry varieties, after 1 year of storage at -18°C

Indicators / Varieties in 2022	D.M (%)	TSS (%)	Total sugars (%)	Inverted sugar (%)	Sucrose (%)	Acids (as malic) (%)	Ascorbic acid (mg %)	Tannins (%)	Total anthocyanins (mg/%)	Pectin (%)	Total polyphenols mg/100 g
Bluecrop	19.39	13	3.70	3.70	0	0.13	10.56	0.109	4.52	0.66	247.37
Bluegold	16.73	12	2.85	2.85	0	0.13	10.56	0.088	13.55	0.42	162.75
Spartan	18.13	13.50	4.05	4.05	0	0.20	8.80	0.088	1.94	0.20	238.24
Toro	15.84	11.50	3.20	3.20	0	0.27	8.80	0.066	1.29	0.20	336.99
Mean	17.52	12.50	3.45	3.45	-	0.18	9.68	0.09	5.33	0.37	246.34
SE	0.78	0.46	0.27	0.27	-	0.03	0.51	0.01	2.83	0.11	35.67
St Dev	1.56	0.91	0.53	0.53	-	0.07	1.02	0.02	5.66	0.22	71.35
CV	8.91	7.30	15.38	15.38	-	36.72	10.50	20.01	106.25	59.30	28.96
Amplitude min - max	15.84-19,39	11.5-13.5	2.85-4.05	2.85-4.05	-	0.13-0.27	8.8-10.56	0.066-0.109	1.29-13.55	0.2-0.66	162.75-336.99

The highest level of refractometric solids is found in Spartan (13.50%) and Bluecrop (13.00%). Their increase is more significant again in Spartan (13.50%), compared to their amount in fresh fruit. The average value of the indicator is 12.50%. A significant decrease is reported in the total sugars of frozen fruit, compared to the fresh ones, which are from 2.85% (Bluegold) to 4.05% (Spartan) (CV-15.38%).

A decrease in the amount of inverted sugar was registered and no sucrose was detected for all frozen fruits.

No significant changes were observed in the values of organic acids compared to those of fresh fruit. A decrease in the ascorbic acid content was reported, compared to those in fresh fruit, which was from 8.80 mg/% (for Spartan and Toro) to 10.56 mg/% (for Bluecrop and Bluegold), with a coefficient of variation of 10.50%.

A strong decrease in the content of tannins compared to the starting raw material was reported, with a high coefficient of variation - 20.01%. The lowest amount is reported for Toro with 0.066%, and the highest for Bluecrop with 0.109%.

The decrease of anthocyanins in frozen fruits is several times and with a very high coefficient of variation, with the lowest values reported for Toro - 1.29 mg/%, Spartan - 1.94 mg/%, and Bluecrop - 4.52 mg/%, and the highest is that of Bluegold - 13.55 mg/%.

A decrease in the pectin level was observed, as it was most pronounced in Bluegold with 0.42%, compared to fresh fruit. Its content is from 0.20% (Spartan and Toro) to 0.66% (Bluecrop).

Regarding the total polyphenols, a decrease in their amount in the frozen fruit was observed only in Bluegold (162.75 mg/100 g), compared to fresh fruit. The most significant increase was found in Toro (336.99 mg/100 g) and Spartan (238.24 mg/100 g), compared to the starting material (VC - 28.96%). The average value of total polyphenols in the varieties is 246.34 mg/100 g.

Comparing the brightness of the color of the blueberry varieties studied, the highest value was reported for the fruits of the Spartan variety (22.68) and the lowest for Bluegold (8.87). The average value for the varieties is 16.78 (Table 3).

The lowest values for the red colour component were measured in Toro (0.50) and the highest in Bluegold (5.97).

For the established blue colour component, the best results were reported for the Bluegold variety and the lowest values for the Bluecrop.

The quality indicator of colour tone has the highest value for fruits of the Spartan variety (15.09).

Table 3. Colour parameters of frozen fruits of different highbush blueberry varieties

Varieties \ Colour parameters	L	a	b	a/b
Bluecrop	19.15	1.54	- 1.60	-5.24
Bluegold	8.87	5.97	- 0.11	-60.46
Patriot	15.94	5.05	- 0.93	-0.31
Spartan	22.68	4.20	- 0.61	15.09
Toro	17.28	0.50	- 0.85	-0.75
Mean	16.78	3.45	-0.82	-10.33
SE	2.28	1.04	0.24	12.99
St Dev	5.10	2.34	0.54	29.06

CONCLUSIONS

The obtained results of the biochemical analyses show that the valuable components in the fruits of highbush blueberries are preserved to a significant extent even after freezing at -18 ^{0}C for one year. The reported differences in biochemical composition and colour parameters are probably due to the diverse soil and climatic conditions during the vegetation combined with the biological characteristics of the varieties within the experimental period.

Fresh Bluegold berries had a higher content of total and inverted sugar, ascorbic acid, tannins, anthocyanins, and pectin of all the varieties studied.

After one year of storage, Bluecrop fruits had a higher content of dry matter weight, ascorbic acid, tannins, and pectin, whereas the Spartan fruits had a higher content of refractometric solids, total and inverted sugar.

Biologically active substances, ascorbic acid, and anthocyanins are best preserved in Toro and Bluegold fruits, respectively, whereas total polyphenols are in Toro.

The Spartan variety is distinguished by the highest values for brightness and color tone of the fruits, and for the red and blue color components those of Bluegold stand out.

The comprehensive analysis of fresh and frozen blueberry fruit can be used in future studies to incorporate health-promoting compounds of fruit into healthy foods, with potential benefits for human health.

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THE IMPACT OF VARIOUS FERTILIZING SCHEMES ON THE BIOCHEMICAL COMPOSITION OF ARONIA FRUITS

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Abstract

The scientific experiment was conducted in a demonstration plantation at the Research Institute of Mountain Stockbreeding and Agriculture of Troyan. A fertilizing scheme was applied to aronia shrubs with various types of fertilizers, as well as combinations among them. The biochemical composition of aronia berries was compared after harvest and after one year of storage at -18°C. High values of dry weight, dry matter by Re, pectin, and total polyphenols were identified in the potassium sulfate fertilizing variant. The combined fertilizing with triple superphosphate, potassium sulfate, and carbamide gave the highest content of total sugars, sucrose and total polyphenols in fruits after the storage period. Statistical differences were proven among fertilizing variants in terms of dry matter by Re, total sugars, sucrose, and ascorbic acid. Organic acids, anthocyanins, and total polyphenols had proven results. The objective of the present study is to analyze the changes in the biochemical composition of aronia berries after the application of various fertilizing variants, after harvest, and one-year storage period.

Key words: *Aronia berries, Aronia melanocarpa L., biochemical composition, fertilizing, storage*

INTRODUCTION

Aronia or black chokeberry (*Aronia melanocarpa* L.) is a perennial shrub with a height of about 2.5-3 m, belonging to the *Rosaceae* family, genus *Aronia*. It originates from North America, from where it was brought to Europe. The fruit type has found wide distribution in Russia, where it is known under the name 'chernoplodnaya ryabina' (T.N. – transliteration from Russian). The first plants were imported into Bulgaria from there in the late 1960s, and early 1970s. The species was originally used in Bulgaria for decorative purposes and the preparation of home products in the past. Its industrial cultivation began in the 80s when numerous plantations were established (Mondeshka, 2005).

Aronia is a precocious species, as it starts fruit-bearing in the second year after planting. Its fruits ripen mainly in August, but the optimal harvesting period is the beginning of September when they have matured enough and the anthocyanin content has reached high levels (Andrzejewska et al., 2015). Their taste is pleasant, slightly sour, slightly astringent, and intensely coloured. They are suitable for fresh consumption, drying, and canning. They are used for wonderful juices, syrups, nectars, jam, purees, pestles, fruit teas, dessert wines, with an attractive dark ruby color, and nutritional supplements (Georgiev & Ludneva 2009; Sidor et al., 2019; Yang et al., 2021). Their characteristic rich content of polyphenolic compounds, anthocyanins, and ascorbic acid, makes them desirable ingredients (components) in the composition of bioactive foods, which has a healing effect on physiological processes in the human body (Hakkinen et al., 1999; Oszmiański & Wojdylo, 2005; Ochmian et al., 2009; Denev et al., 2013; Jurikova et al., 2017; Denev et al., 2018). The therapeutic effect of aronia berries is determined by their hypotensive effect, lowering the cholesterol level in the blood, cardioprotective, immunomodulating, antidiabetic, and anticarcinogenic properties (Simeonov et al., 2002; Yaneva et al., 2002; Hellstrom et al., 2009; Kokotkiewicz et al., 2010; Park & Park 2011; Balansky et al., 2012; Ren et al., 2022).

Typical of aronia berries is the low sucrose content, which makes them suitable for the diet of diabetics (Simeonov et al., 2002; Mondeshka, 2005; Valcheva-Kuzmanova, 2007; Banjari et al., 2017; Catană et al., 2021).

The biochemical composition of its fruits is not constant, but changes depending on the soil and climate conditions in the growing region, the applied agrotechnical events, the harvesting period, the cultivated varieties, and the fruit processing techniques (Misiak & Irzyniec 2009; Tolic et al., 2017; Pop et al., 2022).
The objective of the present experiment is to observe the impact of different fertilizing types on the biochemical composition of fresh aronia berries compared to the changes that occurred in them after one year of storage at -18°C.

MATERIALS AND METHODS

The aronia shrubs were planted in 2019 and were grown with drip irrigation. The planting scheme of the plants is 3.00 m x 2.50 m. Inter-row spacings and intra-row areas are naturally grassed, as mowing is performed when necessary. In contrast, the area around the stem is maintained in black fallow by tillage. The collection plantation is located 450 m above sea level, on an eastern exposure slope. The soils are light gray forest soils with pH varying from 4.6 to 5.6.
In 2022, the vegetation amount of average monthly precipitation was 253.3 l/m^2, which was the lowest reported vegetation precipitation amount for the last five years (Atanasova, 2021).
The following experimental fertilizing variants were set up:
Variant I - control;
Variant II - carbamide (0.150 kg) per shrub;
Variant III - triple superphosphate (0.150 kg) per shrub);
Variant IV - potassium sulfate (0.100 kg) per shrub;
Variant V - triple superphosphate (0.150 kg) and potassium sulfate (0.100 kg) per shrub;
Variant VI - triple superphosphate (0.150 kg), potassium sulfate (0.100 kg) and carbamide (0.150 kg) per shrub;
The biochemical composition of fresh and frozen Aronia berries from the 2022 harvest was analyzed (after one year of storage at -18°C), to which the corresponding fertilizing scheme was applied.
The following biochemical parameters of fresh and dried fruits were studied:

- dry matter weight (%);
- refractometric solids (%);
- sugars (total, invert and sucrose %) and acid, according to the method of Schoorl (Donchev et al., 2001), 25 g of sugars were taken from the sample; chemicals: 10% NaCO3, NaHPO4, Fehling's solution I, Fehling's solution II (made in the laboratory), 30% KI, 1: 6 H2SO4 , titrated by 0.1 n Na 2S2 O3 and starch indicator - sugars; 5 ml of acids were taken from the primary filtrate (as malic) by titration with 0.1 n NaOH (%) and phenolphthalein indicator;
- acids (such as malic) (%) by titration with 0.1 NaOH;
- ascorbic acid (mg %) has been made by the following method: 10 g of the ground sample is quantitatively transferred into a 100 cm3 volumetric flask. Top up with 2% HCL, then filter. Take 20 ml and place in a 100 ml Erlenmeyer flask, adding 10 ml H2O and 5 ml KI. Titrate with KIO3 (in the case of starch indicator) until a deep blue-permanent coloration.
- tannins substances (%) according to the method of Levental (Donchev et al., 2000), 25 g of tannins were taken from the sample. Chemicals: 1: 4 H2SO4, titrated by 0.1 KM4O4 and an indicator (indigo carmine);
- anthocyanins (mg %) by the method of Fuleki & Franciss (1968);
- pectin according to the method of Melitz (Donchev et al., 2000), 12.5 g were taken from the sample. Chemicals: 0.1 n NaOH, 1 n CH_3 COOH, $CaCl_2$, $AgNO_3$;
- total polyphenols (mg/100 g) Folin-Ciocalteu, (Singleton & Rossi, 1965).

The data were statistically processed using the program product MS Excel – 2010, Descriptive statistics. The indicators were calculated - Mean, Standard error, Standard deviation, Minimum-Maximum and Coefficient of variation.

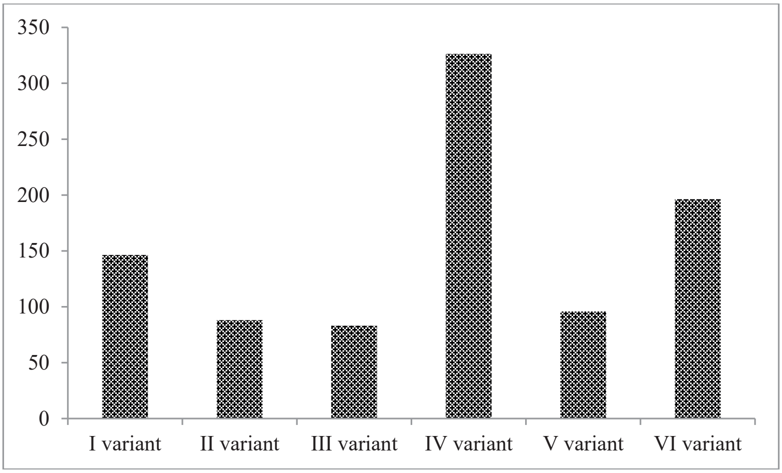
RESULTS AND DISCUSSIONS

Table 1 presents the biochemical composition of fresh aronia berries grown under different fertilizing variants. The dry matter weight is from 26.78% (III variant) to 32.61% (IV

variant). The refractometric solids have the highest value after the application of triple superphosphate (19.00%) and those in the variant with potassium sulfate (24.50%), whereas it has the lowest value after using carbamide (15.00%).
There is a wide range in the amounts of total sugars between the variants. The smallest amount (3.85%) was analyzed in the third variant (compared to the control - 5.00%), and the largest was reported in the second variant with 12.10% (CV% - 38.17).
A greater reduction was observed in the inverted sugar compared to the values of the total sugar in the second and fifth variants. The lowest value was registered after fertilizing with carbamide (1.75%). The variation coefficient for sucrose is very high (CV% - 64.96). The lowest value of this indicator was recorded in the variants with triple superphosphate (1.24%) and the control (1.57%), and it was several times more in fertilizing with carbamide (9.83%).
The analyzed organic acids in fresh aronia berries are in the range of 0.13% - 0.20%. Ascorbic acid was in the range of 12.32 mg/% to 14.08 mg/% and no significant difference was observed between the different experimental variants (CV% - 7.04).
The smallest amount of tannins was recorded in the control variant (0.044%) and more than twice in the shrubs fertilized with triple superphosphate and with the combined application of triple superphosphate, potassium sulfate, and carbamide (0.109%). The calculated coefficient of variation is 37.50%.
The lowest amount of pectin was recorded (0.10%) in the variant with the combined application of triple superphosphate, potassium sulfate, and carbamide. It was slightly higher in fertilizing with triple superphosphate (0.18%), whereas it was the highest in the variant with potassium sulfate (0.95%). The coefficient of variation is very high with 70.59%.
The highest content of total polyphenols was reported in the fourth variant (326.32 mg/ 100 g), lower in the sixth one (196.27 mg/ 100 g) and the first one (146.39 mg/100 g), whereas it was the least in the third variant (83.04 mg/100 g) (Figure 1). The obtained coefficient of variation among the variants is very high – 60.32%.

Table 1. Biochemical composition of fresh aronia fruits, after application of an experimental fertilizing scheme in 2022

Sample No	Dry matter weight %	Refracto-metric solids%	Total sugars %	Inverted sugar %	Sucrose %	Acids (as malic) %	Ascorbic acid mg/%	Tannins %	Pectin %
Variant I	32.00	19.50	5.00	3.35	1.57	0.20	14.08	0.044	0.54
Variant II	27.31	15.00	12.10	1.75	9.83	0.20	12.32	0.066	0.90
Variant III	26.78	19.00	3.85	2.55	1.24	0.13	12.32	0.109	0.18
Variant IV	32.61	24.50	9.20	3.20	5.70	0.13	14.08	0.044	0.95
Variant V	29.93	23.00	8.70	2.55	5.84	0.13	12.32	0.088	0.36
Variant VI	28.94	19.00	9.55	4.20	5.08	0.13	12.32	0.109	0.10
Mean	29.60	20.00	8.07	2.93	4.88	0.15	12.91	0.08	0.51
Minimum-Maximum	26.78-32.61	15-24.5	3.85-12.1	1.75-4.2	1.24-9.83	0.13-0.2	12.32-14.08	0.044-0.109	0.1 0.95
St error	0.98	1.37	1.26	0.34	1.30	0.01	0.37	0.01	0.15
St Dev	2.39	3.36	3.08	0.84	3.17	0.04	0.91	0.03	0.36
CV %	8.08	16.81	38.14	28.70	65.07	23.57	7.04	38.99	71.17



Mean	155.99
Minimum-Maximum	83.04-326.32
St error	38.42
St Dev	94.10
CV %	60.32

Figure 1. Total polyphenols after application of an experimental fertilizing scheme in 2022 (mg/100 g)

Table 2 shows the results for the biochemical composition of aronia berries after a one-year storage period at - 18 ^{0}C. The dry matter weight substance in aronia berries is in a wide range from 17. 67% (II variant) to 28.18 % (V variant), respectively the obtained values are several units lower.

A reduction in the values of refractometric solids was reported after one year of storage. The lowest content was found in the variant with carbamide (12.00%), whereas the highest was reported in the control (16.50%).

The range of variation of total sugars is in close values among the selected variants. The lowest content was found in the control (3.20%), whereas the highest content was found in the fertilizing variant with triple superphosphate, potassium sulfate, and carbamide (4.05%). The variation coefficient is average (10.23%). The decrease compared to the previous year is 57.87%.

Average inverted sugar values were roughly similar for both years: 2.93% (2022) and 2.85% (after storage). The reduction of sucrose in fruits is 89.34%, after one year of storage. In some of the variants, it was not registered at all, whereas the highest was found in the variant with combined fertilizing with triple superphosphate, potassium sulfate, and carbamide (1.57%).

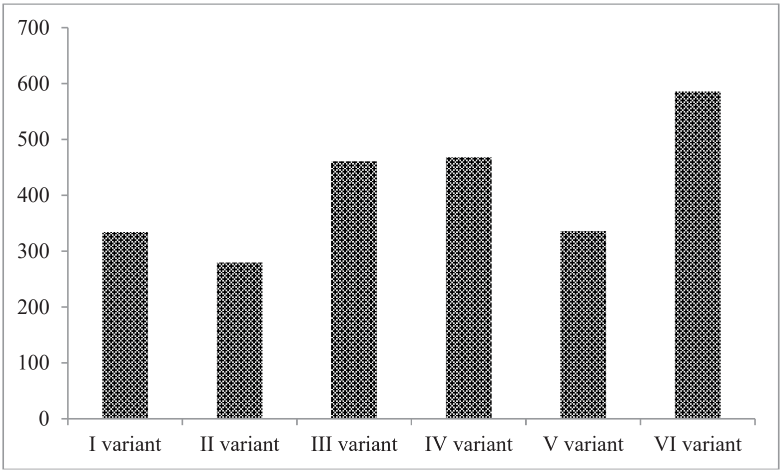
The increase in organic acids is 48.28%, compared to the raw material of the previous year. The average value among variants is 0.29%, and the variation coefficient is 19.48%.

The highest content of ascorbic acid was reported from the variant with carbamide (12.32 mg/%). The average value among the variants is 10.27 mg/%, and the registered reduction is 20.45%, compared to the raw material of the previous year. An analogous trend, as for organic acids, was also found for tannins, as an increase in fruit after storage by 24.53% was reported. The content is in close values among the variants and has a low variation coefficient of 8.77%.

Increased pectin values were found in the control, variant III, variant V, and variant VI. The average value for the variants is 0.78%, and the increase is 34.62%, compared to the starting raw material. An increase in the content of total polyphenols in the fruit was reported in all variants, after storage, which was 62.02%. The highest value was recorded after the application of combined fertilizing with triple superphosphate, potassium sulfate, and carbamide (585.84 mg/100 g), whereas the lowest was in fertilizing with carbamide (279.56 mg/100 g) (Figure 2).

Table 2. Biochemical composition of aronia berries, with a different fertilizing scheme applied, after one year of storage – 2023

Sample No	Dry matter weight %	Refractometric solids%	Total sugars %	Inverted sugar %	Sucrose %	Acids (as malic) %	Ascorbic acid mg %	Tannins %	Pectin %
Variant I	24.19	16.50	3.20	3.20	-	0.34	10.56	0.094	0.96
Variant II	17.67	12.00	3.55	2.70	0.81	0.34	12.32	0.094	0.66
Variant III	26.75	14.50	3.20	3.20	-	0.27	8.80	0.112	0.98
Variant IV	27.63	13.00	3.20	2.40	0.76	0.27	10.56	0.112	0.96
Variant V	28.18	16.00	3.20	3.20	-	0.34	10.56	0.112	0.60
Variant VI	21.75	14.50	4.05	2.40	1.57	0.20	8.80	0.112	0.52
Mean	24.36	14.42	3.40	2.85	0.52	0.29	10.27	0.106	0.78
Minimum-Maximum	17.67-28.18	12-16.5	3.2-4.05	2.4-3.2	0-1.57	0.2 0.34	8.8-12.32	0.094-0.112	0.52-0.98
St error	1.66	0.70	0.14	0.16	0.26	0.02	0.54	0.00	0.09
St Dev	4.06	1.72	0.35	0.40	0.64	0.06	1.32	0.01	0.21
CV %	16.68	11.90	10.23	13.99	122.51	19.48	12.90	8.77	26.84



Mean	410.75
Minimum-Maximum	279.56-585.84
St error	46.58
St Dev	114.10
CV %	27.78

Figure 2. Total polyphenols with a different fertilizing scheme applied, after one year of storage (mg/100 g)

Table 3. Percentage decrease and increase in the indicators of the biochemical composition of aronia berries after one year of storage

Indicators	Dry matter weight %	Refractometric solids%	Total sugars %	Inverted sugar %	Sucrose %	Acids (as malic) %	Ascorbic acid mg /%	Tannins %	Pectin %	Total polyphenols mg/100 g
Percentage (%) decrease / increase, compared to 2022	Decrease 17.70%	Decrease 27.9	Decrease 57.87	Decrease 2.73	Decrease 89.34	Increase 48.28	Decrease 20.45	Increase 24.53	Increase 34.62	Increase 62.02

CONCLUSIONS

Aronia, as a representative of the small-fruited fruit species, is a more unpretentious crop to growing conditions.

The biochemical composition of fruits was evaluated depending on the applied fertilization. The values of the ascorbic acid index in the fresh fruits are higher in the control than in the variant with added potassium sulfate. The highest content of total sugars and sucrose was recorded in a urea fertilization scheme. The highest values of dry weight, dry refractometric substance and pectin were found in the variant with added potassium sulfate.

Freezing as a storage method for aronia berries ensures the preservation of their biological activities and provides an opportunity for their longer use and inclusion in organic products and nutritional supplements.

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EVALUATION OF SOME BLACKCURRANT CULTIVARS ACCORDING TO FRUIT QUALITY PARAMETERS

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Abstract

Ribes nigrum L. (blackcurrant) is widely cultivated across temperate Europe, Russia, New Zealand, parts of Asia, and to a lesser extent, North America. Blackcurrants are a valuable source of bioactive compounds like anthocyanins and polyphenols. The berries are used in the production of juices, nectars, jams and functional food. Cultivars with enhanced levels of anthocyanins are currently requested by juice processors and consumers due to their health benefits. The experiment has been carried out at the Research Institute for Fruit Growing Piteşti - Mărăcineni, Romania, using six cultivars with different origins: 'Tiben', 'Ruben', 'Tisel', 'Gagatai', 'Titania', and 'Kalinka'. The following physical parameters were measured: yield, berry weight (BW), diameter, firmness, soluble solids content (SSC) and total sugar content (TSC). Also, a series of biochemical parameters were determined, such as: total polyphenols content (TPC), total flavonoids content (TFC), total tannins content (TTC), total anthocyanins content (TAC), and lycopene and β-carotene contents. All the determined parameters were correlated with the antioxidant activity values.

***Key words**: blackcurrant, breeding, antioxidant activity, total polyphenol content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC), lycopene content, β-carotene content.*

INTRODUCTION

Blackcurrant (*Ribes nigrum* L.) is a significant fruit crop grown extensively in northern Europe (Griffiths et al., 1999; Brennan et al., 2008) and Russia (Mitchell et al., 2011) with a history of domestication dating back to at least 400 years ago. Poland has been the leading producer of blackcurrants in Europe (Pluta et al., 2012) for many years.

Blackcurrants are small, dark purple fruits that originate from medium-sized woody shrubs (Törrönen et al., 2012; Corrigan et al., 2014). The berries are highly valued for their high vitamin C and anthocyanin content (Szajdek et al., 2008), and are used in large-scale production of fruit juices, nectars, jams and functional food products (Griffiths et al., 1999; Brennan et al., 2008).

Various breeding programs in Romania and other countries have mainly concentrated on enhancing agronomic traits like: yield, pest resistance, and disease resistance (Corrigan et al., 2014). These programmes generate large numbers of offspring, which are generally screened by the breeders to select candidates that meet the specific industry requirements for flavour typicality (Currie et al., 2013). The phenolic compounds dominantly locate in the berry skin whereas most sugars and organic acids are in the pulp (Sandell et al., 2009). Consequently, the berry pulp has a more intense flavour than the skin because the potentially astringent and bitter compounds are bound by polysaccharides.

The study aims at comparing the physical characteristics and chemical composition of blackcurrants, specifically focusing on: yield, berry weight, shape index, firmness, soluble solids content, total sugar content, total polyphenol content, total flavonoid content, total tannin content, total anthocyanin content, as well as on lycopene and β-carotene contents. The goal of the current study is providing comprehensive information as regards the selection of appropriate blackcurrant genotypes for breeding and fruit production toward a potential development at the Research Institute for Fruit Growing in Piteşti.

MATERIALS AND METHODS

Plant material

The experiment was carried out at the Research Institute for Fruit Growing (RIFG) Piteşti-Mărăcineni in South of Romania 44°54'12" Northern latitude, and 24°52'18" Eastern longitude, 284 m altitude, over a period of 2 years (2022-2023).

The study included the following genotypes:

- three Polish cultivars: 'Tiben' obtained from crossing 'Titania' with 'Ben Nevis'; 'Tisel' obtained from crossing 'Titania' with itself; 'Ruben' obtained from crossing 'Bieloruskaja Slodkaja' with 'Ben Lomond';
- a Swedish cultivar 'Titania' was developed by Tamas (1984) from crossing 'Altayskaya Desertnaya' with 'Consort' × 'Kajaanin Musta',
- a Lithuanian cultivar: 'Gagatai' obtained from crossing 'Minaj Shmyriov' with 'Ojebyn';
- a Belarusian cultivar: 'Kalinka' with unknown genitors.

Fruit quality parameters

During the peak fruit harvesting period we recorded various indicators from a sample of 50 fruits, with each measurement and analysis repeated 3 times.

Fruit production (g/plant) was calculated by weighing the fruits on each plant at every harvest and summing the yield.

The average ***fruit weight*** was determined by using the HL-400 digital balance to weigh each fruit. Additionally, the ***fruit diameter*** was measured using a digital calliper, and the ***berry firmness*** was assessed non-destructively using a Bareiss HPE II Fff penetrometer for each sample.

Various quality parameters were measured, such as total soluble solids, total sugars content, total polyphenol content, total flavonoid content, total tannin content, total anthocyanin content, as well as lycopene and β-carotene contents.

Total soluble solid content **(TSS)** was measured using a Kruss DR201-95 refractometer, and the findings were expressed as °Brix at 20°C.

Total sugar content **(TSC)** was determined by the methodology suggested by Dubois et al. (1956).

Total polyphenol content **(TPC)** was measured using the method recommended by Matić et al. (2017). This involved the reaction of polyphenols with phosphotungstic acid in an alkaline medium, resulting in the formation of a blue-coloured compound. The newly formed compound has maximum absorption at 760 nm. The results were reported as mg gallic acid equivalent (GAE) per 100 g fresh weight (FW).

Total flavonoid content (TFC) was measured using the method proposed by Giosanu et al. (2016). Flavonoids reacted with aluminum chloride to form a yellow-orange compound which exhibited maximum absorption at 510 nm. The findings were reported as mg catechin equivalent (CE)/100 g fresh weight (FW).

To determine the ***total anthocyanin content*** **(TAC)**, the pH differential method suggested by Di Stefano and Cravero (1989) was used. According to this method, the total monomeric anthocyanin content is measured by taking into account the reversible structural transformation of the anthocyanin chromophore based on the pH. The absorbance at 520 nm was measured 30 minutes after preparing the blueberry extract samples in pH 0.6 (2% hydrochloric acid) and pH 3.5 (a phosphate buffer with 0.1 M citric acid and 0.2 M disodium phosphate). The results were expressed as cyanidin-3-O-glucoside equivalent (C3GE) per 100 g fresh weight (FW).

Total tannin contents **(TTC)** were measured following the method described by Cosmulescu et al. (2023). The results were reported as mg gallic acid equivalent (GAE) per 100 g fresh weight (FW).

The levels of ***lycopene and β-carotene*** were determined using the method proposed by Tudor-Radu et al. (2016). Carotenoids were extracted using a mixture of hexane, ethanol and acetone in a 2:1:1 ratio. The results were expressed in milligrams of lycopene or β-carotene per 100 grams of fresh weight (FW), utilizing the molar extinction coefficients of both compounds at 470 and 503 nm (Tudor-Radu et al., 2016).

The antioxidant activity was assessed using the DPPH assay method (Vîjan et al., 2023). The results were expressed as a percentage of the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH I%).

Chemicals and Reagents

All the chemicals and reagents were purchased from Merck, Darmstadt, Germany. They include 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium hydroxide, sodium carbonate, sodium nitrite, disodium phosphate, aluminum chloride, acetone, n-hexane, ethanol, gallic acid, catechin, cyanidin-3-O-glucoside, and Folin-Ciocalteu reagent.

Statistical Analysis

The analyses were conducted with three repetitions, and the data were presented as mean ± standard deviation (SD). Excel 2021 (XLSTAT) was utilized for statistical analysis of the data. One-way analysis of variance (ANOVA), two-way ANOVA and Duncan's multiple range tests were carried out.

RESULTS AND DISCUSSIONS

The Tables 1-3 show the values for yield, berry weight, diameter, firmness, total soluble solids (TSS), total sugar (TSC), total polyphenols (TPC), total flavonoids (TFC), total tannins (TTC), total anthocyanins (TAC), lycopene and β-carotene contents, and the antioxidant activity (DPPH%) with indication of the values of mean and standard deviation for six blackcurrant cultivars.

The fruit mass ranged from 0.8 kg for 'Tiben' to 1.78 kg for 'Kalinka' (Table 1), with no significant variation observed among the different cultivars.

The weight of the blackcurrant is a crucial quality factor significantly influenced by the genotype, and also impacted by the growing conditions (Woznicki et al., 2016). The weight of the berries ranged from 0.73 g/fruit for 'Tisel' to 1.55 g/fruit for 'Titania'. A similar finding was reported by Kikas and Libek, 2023 for the Estonian blackcurrant cultivar 'Elmar'.

The fruit size is a key factor that determines the quality of the yield (Ochmian et al., 2014). 'Gagatai' had the largest fruit diameter, a diameter of 13.02 mm, while 'Tisel' had a diameter of 9.93 mm, which was the smallest fruit diameter.The firmness, susceptibility to damage, and the force needed to detach the fruit from the stem are key factors that influence the suitability of different cultivars for mechanical harvesting (Ochmian et al., 2014).

Table 1. Fruit biometric characteristics of blackcurrant genotypes (average 2022-2023)

Genotype	Yield (kg/plant)	Berry weight (g/fruit)	Diameter (mm)	Firmness (N)	TSS (°Brix)
Ruben	1.22±0.78[a*]	0.84±0.09[c]	11.66±0.60[b]	26.97±3.23[b]	19.13±2.00[ab]
Tisel	1.68±0.89[a]	0.73±0.10[c]	9.93±0.91[d]	29.13±7.27[bc]	22.93±2.14[a]
Kalinka	1.78±0.31[a]	0.86±0.22[c]	10.38±0.69[cd]	30.90±7.04[b]	21.23±4.48[a]
Gagatai	1.24±0.90[a]	1.29±0.12[ab]	13.02±0.48[a]	46.53±4.75[a]	15.23±1.46[b]
Titania	1.62±0.13[a]	1.55±0.35[a]	11.08±0.28[bc]	37.97±5.75[ab]	18.53±1.78[ab]
Tiben	0.80±0.09[a]	1.07±0.19[bc]	11.53±0.10[b]	36.40±5.30[ab]	16.03±2.10[b]

Note: [*]Different letters between cultivars denote significant differences (Duncan test. $p < 0.05$).
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. $P < 0.05$).

The berry firmness oscillated between 26.97 N for 'Ruben' and 46.53 N for 'Gagatai'.

Over the two years, the highest TSS concentration was found in the fruits of 'Tisel' (22.93°Brix), and the lowest in the fruits of 'Gagatai' (15.23°Brix).

Other parameters such as: total sugar (TSC), total polyphenols (TPC), total flavonoids (TFC), total tannins (TTC), total anthocyanins (TAC), along with lycopene and β-carotene contents and the antioxidant activity (DPPH%) are vital in assessing the taste of fruits and their suitability for fresh consumption. Blackcurrants are rich in phytochemicals, thus possessing strong antioxidant, anti-inflammatory, and anti-microbial properties (Tabart et al., 2006; Nour et al., 2014).

The impact of environmental conditions on the concentration of active components in different plants is well-documented. Factors like light, temperature, water availability, mineral nutrition, grafting, increased atmospheric CO_2, elevated ozone levels, and agricultural practices directly influence biochemical processes, consequently impacting the production of these secondary metabolites. Additionally, genetic makeup can significantly influence the antioxidant, micro-nutrient, and phytochemical composition of plants (Tabart et al., 2006; Treutter, 2010; Nour et al., 2014). Total sugars content (TSC) of blackcurrant fruit varied from 33.37 g glucose/100 g for 'Tiben' to 80.53 g glucose/100 g for 'Tisel' (Table 2).

Table 2. Fruit biochemical characteristics of blackcurrant genotypes (average 2022-2023)

Genotype	TSC (g glucose/100 g)	TPC (mg GAE/100 g)	TFC (mg CE/100 g)	TTC (mg GAE/100 g)	TAC (mg C3GE/100 g)
Ruben	41.55±33.88[b*]	718.03±7.80[a]	161.8±0.82[b]	541.08±9.62[c]	40.98±3.81[b]
Tisel	80.53±0.25[a]	727.11±0.10[a]	164.06±0.65[b]	530.05±0.04[d]	36.87±0.11[bc]
Kalinka	49.34±19.92[b]	687.18±22.47[b]	216.08±50.56[a]	579.68±8.30[b]	62.45±14.44[a]
Gagatai	46.78±0.09[b]	712.92±0.11[a]	143.48±0.67[b]	596.36±0.18[a]	14.73±0.01[de]
Titania	55.70±0.07[b]	493.09±0.12[d]	80.28±0.40[c]	389.20±0.10[f]	9.18±0.18[e]
Tiben	33.37±0.04[b]	653.71±0.22[c]	125.15±0.67[bc]	440.73±0.04[e]	23.81±0.15[cd]

Note: [*]Different letters between cultivars denote significant differences (Duncan test. $p < 0.05$).
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. $P < 0.05$).

During the two years of experimentation, the total polyphenols content (TPC) in the fruits of the blackcurrant cultivars ranged from an average of 493.09 mg GAE/100 g for 'Titania' to 727.11 mg GAE/100 g for the 'Tisel'. Similar results for TPC were reported by Kikas et al., 2020 for 37 blackcurrant cultivars of different geographical origin (Belarus, Estonia, Finland, Latvia, Lithuania, Norway, Poland, Russia, Scotland, Sweden, and Ukraine) grown in Estonia.

The flavonoid content (TFC) in the analyzed blackcurrants varied from 80.28 mg CE/100 g for 'Titania' to 216.08 mg CE/100 g for 'Kalinka'. The TFC results obtained in our study fall between the previously reported data by Mikkonen et al. (2001), Laczkó-Zöld et al. (2018), and Milić et al. (2022) for blackcurrants and redcurrants.

The total tannin content (TTC) ranged from 389.20 mg GAE/100 g for 'Titania' to 596.36 mg GAE/100 g for 'Gagatai'. Our results revealed that blackcurrants have high levels of tannins and low levels of flavonoids.

The total anthocyanin content (TAC) in the fruits of the blackcurrant cultivars showed significant differences over the two years of research. The average total anthocyanin content (TAC) was 9.18 mg C3GE/100 g for 'Titania' and 62.45 mg C3GE/100 g for 'Kalinka'. Sellappan et al. (2002) noted significant differences and inconsistencies among cultivars over different periods of time. These variations may be attributed to genetic disparities, harvest maturity, agricultural techniques, as well as to variations in extraction and laboratory procedures (Clark et al., 2002).

As regards the content of carotenoids (Table 3), the lowest level of β-carotene was of 0.20 mg/100 g in 'Tisel', while the highest level was of 0.39 mg/100 g in 'Kalinka'. The lycopene content ranged from 0.003 mg/100 g for 'Titania' to 0.03 mg/100 g for 'Kalinka' and 'Tiben'.

Table 3. Fruit biochemical characteristics of blackcurrant genotypes (average 2022-2023)

Genotype	β-carotene (mg/100 g)	Lycopene (mg/100 g)	DDPH %
Ruben	0.24±0.04[b*]	0.01±0.01[c]	93.44±1.12[ab]
Tisel	0.20±0.01[b]	0.02±0.01[c]	94.65±0.04[a]
Kalinka	0.39±0.05[a]	0.03±0.01[a]	91.78±2.14[b]
Gagatai	0.34±0.01[a]	0.02±0.01[bc]	95.52±0.05[a]
Titania	0.22±0.01[b]	0.003±0.01[d]	95.41±0.05[a]
Tiben	0.25±0.01[b]	0.03±0.01[ab]	94.64±0.05[a]

Note: [*]Different letters between cultivars denote significant differences (Duncan test. $p < 0.05$).
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. $P < 0.05$).

The antioxidative activity of all the tested berry extracts showed appropriate values, ranging from 91.78% for 'Kalinka' to 95.52% for 'Gagatai'. No significant differences were observed among the six cultivars. Similar values were also reported by Anisimovienė in 2013.
In our study, TPC was positively correlated with TTC (r=0.834**), TFC (r=0.696**), lycopene (r=0.578*), and TAC (r=0.524*) (Table 4). TFC presented a positive correlation with TAC (r=0.931**), TTC (r=0.753**), and both carotenoids: lycopene (r=0.780**), and β-carotene (r=0.704**). In terms of antioxidant activity (DPPH %), there was a significant negative correlation with TFC (r=-0.867**), TAC (r=-0.931**), both lycopene (r=-0.655**) and β-carotene (r=-0.629**).

Table 4. Correlation matrix of TSC, TPC, TFC, TTC, TAC, β-carotene, lycopene and DPPH % in blackcurrant fruits

		TSC	TPC	TFC	TTC	TAC	β-carotene	lycopene	DPPH %
TSC (g/100 g)	Pearson Correlation	1	-0.047	-0.148	-0.011	-0.136	-0.380	-0.331	0.416
TPC (mg GAE/100g)	Pearson Correlation		1	0.696**	0.834**	0.524*	0.347	0.578*	-0.351
TFC (mg CE/100 g	Pearson Correlation			1	0.753**	0.931**	0.704**	0.780**	-0.867**
TTC (mg GAE/100 g)	Pearson Correlation				1	0.559*	0.685**	0.520*	-0.422
TAC (mg C3GE/100 g)	Pearson Correlation					1	0.568*	0.684**	-0.931**
β-carotene	Pearson Correlation						1	0.709**	-0.629**
lycopene	Pearson Correlation							1	-0.655**

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

CONCLUSIONS

The cultivar significantly influenced the composition of blackcurrant fruits regarding the total phenolic content, in general, and tannins, flavonoids and anthocyanins, in particular. In addition, a significant fluctuation in lycopene content was observed. The antioxidant activity of all the tested blackcurrant extracts showed high values (91.78-95.52%), but no significant differences among the six cultivars.
The current study indicated that 100 g of fresh blackcurrant from 'Ruben', 'Tisel', 'Kalinka', 'Gagatai', 'Titania' and 'Tiben' contained 33.37-80.53 g of total sugars, 493.09-727.11 mg GAE of polyphenolic compounds, 389.20-596.36 mg GAE of tannins, 80.28-216.08 mg CE of flavonoids, 9.18-62.45 mg C3GE of monomeric anthocyanins, 0.003-0.03 mg of lycopene, and 0.20-0.39 mg of β-carotene. These results indicate that blackcurrants have high level of tannins, medium level of flavonoids, low levels of anthocyanins and especially of carotenoids. Presented data confirmed the usefulness of *Ribes nigrum* L. species as a rich source of bioactive phenolic compounds with the potential to be used in food and pharmaceutical industries.
Regarding all the studied cultivars, 'Tisel' stood out with the highest values for weight/plant, TSS, TSC and TPC. Also, 'Kalinka' stood out with the highest values for TAC and carotenoids. The interest in breeding programs will increase due to their potential as sources of genetic variability.
However, further research is needed to determine the other antioxidant compounds from the blackcurrant fruits.

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EVALUATION OF BLUEBERRY FRUITS AND JUICE OF SOME CULTIVARS GROWN IN PITEŞTI - MĂRĂCINENI, ROMANIA

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Abstract

Highbush blueberry is a popular berry with an attractive flavour and colour. It provides health benefits due to an important number of bioactive compounds with antioxidant, antitumor, antimutagenic, and antidiabetic effects, as well as to its ability to prevent cardiovascular disease. Blueberry juice is one of the most widespread blueberry products and it is demanded by consumers since it is tasty and it preserves most of the nutrients. The aim of this study is the evaluation of the blueberry fruits and juice of some cultivars grown at the Research Institute for Fruit Growing Piteşti - Mărăcineni, Romania. Since heat processing affects the nutritional and sensory properties, blueberry juices have been prepared by cold pressing the fruit. In order to inhibit the development of the fermentation microbiota and improve the organoleptic characteristics of blueberry juices, the samples of each juice were prepared in a 1:1 (w:w) mixture with acacia honey. Total tannin content (TTC) and total flavonoid content (TFC) were periodically determined for all juices. The results revealed very significant effects of the three factors (genotype, storage period and sweetener) on TTC and TFC, with acacia honey showing the lowest effect size.

***Key words**: highbush blueberry, total tannins content (TTC), total flavonoids content (TFC.)*

INTRODUCTION

Analysis of public health shows an alarming increase in the number of people who suffer from or are prone to cardiovascular diseases, cancer, disorders of lipid metabolism (increased cholesterol and blood sugar, weight gain), allergic diseases, or various nervous system diseases (Hera et al., 2023; Mazilu et al., 2022). Nutritionists strongly recommend a diet rich in fruits and vegetables so as to prevent premature aging, metabolic diseases, and even to fight obesity (Lavefve et al., 2020).
Since fruits and vegetables are seasonal and perishable, they are transformed into functional foods so that they can keep their nutritional value closer to that of fresh products while being available beyond the harvest season (Slavin and Lloyd, 2012).
Among these, the blueberry juice is one of the most widespread blueberry products, and it is demanded by consumers since it is tasty and it preserves most of the nutrients (Tobar-Bolaños et al., 2021).
Blueberries are a valuable crop that can thrive in acidic, poorly drained sandy soils, which may otherwise be considered unsuitable for agricultural production. North America is the primary producer of blueberries.
Cultivated blueberries belong to the *Cyanococcus* section of the *Vaccinium* genus in the heath family *Ericaceae* (Ballington et al., 1997). Species within this section are often referred to as the "true" or cluster-fruited blueberries (Camp, 1945). Wild *Cyanococcus* species are exclusively found in North America (Hancock and Draper, 1989).
Blueberry species are commonly classified as lowbush, highbush, and rabbiteye types based on their stature. Lowbush plants are rhizomatous, with stems ranging from 0.3 m to 0.6 m. Highbush plants are crown-forming shrubs and they are generally 1.8-2.5 m tall. Rabbiteye plants are also crown-forming shrubs; normally, they are 2-4 m tall (Ballington et al., 1997). Among major fruit crops, blueberries have been domesticated recently, during the 20th century.

The berries that cannot be sold on fresh markets are used to make juices, which decreases waste and allows the obtaining of some blueberry products that can still maintain the flavour and nutrients of the fruit (Bates et al., 2001).
The blueberry juice is a popular product demanded by consumers for its appealing taste and ability to preserve the nutrients from fresh fruit (Hotchkiss et al., 2021). Due to its high level of anthocyanins and other antioxidant compounds like polyphenols, vitamins C and E, the blueberry juice is recommended to maintain human health (Nindo et al., 2005; Tobar-Bolaños et al., 2021). It can be made from fresh or frozen berries, using a juice squeezer. Since blueberries have a balanced composition in sugars and acids (Hera et al., 2023), the preparation of blueberry juices does not require the dilution with water or the addition of sugar or other sweeteners. The abundance of blueberry juice in bioactive phenolic compounds is correlated with the synthesis of secondary metabolites by the plant, and with the processsing juice technology (Brambilla et al., 2008).
The impact of juice processing on fresh berry bioactive compounds content has been studied and the results showed a detrimental effect of the processing technology of juice explained through oxidative degradation because of tissue disorganization (Lee et al., 2002; Skrede et al., 2000). It has been proven that the steam-blanching of berries before milling is effective in improving stability and in the recovery of the phenolic bioactive compounds (Rossi et al., 2003). It seems that blanching acts by inactivating the oxidative enzymes of the processed fruits and by physically improving the permeability of the pigmented pericarp cells (Pizzocaro et al., 1988). The juice needs to be clarified, filtered, and pasteurized to ensure that it becomes a microbiologically stable food product with an appealing colour, a quality highly valued by consumers (Girard and Sinha, 2006; Siddiq et al., 2018; Song et al., 2018).
It is in our interest to improve knowledge as regards the bioactive compounds in raw fruit and to preserve these compounds in the end-product. To this end, four blueberry cultivars were grown in the same environmental conditions (at the Research Institute for Fruit Growing Piteşti - Mărăcineni), and they were individually processed into juice after an initial blanching step, with a special focus on the cultivar influence upon the phenolic content of the juice. To preserve the organoleptic characteristics and inhibit the fermentation microbiota, simple juices and samples mixed 1:1 (w:w) juice with acacia honey were prepared from the fruits of each variety. Total tannin content (TTC) and total flavonoid content (TFC) were periodically determined for all juices.

MATERIALS AND METHODS

Chemicals and Reagents
All the chemicals and reagents were purchased from Merck, Darmstadt, Germany.
Plant material
The study was carried at the Research Institute for Fruit Growing in Piteşti-Mărăcineni (RIFG) in southern Romania, located at 44°54’12” north latitude, and 24°52’18” east longitude, with an altitude of 284 meters.
The study included the following genotypes:
(1) two American cultivars:
- 'Duke' obtained from crossing ('Ivanhoe × Earliblue') with 192–8 (E-30 × E-11),
- 'Northblue' obtained from crossing *Vaccinium corymbosum* with *Vaccinium angustifolium,*
(2) two Romanian advanced selections:
- '4/6' open-pollinated 'Spartan',
- '6/38' obtained from crossing 'Pemberton' with 'Bluecrop'.

Figure 1. The blueberry genotypes

Acacia honey was produced by bee populations of the *Apis mellifera Carpatica* race in a small apiary of Southern Romania (Costesti, Arges). Physicochemical analysis of some honey samples (Vîjan et al., 2023) showed the acacia honey had a safe moisture content (14.36%) for storage and consumption, an average level of ash (0.14%), free acidity (7.50 mEq/kg) and sugar (63.79 g glucose/100 g), the lowest 5-hydroxylmethylfurfural (16.07 mg/kg), phenolic content (90.18 mg GAE/100 g), and flavonoid content (11.83 mg CE/100 g), but a high level of tannins (62.73 mg GAE/100 g).

Fruit quality parameters

The average fruit weight was determined by weighing a sample of 50 fruits from each repetition at each harvest, using a scale (in grams). After the harvesting work, depending on the ripening period of each genotype, a weighted-average weight was calculated.

The length and diameter of the fruit were determined by measuring the fruit with a digital calliper. The size index of the fruit was calculated as the ratio of these two dimensions (Titirică et al., 2023).

The total soluble solids content was measured using a digital refractometer Haana Instruments 96801, and values were recorded in °Brix.

The fruit firmness was assessed non-destructively using a Bareiss HPE II Fff penetrometer for each sample.

Blueberry juice preparation and treatments

Fully mature and good-quality blueberries were picked from Mărăcineni, Romania. After washing with distilled water, the fruits were poured into a motorized juice extractor (Midea WJE2802D, Midea, Guiyang, China) for about 10 s to obtain the juice. Afterwards, the juice was filtered by a sterile muslin cloth, necessary to provide uniform consistency. The extracted juice was separated into two, depending on the two treatments to which it was exposed: simple juices obtained without adding honey and juices to which acacia honey was added to improve the organoleptic characteristics of the juice and inhibit the fermentation microbiota.

The blueberry juice samples were kept at 4°C throughout the current study, in hermetically sealed glass containers. The samples were analysed at 5 different time points: I (immediately after obtaining the juice), II (after one month), III (after 3 months), IV (after 4 months), and V (after 6 months).

Blueberry juice biochemical parameters

The total tannins content (TTC) was measured using the method recommended by Matić et al., 2017. This involved the reaction of tannins with phosphotungstic acid in an alkaline medium, resulting in the formation of a blue-coloured compound. The newly formed compound has maximum absorption at 760 nm. The results were reported as mg gallic acid equivalent (GAE) per 100 g fresh weight (FW).

The total flavonoid content (TFC) was measured using the method proposed by Giosanu et al., 2016. Flavonoids reacted with aluminium chloride to form a yellow-orange compound which exhibited maximum absorption at 510 nm. The findings were reported as mg catechin equivalent (CE) per 100 g fresh weight (FW).

Statistical Analysis

The analyses were conducted in triplicate, and the data were presented as mean ± standard deviation (SD). Excel 2021 (XLSTAT) was used for the statistical analysis of the data. One-way analysis of variance (ANOVA), two-way and three-way ANOVA (factorial ANOVA), and Duncan's multiple range tests were carried out.

RESULTS AND DISCUSSIONS

Biometric characteristics of the fruit

The main biometric characteristics of the blueberry fruit refer to the size, shape, and texture of the pulp, and are expressed through: average weight, size index, and firmness. Significant differences have been observed between genotypes as regards the above-mentioned biometric indicators. The average fruit weight is a genetically determined characteristic, influenced by technical and cultural conditions and it has recorded different values in the current study.

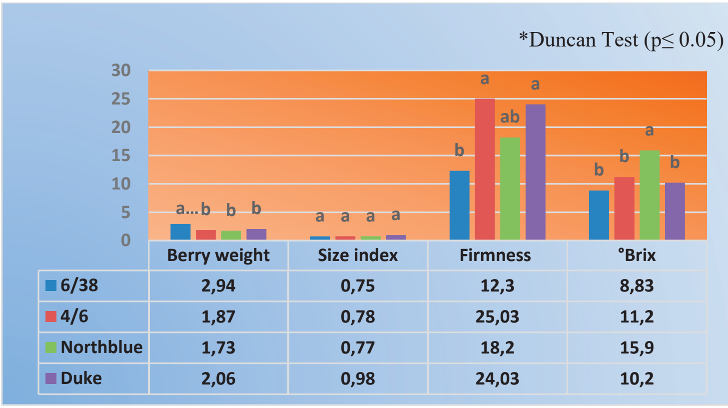
Figure 2 shows that the values for average berry weight oscillated between 1.73 g for 'Northblue' to 2.94 g for '6/38'.

Regarding the size index, no significant differences were recorded between genotypes.

The fruit firmness is a crucial parameter for fresh blueberries because it affects the quality and the post-harvest storage potential.

In horticultural studies, the fruit firmness is also referred to as a textural or mechanical quality that can indicate differences in fruit ripeness or horticultural product quality (Abbot, 1999; Musacchi and Serra, 2018). The firmest fruits were harvested from '4/6' and 'Duke'.

The fruit ripening is influenced by total soluble solids, which also affects the nutrient absorption and economic outcomes in fruit trade (Li et al., 2016). The total soluble solids oscillated between 15.9°Brix for 'Northblue' to 8.83°Brix for '6/38'.



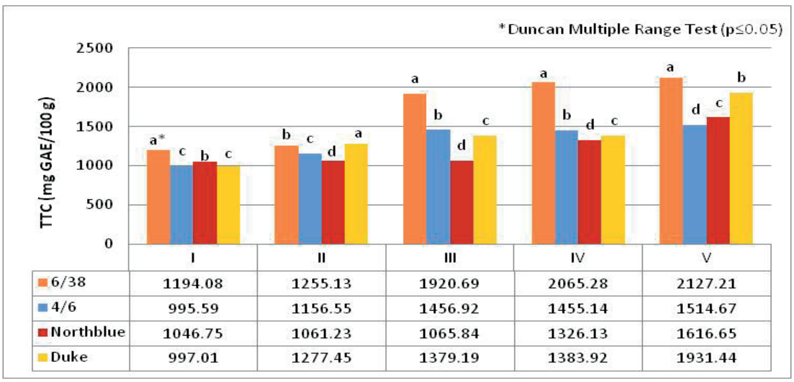
	Berry weight	Size index	Firmness	°Brix
6/38	2,94	0,75	12,3	8,83
4/6	1,87	0,78	25,03	11,2
Northblue	1,73	0,77	18,2	15,9
Duke	2,06	0,98	24,03	10,2

Figure 2. The biometrical parameters of blueberry genotypes
Note: *Different letters between cultivars denote significant differences (Duncan test. p < 0.05);
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. P < 0.05)

Juice's Total Tannins Content

The bioactive substances in blueberries, particularly polyphenols and anthocyanins, are subject to various post-harvest factors that influence their content and functionality in different ways (Michalska and Łysiak, 2015). It has been observed that tannins accumulation continues in overripe berries and it may even increase after storage. This is due to the outer layer which by losing firmness, it allows water to evaporate more quickly (Kalt et al., 2001). The blueberry juice contains multiple phenolic compounds which makes it a potent solution that manages to enhance the nutritional value of this food (Seraglio et al., 2023).

In all the studied genotypes, the content of tannins (TTC) in the juice has increased from the first chemical analysis on August 1, 2023, to the last one on February 1, 2024 (Figures 3 and 4).



	I	II	III	IV	V
6/38	1194.08	1255.13	1920.69	2065.28	2127.21
4/6	995.59	1156.55	1456.92	1455.14	1514.67
Northblue	1046.75	1061.23	1065.84	1326.13	1616.65
Duke	997.01	1277.45	1379.19	1383.92	1931.44

Figure 3. The influence of genotype on TTC depending on the storage period in blueberry juices without honey
Note: *Different letters between cultivars denote significant differences (Duncan test. p < 0.05);
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. P < 0.05)

At the initial time (I), by adding acacia honey to blueberry juice, TTC in juices of '6/38' and 'Northblue' decreased, while in the other two juice samples there was an increase. This result is certainly influenced by TCC of acacia honey (62.73 mg GAE/100 g, Vîjan et al., 2023). The highest increase in TTC values was recorded for 'Duke' (93.72%) and '6/38' (78.15%) in juice samples without honey and for '6/38' (66.05%) in juice samples with acacia honey. The smallest increase in TTC values was recorded for 'Northblue' in the sample of blueberry juice with acacia honey (49.53%), followed by the sample without honey (54.44%). Statistically, very significant differences were recorded in all cases.

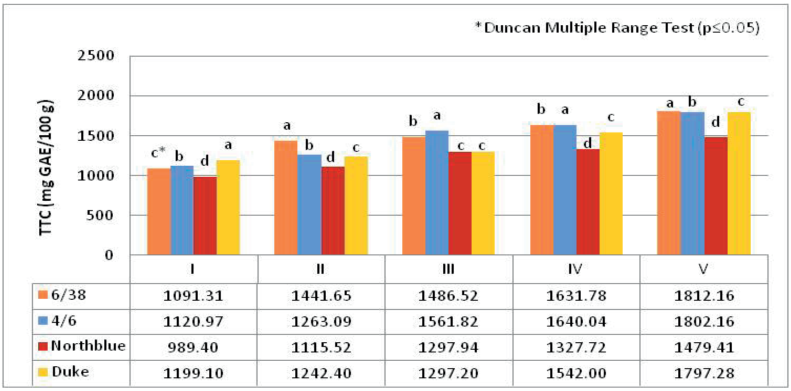


Figure 4. The influence of genotype on TTC depending on the storage period in blueberry juices with acacia honey
Note: *Different letters between cultivars denote significant differences (Duncan test. p < 0.05);
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. P < 0.05)

Regarding the influence of the storage period on TTC depending on genotype (Figures 5 and 6), a very significant increase was observed from the beginning of the study to the final storage moment in all blueberry juices.

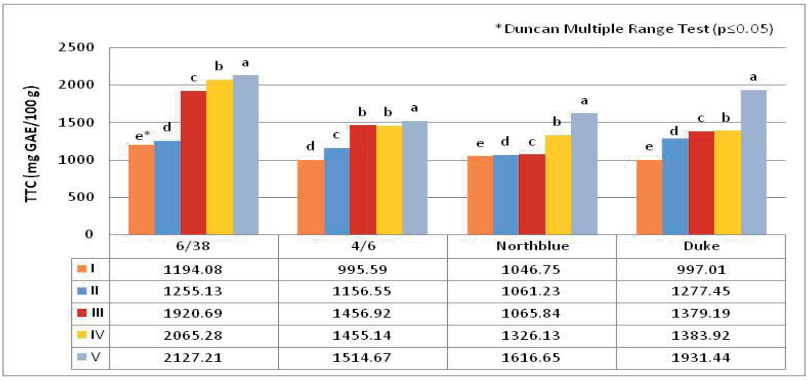
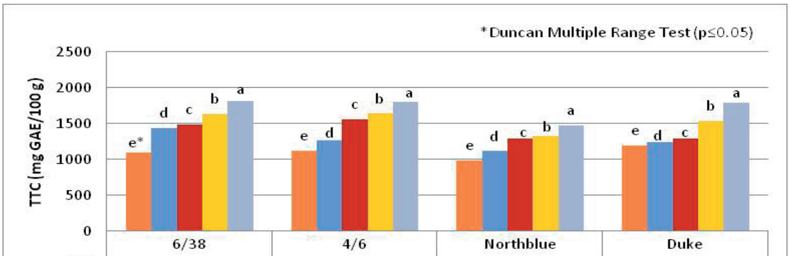


Figure 5. The influence of the storage period on TTC depending on blueberry genotype in juices without honey
Note: *Different letters between cultivars denote significant differences (Duncan test. p < 0.05);
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. P < 0.05)



	6/38	4/6	Northblue	Duke
I	1091.31	1120.97	989.40	1199.10
II	1441.65	1263.09	1115.52	1242.40
III	1486.52	1561.82	1297.94	1297.20
IV	1631.78	1640.04	1327.72	1542.00
V	1812.16	1802.16	1479.41	1797.28

Figure 6. The influence of the storage period on TTC depending on blueberry genotype in juices with acacia honey
Note: *Different letters between cultivars denote significant differences (Duncan test. p < 0.05);
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. P < 0.05)

In the case of blueberry juices without honey (Figure 5), TTC was 995.59-1194.08 mg GAE/100 g at time point I, and 1514.67-2127.21 mg GAE/100 g at time point V. The same increase was observed in the juices to which acacia honey was added, but the percentage increase for the entire storage period was lower compared to juices without honey. Similar results were found in the blueberry juice (Bett-Garber et al., 2015; Li et al., 2021), ginkgo kernel juice (Wang et al., 2019), sea buckthorn and sea buckthorn-apple juices (Tkacz et al., 2020), and mulberry juice (Kwaw et al., 2018).

Regarding the influence of sweetener on TTC depending on genotype and the storage period (Figure 7), a very significant effects between the juices without honey and the juices with acacia honey was observed at each time point for all blueberry genotypes. However, of the three factors (genotype, storage period and sweetener) the sweetener showed the lowest effect size on TTC values (data not presented).

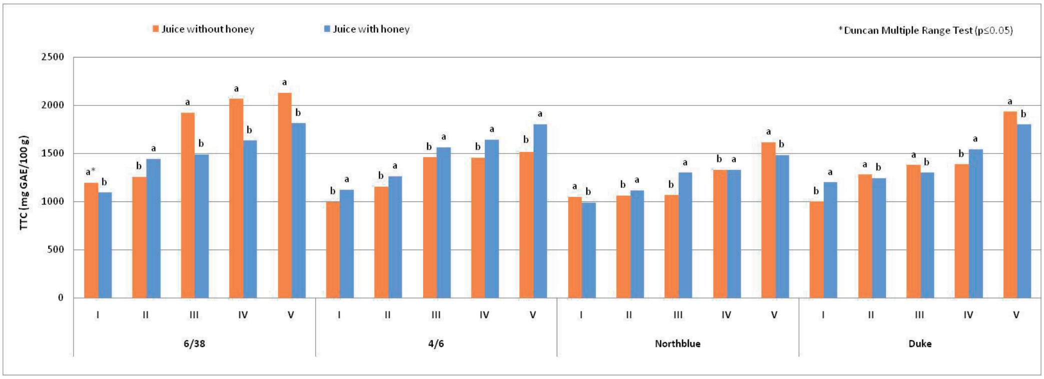


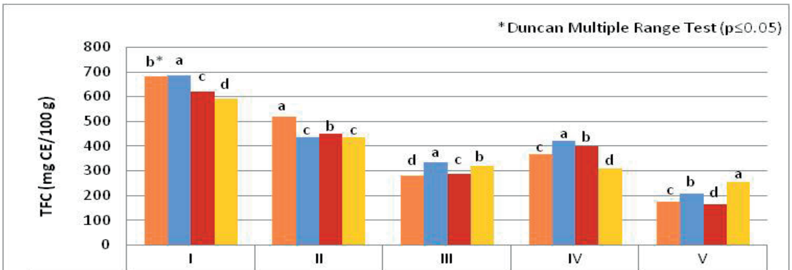
Figure 7. The influence of sweetener on TTC depending on genotype and storage period in blueberry juices
Note: *Different letters between cultivars denote significant differences (Duncan test. p < 0.05);
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. P < 0.05)

Total flavonoid content in juice

Flavonoids in blueberry are well-known for being synthesized in specific locations. They contribute to the colour and fragrance of flowers, as well as to an effective fruit dispersion that attracts pollinators since they support the spore germination, as well as the growth and development of seedlings (Panche et al., 2016).

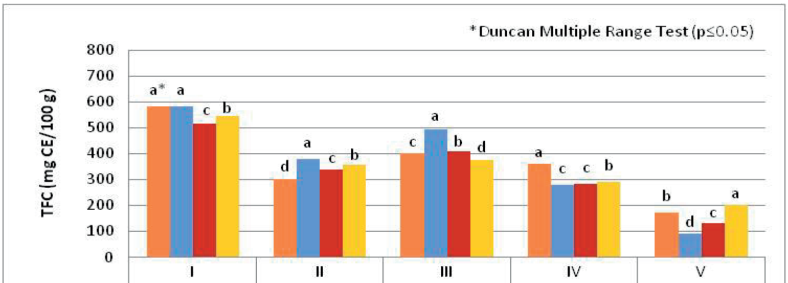
Regarding the influence of the genotype on TFC depending on the storage period in juices obtained without adding honey and the juices to which acacia honey was added, a decrease is observed continuously over the entire storage

period for each genotype with very significant differences between genotypes (Figures 8 and 9).
At the initial time (I), by adding acacia honey to blueberry juices, the TFC values decreased in all juices regardless of blueberry genotype, this decrease being explained by the small intake of the acacia honey, which presented a TFC value of 11.83 mg CE/100 g (Vîjan et al., 2023).
The highest decrease in TFC values registered between de time I and the last storage moment was recorded for 'Northblue' (73.96%) and '6/38' (74.46%) in juice samples without honey and for '4/6' (84.30%) and 'Northblue' (74.85%) in juice samples with acacia honey. The smallest decrease in TFC values was recorded for 'Duke' in the sample of blueberry juice without acacia honey (57.16%), followed by the sample with honey (63.05%), this genotype proved a superior stability of TFC both in the unsweetened juice and in the juice with honey.
As regards the influence of the storage period on TFC depending on genotype (Figures 10 and 11), very significant differences were noticed for all juices. For blueberry juices without honey, the highest TFC was recorded at Time point I: 588.99-685.68 mg CE/100 g, and it decreased to 161.14-252.31 mg CE/100 g at Time point V. The same decrease was observed in the juices to which acacia honey was added, the percentage decrease being comparable between the two types of juices: 57.16-74.46% in the juices without honey and 63.05-84.30% in the juices with acacia honey.



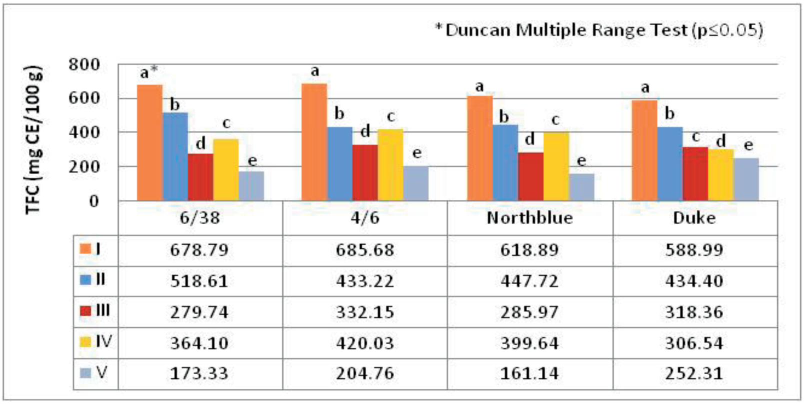
	I	II	III	IV	V
6/38	678.79	518.61	279.74	364.10	173.33
4/6	685.68	433.22	332.15	420.03	204.76
Northblue	618.89	447.72	285.97	399.64	161.14
Duke	588.99	434.40	318.36	306.54	252.31

Figure 8. The influence of genotype on TFC depending on the storage period in blueberry juices without honey
Note: *Different letters between cultivars denote significant differences (Duncan test. p < 0.05);
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. P < 0.05)



	I	II	III	IV	V
6/38	583.21	300.75	400.76	360.63	172.31
4/6	583.94	379.63	495.34	280.18	91.67
Northblue	517.22	339.33	407.98	281.88	130.06
Duke	545.21	355.12	373.66	290.12	201.46

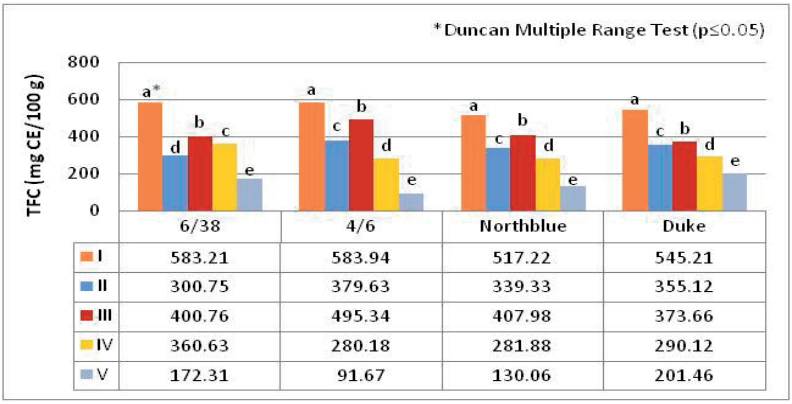
Figure 9. The influence of genotype on TFC depending on the storage period in blueberry juices with honey
Note: *Different letters between cultivars denote significant differences (Duncan test. P < 0.05);
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. P < 0.05)



	6/38	4/6	Northblue	Duke
I	678.79	685.68	618.89	588.99
II	518.61	433.22	447.72	434.40
III	279.74	332.15	285.97	318.36
IV	364.10	420.03	399.64	306.54
V	173.33	204.76	161.14	252.31

Note: *Different letters between cultivars denote significant differences (Duncan test. p < 0.05).
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. P < 0.05)

Figure 10. The influence of the storage period on TFC depending on blueberry genotype in juices without honey



	6/38	4/6	Northblue	Duke
I	583.21	583.94	517.22	545.21
II	300.75	379.63	339.33	355.12
III	400.76	495.34	407.98	373.66
IV	360.63	280.18	281.88	290.12
V	172.31	91.67	130.06	201.46

Figure 11. The influence of the storage period on TFC depending on blueberry genotype in juices with honey

Note: *Different letters between cultivars denote significant differences (Duncan test. p < 0.05);
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. P < 0.05)

Regarding the influence of sweetener on TFC depending on genotype and the storage period (Figure 12), a very significant effects between the juices without honey and the juices with acacia honey was observed at each time point for all blueberry genotypes.

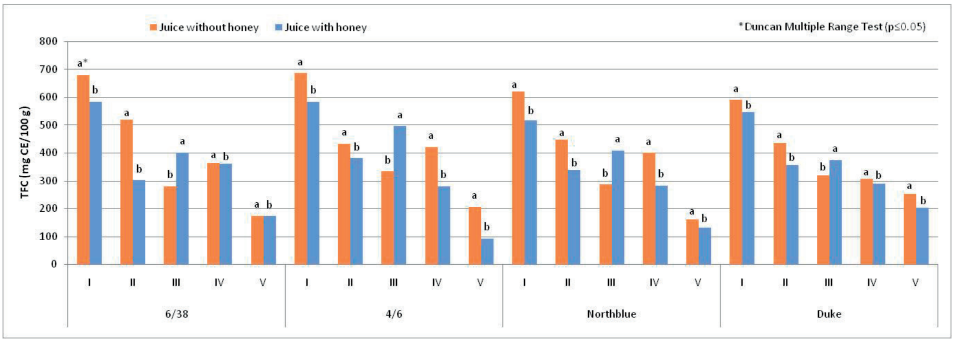


Figure 12. The influence of sweetener on TFC depending on genotype and storage period in blueberry juices

Note: *Different letters between cultivars denote significant differences (Duncan test. p < 0.05);
Different letters between susceptible and resistant cultivars denote significant differences (LSD test. P < 0.05)

CONCLUSIONS

The results revealed very significant effects of the three analysed factors (genotype, storage period and sweetener) on the total tannin content and the total flavonoid content, with the sweetener (acacia honey) showing the lowest effect size.

Extending the storage time led to opposite trends on TTC and TFC as from the first analysis to the fifth, the tannins content increased, while the flavonoids content decreased.

Among the genotypes, 'Duke' stood out, from which juices were obtained (unsweetened, but also with honey) that presented at the end of the study the highest levels of flavonoids. 'Northblue' was also noted, which at the end of the storage period accumulated the lowest level of tannin. Top-quality blueberry juices can be obtained only by taking into account the fruit compositional variability and its preservation along the processing chain.

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IN VITRO CULTURE OF KIWIFRUIT SPECIES (*ACTINIDIA* SP.) - A REVIEW

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Abstract

Actinidia deliciosa, known as kiwifruit, has gained a great popularity and demand due to its nutritional and medicinal value. Actinidia genus has at least three cultivated species with an important economic role. In vitro culture of kiwifruit species was an important tool for plant micropropagation and for unconventional breeding (direct organogenesis, callogenesis, somatic embryogenesis, cells culture, etc). The actual paper presents a comprehensive image of the main results that were achieved in different in vitro cultures of kiwifruit species. New alternative developments are being proposed in order to obtain efficient protocols of the species for production of clonal planting materials.

Key words: *callus, culture media, micropropagation, organogenesis, somatic embryos.*

INTRODUCTION

Actinidia deliciosa, commonly known as kiwi fruit and belonging to the *Actinidiaceae* family, is a dioecious woody climbing shrub. It thrives in temperate and cold regions as well and is cultivated for its flavourful fruit. Among the various species within the *Actinidia* genus, *A. deliciosa, A. arguta* and *A. chinensis* are the three specifically grown for their fruits.

Rugini and Gutierrez-Pesce (2003) note that kiwifruit is notable for its high vitamin C content, with *A. deliciosa* providing 140 mg per 100 g of fresh weight. The fruit also contains pretty high levels of minerals, mainly potassium and magnesium, as well as trace elements such as copper, zinc, and manganese. While kiwifruit is commonly consumed fresh within 1 to 6 months post-harvest, it is also processed for various food products, including jams, juices, and syrups.

In Romania, kiwi is recognized as an imported fruit and is predominantly available in supermarkets at a relatively high cost. The experimental orchard belonging to the Faculty of Horticulture in Bucharest, Romania, has kiwi fruits planted since 1993, and since then, efforts have been made to develop optimal techniques for cultivation, management, and micropropagation, resulting positive outcomes. (Stănică et al., 2007)

The successful marketing of *A. deliciosa* cultivars has motivated the initiation of breeding programs with the goal of enhancing the species value. Nevertheless, the species' dioecious nature, prolonged juvenile period, and inadequate rooting, are constraints to these breeding initiatives.

Kiwifruit plants needs approximately 25 weeks from flower bloom to achieve physiological maturity (Mardiana et al, 2018). Consequently, obtaining large quantities of kiwi seeds out of seeds would require a considerable amount of time.

The most cost-effective technique for capturing genetic gains in horticulturally important plants is the production of clonal planting material from vegetative tissue through conventional methods. However, this method is often limited by the poor rooting of cuttings. In contrast, tissue culture techniques provide a fast and reliable alternative for producing genetically uniform clonal material in a shorter time frame, making them highly effective for the propagation and conservation of species with reproductive challenges.

Actinidia sp. is commonly propagated through grafting and stem cuttings, but the limited cuttings rooting success restricts the production of clonal planting materials.

The initiation of an *in vitro* culture, using plant materials sourced from the field is a crucial step in establishing a clonal propagation process. The effective establishment of *in vitro* cultures is influenced by factors such as genotype, health of the mother plant grown in the field, preparation of the explant, surface sterilization procedures, medium composition, and growth conditions, etc.

The purpose of this paper is to provide a brief overview of the achievements and progress made in the recent years regarding the *in vitro* micropropagation of *Actinidia* species.

HISTORY

Tissue culture with its *in vitro* manipulation of plant cells, tissues, and organs, stands as a prominent biotechnological technique. Several tissue culture methods have been applied to overcome the challenges associated with traditional reproduction in *Actinidia*. Harada (1975) was the first to propose a micropropagation protocol for *Actinidia*, which was later refined by Standardi (1983), Wessels et al. (1984), and Monette (1986). The Murashige and Skoog (MS) method has become widely used for preparing the culture medium, demonstrating benefits for both regeneration and callus formation. Additionally, other media such as Gamborg B5 and N6 have demonstrated success in this context.

STERILIZATION

The sterilization of explants is a critical stage in plant tissue culture, as it is necessary to eliminate all microorganisms, including bacteria and fungi. This step is essential for achieving successful initiation, growth, and development of cultured tissues in vitro. Without proper sterilization, the cultured tissues would be susceptible to contamination and compromised in their development. Deb et al. (2019) described a successful sterilization protocol involving the collection of nodal segments from branches, which were then wrapped in fresh banana leaves and moistened with sprinkled water to retain humidity. The scales and other impurities were carefully removed, followed by washing under running tap water. The segments were stored at 4°C in a refrigerator until needed. For surface sterilization, 2 cm nodal segments were gently scrubbed with a soft brush using “Labolene” (a commercial laboratory detergent at 1:100, v/v). This was followed by washing under running tap water for 10-15 minutes, sterilization with an aqueous solution of $HgCl_2$ (0.3%, w/v) for 3-5 minutes, and subsequent washing 4-5 times with sterile water.

In a study done by Zhong et al (2021), the stem segment containing a single bud from the kiwifruit underwent disinfection using 70% alcohol and 15% NaClO. The outcomes demonstrated that the rates of contamination and browning could be maintained below 20%, and the survival rate could surpass 70% when the single bud stem segment of the kiwifruit was sterilized with 70% alcohol for 30-60 seconds and 15% NaClO for 15 minutes, respectively.

MULTIPLICATION STAGE

Various vegetative explants, such as leaves, bulbs/pseudo-bulbs, roots, axillary buds, nodal segments, and inflorescences have been utilized for the propagation of various agronomical significant species. Deb et al. (2019) identified nodal segments with axillary buds as the most effective method for producing clones among various propagation techniques.

Based on the previously achievements in what is currently referred to as *A. deliciosa* (mainly in cv. ‘Hayward’) and *A. chinensis*, plant regeneration has been achieved in *A. arguta* using young leaves (Zhang and Qian, 1996; Zhu, 1997) and mature embryos (Xiao et al, 1990). Similarly, in *A. deliciosa,* regeneration has been accomplished from mature endosperm (Xiao et al, 1990), cell suspensions (Zhang et al, 1991), long-term stored stems (Ding et al, 1997), and in interspecific hybrids of *A. deliciosa* x *A. arguta* (Famiani et al, 1997).

Factors beyond traditional plant growth regulators and hormones that impact plant regeneration have been investigated. Coconut milk has been demonstrated to enhance axillary shoot growth (Boase et al, 1993), while vermiculite is noted to help in the rooting of regenerated shoots, either alone or in combination with agar *in vitro* (Chen 1997, Wang 1997). Feito et al (1994) discovered that the metabolic activity of BA and endogenous cytokinins is influenced by the physical composition of the culture medium.

Pedroso's et al. (1992) discovered that inoculating nodal segments and shoot tips on Murashige and Skoog (MS) media without growth regulators, it results in a good plant development at a reduced cost and minimal manipulation.

In recent years, increased efforts have been directed towards comprehending the mechanism of plant growth regulator uptake, particularly cytokinins. The absorption and metabolism of 6-benzyladenine (BAP) were investigated in *A. deliciosa* explants cultivated in ventilated cultures, a method employed to prevent the formation of hyperhydric shoots during propagation in liquid medium. Cañal et al. (2000) reported that 65% of the initial amount of BAP disappeared within the first half-hour of culture, converting into seven distinct glucosides. Moncaleon et al. (2001) further demonstrated that 6-benzylaminopurine (BAP) plays a critical role throughout various phases of micropropagation and influences the development of regenerants. The highest quality shoots, measured by multiplication index, weight, length, and callus formation, were achieved after one day of cultivation with 4.4 mg L^{-1} of BAP. Additionally, zeatin was identified as the most effective cytokinin for inducing shoot regeneration (Akbaș et al., 2007). Herrera et al. (2005) obtained optimal results in shoot multiplication and elongation using Cheng K medium supplemented with 0.05 µM NAA, 22 µM BA, and 1.4 µM GA3 (Prado et al., 2002). In an experiment conducted by Akbaș et al (2007), the use of BAP resulted in a more favourable outcome for shoot production compared to the kinetin treatments. Optimal results for kiwifruit shoot production were achieved with 0.5 mg L^{-1} BAP. Consistent with these studies, Moncaleon et al. (2001) noted that BAP significantly affects multiple stages of micropropagation and also plays a role in regulating the development of regenerants. A comparison is summarized in Table 1. In analogous findings concerning cv. Hayward kiwifruit, zeatin appears to be the most effective in inducing shoot regeneration from callus, while BAP is typically employed for shoot proliferation (Rugini et al., 1991). Marino and Bertazza (1990) found that BAP caused hyperhydricity in older leaves, an effect that was not seen with zeatin, even though zeatin resulted in higher proliferation rates. A significant increase in shoot proliferation was documented when employing a liquid medium containing 8.9 µM indole butyric acid (IBA), with chilled lateral buds proving to be the most effective for multiplication (Lionakis & Zirari, 1991). Pais et al. (1987) reported successful proliferation by employing a combination of 50% MS macro- and micronutrients, full-strength MS vitamins, and 5 mg L^{-1} ascorbic acid as an antioxidant, along with 2.3 µM zeatin and 0.3 µM IAA during a 4-week subculture period. The most favourable average root number and length per shoot were achieved with media supplemented with 1.0 mg L^{-1} NAA (Akbaș, et al. 2007).

Table 1. Plant growth regulators and hormons aiming successful shoot multiplication for *Actinidia* sp.

Type of growth regulator	Author	Year
BAP	Rugini et al.	1991
BAP	Moncaleon et al.	2001
Zeatin	Moncaleon et al.	2001
Cheng K+NAA+BAP+GA_3	Herrera et al.	2005
0.5 mg L^{-1} BAP	Akbaș et al.	2007

Mardiana et al. (2018) demonstrated the significance of gelling agents in influencing the growth of kiwi shoots. Based on the findings of this study, the results indicated that the treatment with Swallow Globe Agar at 4 g/l combined with Nutrijell Agar at 4 g/l resulted in the highest average number of shoots, as well as the highest number of leaves and roots. This substantiates that the combination of Swallow Globe and Nutrijell agar creates favorable conditions for the growth of kiwi shoots.

Deb et al. (2019) successfully established an efficient *in vitro* propagation protocol, utilizing nodal segments as *in vivo* sources, implementing an effective rooting technique, and conducting primary hardening with cost-effective substrates as an alternative to agar.

Fortified with 3% (w/v) sucrose, Polyvinylpyrrolidone (300 mg/L), and benzyl adenine (BA) (6 μM), 7-week-old nodal segments were established on MS medium, resulting in an average formation of 4.2 shoot buds in 75% of cultured segments. Additionally, using MS medium supplemented with 3% sucrose and 3 μM BA produced 2 micro shoots in 58.3% of cultures per cycle. The activation of axillary buds relies on a comparatively high concentration of cytokinin, which inhibits apical dominance and facilitates the growth of buds (Pierik, 1987). Velayandom et al. (1985) and Monette (1986) identified this method, which uses nodal segments and shoot tips, as simpler than other micropropagation techniques. It consistently generates a substantial number of uniform shoots from a single explant in a short period, maintaining genetic stability. Zuccherelli (1981) noted that for *Actinidia* species, particularly in large-scale cultivation, this approach is the most practical propagation method. Standardi (1981, 1983) and Wessels et al. (1983) emphasized the importance of carefully avoiding the propagation of shoots derived from basal callus, as *Actinidia* species easily regenerate from undifferentiated cells and are prone to somatic variation. For root induction, various techniques are utilized, many of which rely on indole-3-butyric acid (IBA) as an effective root inducer. A common method involves briefly immersing the base of the shoot in a high-concentration IBA solution (50 mg/L) to promote root formation. An alternative method includes the overnight immersion of the shoot's basal part in solutions with low IBA content (0.3, 1, 2 mg/l), resulting in a high rate of successful rooting (adapted from Standardi, 1981; Wessels et al., 1983).

CONCLUSIONS

In vitro propagation through the development of axillary buds is quicker than conventional methods and needs a small quantity of starting material, the *in vitro* plants propagated in this way in many species have proved to be true to type (Murashige, 1974; Navarro et al., 1975; Styer, 1983). Therefore, it is the best way for propagation for new cultivars or clones for fruit production. Before this, *in vitro* propagation technique is to be used for propagation of *Actinidia sp.*, the mother plants of this origin must be evaluated, but can be used immediately for breeding purposes.

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POPULATION DYNAMICS OF *LEUCOPTERA MALIFOLIELLA* COSTA IN CHERRY ORCHARD FROM CLUJ COUNTY

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Abstract

Leucoptera malifoliella is a polyphagous leaf miner, which is present across Europe and Asia finding its host plants mostly from the Rosaceae or Betulaceae families. It can cause severe damage in various orchards, especially, pear and apple due to larvae which are feeding on the mesophyll tissue of leaves and cause premature leaf fall and serious yield reduction. Therefore, the main aim of this research was to monitor and investigate the life cycle of the species and the damage caused during 2021 and 2022 on two sweet cherry tree varieties 'Regina' and 'Regina'. The results show that the pear leaf blister moth has three peaks in flight dynamics, which could correspond to the development of three and one generation during the years 2021 and 2022. The flight of the overwintering males (1st generation) began in May and the 3rd generations flew until the 28th of September. The peak of the flight curve for each generation was recorded 2-3 weeks after the start of the flight. The frequency of the attack increased from one generation to another, reaching up to 23.64% in 'Kordia' and 19.04% in 'Regina'. The intensity of the attack increased as well from one generation to another up to 90% at the end of the season. The average number of mines/leaf was over 7 mines, with a maximum of 23 mines for the 'Regina' variety and 28 mines for 'Kordia' leading to a damage level of 17.13% and 21.27% for the two sweet cherry varieties. In 2022 the number of pest generation decreased proving the efficiency of pheromone traps.

Key words: *pear leaf blister moth, cherry trees, monitoring, AtraScit pheromone, damage.*

INTRODUCTION

In many fruit orchards among the rich complex of pests that attack at the leaf level, the species of mining microlepidopterans are often found. While worldwide over 300 leaf miner species are known, the most frequently reported species are: *Leucoptera malifoliella* (Costa, 1836) (Lyonetiidae), *Lyonetia clerkella Phyllonorycter blancardella*, *Phyllonorycter corylifoliella*, *Callisto denticulella*, *Stigmella malella* (Tešanović, 2011; Płóciennik et al., 2011; Tešanović and Spasić, 2015; Rather and Buhroo, 2015; Kholboevich et al., 2020). Leaf mining moth can easily be recognized primarily, based on the pattern of feeding tunnels they create and the mined leaves which can often contain frass and droppings. The shape of the mine, the pattern of frass deposition and the host plant itself are also important indicators in the determination of these species (Sepros, 1986; Seven, 2006). However, some mining insects are feeding on other parts of the plants such as fruit surfaces or flower petals (Saidov et al., 2018; Lopez-Vaamonde et al., 2021).

Leaf-mining insects are solitary miners whose larvae feed inside the leaves producing concentric galleries, which can reach a diameter of 5-6 mm and are called leaf mines. The mines change their size and colour over time from small and whitish to large with brown spots. Frass may be visible, grouped in dark, concentric circles. In the case of heavy infestation, the leaf surface is severely damaged by the presence of mines affecting photosynthesis and crop production (Nawaz et al., 2021).

In fruit orchards, the dominant leaf-miner species is *L. malifoliella* most commonly known as pear leaf blister moth, pear leaf miner, apple leaf miner or ribbed apple leaf miner (Maciesiak, 1999; Radoslav et al., 2001; Fekete et al., 2005; Molet, 2011).

In Romania, *L. malifoliella* was first reported by Caradja in 1901 in Neamţ County, at Grumăzeşti (Oltean, 2000). Between 1960 and 1970 insect pest outbreaks were observed across the country. Due to the large gene pool of insect populations, their high reproductive rate and the lack of efficient pest management strategies at that time, *L. malifoliella* damaged several orchards including apple, pear and cherry orchards in Arad, Timiş, Hunedoara, Alba, Sibiu, Bihor, Satu-Mare, Maramureş, Sălaj, Cluj, Bistriţa-Năsăud, Mureş, Caraş-Severin, Mehedinţi, Dolj, Olt, Gorj, Argeş, Vâlcea, Vrancea, Buzău, Dâmboviţa, Suceava and Călăraşi counties (Nicolae et al., 2011).

L. malifoliella is a polyphagous species and has many hosts but the most preferred host plants are members of the Rosaceae family (Béguinot, 2017). It occurs in temperate climates causing severe damage in fruit orchards as well (Koç et al., 2014). Among fruit trees this pest prefers the most *Malus domestica* Borkh, *Pyrus sativa* Lam., *Prunus avium* L., *Cydonia oblonga* Mill., *Prunus domestica* L., *Prunus persica* L., *Prunus armeniaca* L. (Bajec et al., 2009; Barić et al., 2010), and *Mespilus germanica* L. (Janick, 2008). This first attack of *L. malifoliella* in a cherry orchard was first reported in 2004 by Balazs and Jenser, followed by other authors in the next years (Nicolae et al., 2011; Mishchenko and Prakh, 2016). Besides fruit trees, this pest finds hosts in different trees and shrubs as follows: *Crataegus monogyna* Jacq., *Clematis vitalba* L., *Cornus mas* L., *Cotoneaster melanocarpus* Lodd. (Kirichenko et al., 2017; Hellers, 2019; Fichtner and Wissemann, 2021) and even in *Alnus, Betula, Quercus* and *Salix* spp. (Celli and Burchi, 1983). Ecevit et al., in 1987, reported this species as a new pest of hazelnuts.

The species is multivoltine, developing multiple generations (1-5) each year depending on the geographical location (Mey, 1988; Sáringer and Csontos, 1998; Tălmaciu et al., 2008).

A warm climate and the increasing number of generations of this species can be extremely dangerous, especially in areas where large orchards of various fruits are located and from where they could be spread toward other locations (Maciesiak and Olszak, 2006; Béguinot, 2017). In some cases, favourable climatic conditions and unsuitable use and application of pesticides can stimulate more the outbreak of this pest.

According to previous research results, in fruit orchards, the frequency and intensity of the attack of *L. malifoliella* is influenced first by the host plant (Andreev et al., 2001; Jenser et al., 2001; Enzsoly and Kuroli, 2003; Tešanović and Spasić, 2010) and then, by pest monitoring methods and pest detection. Due to the very small size of this pest and its hiding behaviour are the main reasons why this pest is not observed in time and treatments are applied too late which could lead to even worse results (Ivanov, 1976; Šubić, 2015a). The average size of the mine is 0.88-1.04 cm^2, depending on the attacked plant and variety (Baufeld and Freier, 1992). The number of mines on one leaf differs and it is correlated with the population dynamics of the species generally (Górska-Drabik, 2006). A large number of mines cause heavy premature leaf loss during August or early September (Bajec et al., 2009). Early foliage drop adversely affects fruit size, quality and total yield. Heavy leaf loss also influences the differentiation of flower buds and the maturation of the shoots (Šubić, 2015b).

Various studies from the scientific literature revealed it is that 28 insects were reported to attack the final larval instars; most of them belonging to the Eulophidae, Pteromalidae and Braconidae families.

Previous studies also show that the rate of parasitism is low in the first generation of the pest but increases during the second generation (Mey, 1993).

Regarding chemical control management, first and foremost is very important to take into account the phenological stages of the pest in order to identify its sensitive stages and apply the pesticide needed. Several insecticides have already been tested to overcome pear-leaf blister moths (Injac, 1981; Injac and Dulic, 1983; Maciesiak, 1996), but the results showed that their effectiveness is strongly correlated with the moment of treatment application and the coverage area (Pénzes, 1985; Marinkov, 1986).

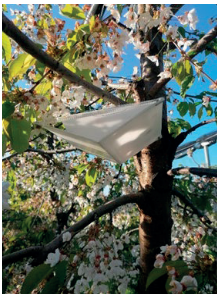
In this regard, field monitoring and forecasting remain the most reliable methods to use to follow the phenological stages of *L. malifoliella* and apply effective treatments. Pheromone traps 5.9-Dimethylheptadecane became of great importance providing accurate information about the pest population dynamics (Francke et al., 1987; Ciocan-Tarta et al., 1998; Liang et al., 2000) showing good attractiveness and selectivity (Kutinkova and Andreev, 2002). These traps are used for population monitoring and to indicate the best time to apply (Koutinkova et al., 1999; Nikolic et al., 2011; Rosu-Mares et al., 2021) chemical treatments (Nicolae et al., 1996; Drosu, 2010; Grünig et al., 2021).
Between 1985 and 2000, *L. malifoliella* was identified in the fruit-growing basin of Cluj County as a pest that caused severe damage, especially in apple orchards. After this period a significant decrease of the pest population density was reported. In 2019, the species was first reported in cherry orchards in Cluj County and in the following year, a significant pest outbreak was detected in an urban cherry orchard (Steluța farm) in the city of Cluj-Napoca.
Therefore, the main aim of this research was to monitor pest population development, its' density and the intensity of the attack of this species upon the urban sweet cherry orchard in the climatic conditions of Cluj-Napoca. Furthermore, this study aimed also to serve as a forewarning for fellow farmers to help them develop efficient integrated management strategies against this yield-devastating pest.

MATERIALS AND METHODS

The current research has been carried out in an urban sweet cherry orchard (a commercial family orchard) situated in Cluj-Napoca (46°8'1" N, 23°6'0" E) established in 2010 laying out on five hectares. The cherry cultivars were grafted on Gisella 5. All the fruit trees were planted in high density system at 4 x 1.5 m. The inter-rows were grassed and maintained through frequent mowing combined with herbicide strips along the tree rows. The orchard is equipped with a drip irrigation system and protection covers against rainfall and hale.

L. malifoliella (Figure 1) was detected first in 2021 attacking 'Regina' and 'Kordia' sweet cherry cultivars. In order to monitor pest population pheromone traps were installed in the orchard. To determine the exact time of trap placement on the 4th of March in both experimental years. For orchard monitoring and mass capture, 20 AtraSCIT pheromone traps/ha were installed in the orchard to detect and monitor pear blister moth activity (Figure 2a).

Figure 1. *Leucoptera malifoliella* adults



a

B

Figure 2. AtraSCIT pheromone traps in the sweet cherry orchard (a) the monitoring population of *L. malifoliella* by counting the males caught in the trap (b)

The monitoring period of this pest started in May and ended in September. During this period 10 readings of the pheromonal traps were carried out, recording the number of males caught in the installed traps (Figure 2b). Based on the data recorded, the flight pattern was determined. To define the frequency and intensity of the attack leaves from 'Regina' and 'Kordia' sweet cherry tree leaves were collected at two different moments during the growing season. The first set of leaves was harvested on the 23rd of July, while the second set was on the 1st of September. In total, 100 leaves/trees were randomly picked from the bottom, middle and upper parts of the tree and transferred to the laboratory for further analysis. To determine the frequency of the attack the following formula was used:

$$F\ (\%) = \frac{\mathrm{n\ x\ 100}}{\mathrm{N}}$$

where:
n is the number of affected leaves;
N is the total number of leaves examined.

After the calculation of the frequency of the attack, the number of mines was recorded. Afterwards, the leaves were further investigated using WinDIAS 3 Leaf Image Analysis System to determine the intensity of the attack by scanning the leaves and measuring the damaged area of the leaf area.
Regarding the intensity of the attack and damage level, these were determined based on the number of mines recorded on the leaves and the damaged area of the leaves and expressed as percentages. Therefore, the results of the analysis carried out on the leaves harvested on the 23rd of July, split the data into four groups based on the number of mines recorded per leaf (Figure 3).

'Kordia' 'Regina'

Figure 3. Damaged leaves of 'Kordia' and 'Regina' varieties by *L. malifoliella*

Based on the data recorded, the intensity of the attack was calculated using the following formula:

$$I\ (\%) = \frac{\sum(ixf)}{n}$$

where:
i is the damage score for the sample leaf;
f is the no. of leaves scored;
n is the total number of affected leaves examined.

The damage level of the attack was then calculated according to the following formula:

$$Damage\ level\ (\%) = \frac{\mathrm{F\ (\%)\ x\ I\ (\%)}}{100}$$

In cases when the mines were joined due to overlapping of the larvae activity, the number of the mines was counted separately based on the imprint of the larval excrement.
The data were analyzed using the analysis of variance (ANOVA) to detect significant differences between the means at $p<0.05$. When the null hypothesis was rejected, Duncan's multiple range test was performed to indicate significant differences between the means. Pearson's correlation was also assessed to measure the linear associations between the variables under study.

RESULTS AND DISCUSSION

The results of this research revealed that *L. malifoliella* developed three generations in 2021 and one generation in 2022. Regarding the pest dynamics, it was observed that the first overwintering adult month emerged on the 9th of May (Figure 4) and began its flight. The duration of the flight period was approximately 6 weeks, reaching the peak of the flight pattern within 2 weeks from pest occurrence. In total, 6390 individuals were caught in all traps during the entire flight period in 2021 and 4522 in 2022. On the 25th of May, 1528 individuals were counted in the pheromone trap being considered the peak of the flight pattern in 2021 and 2612 in 2022 (Figure 4).
The maximum number of captured adults recorded between two consecutive readings in one trap was 135. The flight of the second generation spanned for approximately one month (from the first decade of July to the first decade of August). The peak of the flight curve was recorded in two weeks, similar to the first generation, with 921 captured individuals on the 20th of July (Figure 4). During the flight period of this generation, 1062 individuals were captured in traps, with a maximum number of 110 adults in a single trap between the two consecutive readings.
The flight of the third generation started at the beginning of the second decade of August and continued until the end of September 2021. The peak of the flight curve was recorded after three weeks, on the 31st of August when 1993

captures were recorded (Figure 4). The total number of males caught in the pheromone traps of this generation was 3001, and the maximum number of captures by a single trap between two consecutive readings was 217. During the 2021 year, the total number of *L. malifoliella* male adults captured in the AtraScit pheromone traps was 6390. The mean number of adults captured in traps was 319.5, while the number of adults captured in one trap ranged between 131 and 517.

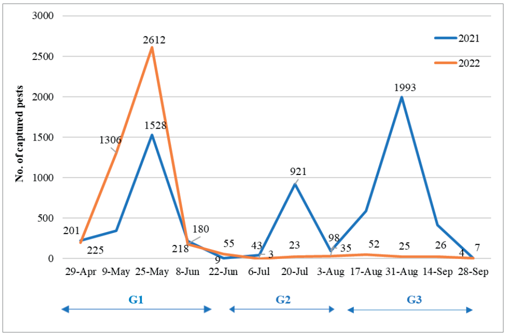


Figure 4. The flight pattern of *Leucoptera malifoliella* in 2021 and 2022. The data presented are the mean values of the 20 traps

The observations made on *L. malifoliella* in the 2022 year, revealed that the highest number of individuals caught in traps was 2612 on the 25th of May and after this date, the number of individuals caught decreased. In this year only two generations were recorded (Figure 4). The total number of *L. malifoliella* male adults captured in the AtraScit pheromone traps was 4522. The lack of the third generation might be explained by the high efficiency of the pheromone traps installed in the previous year which led to the decrease of the *L. malifoliella* population.

The high number of captures during the experimental years demonstrates that AtraScit pheromone traps were suitable not only for pest monitoring but also for mass trapping. Our findings are in accordance with previous research results which enhance the efficiency of this pheromone trap for *Leucoptera scitella* in apple orchards capturing all the existing individuals from the orchard (Rosu-Mares et al., 2021).

To understand better the flight pattern of this species during 2021 and 2022, the number of captured males was correlated with the most important meteorological parameters as follows: minimum, maximum and average temperature, precipitation and wind speed. Therefore, the results of Pearson's correlation indicated a negative linear association between air temperature and the number of captures. As air temperature increased, the number of captures decreased, suggesting that *L. malifoliella* adults preferred lower temperatures, humidity and windy conditions more for their flight and population spread in both years (Table 1).

Table 1. Pearson's correlation coefficients (r) between weather conditions and the number of captured male adults from the 9th of May to the 28th of September 2021 and 2022

Met. Param./Year	Avg. temp. (°C)	Avg. min. temp. (°C)	Avg. max. temp. (°C)	Avg. precip (mm)	Wind speed (km/h)
2021	-0.2982	-0.1097	-0.3256	0.0587	0.0629
2022	-0.1444	-0.1703	-0.2634	0.8202	0.0672

During the monitoring period, it has been observed that pupation of the first and second generations took place predominantly on foliage and fruits (Figure 5) of the sweet cherry trees, but cocoons of the third generation were observed only in more protected (hidden) areas such as under-bark, on stems, twigs and even in the vicinity of flower buds (Figure 6).

Figure 5. Pupae of *Leucoptera malifoliella* on leaves and fruits (G1 and G2)

Figure 6. Overwintering cocoons of *Leucoptera malifoliella* on woody parts of sweet cherry trees

Thus, in 2021 the results, of the leaf analyses revealed that 48% and 38% of the leaves of 'Regina' and 'Kordia' varieties respectively, had up to 5 mines/leaf. Leaves with 6 to 10 mines accounted for 29% of 'Regina' and 43% of 'Kordia' leaves. Between 11 and 15 mines were found in 4% of 'Regina' leaves and 9% of 'Kordia' leaves. Leaves with 16 and 20 mines were found only on the leaves of the 'Kordia' variety. The maximum number of mines on a single damaged leaf was 23 mines in 'Regina' and 28 mines for the 'Kordia' variety (Figures 7 and 8).

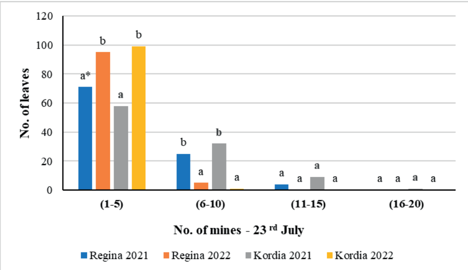


Figure 7. The number of mines per leaf recorded in 'Regina' and 'Kordia' sweet cherry varieties on the 23rd of July 2021 and 2022. The data shown are means. Lowercase letters above the bars indicate significant differences between the experimental years within the same sweet cherry variety while asterisk denotes significant differences between the varieties from the same experimental year according to Duncan's multiple range test at P<0.05

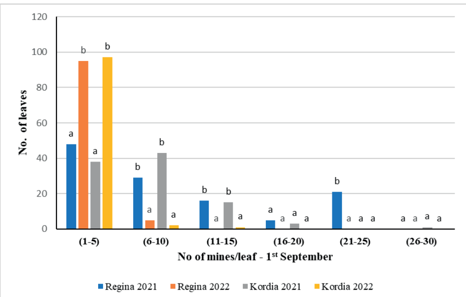


Figure 8. The number of mines per leaf recorded in 'Regina' and 'Kordia' sweet cherry varieties on the 1st of September 2021 and 2022. The data shown are means. Lowercase letters above the bars indicate significant differences between the experimental years within the same sweet cherry variety. No significant differences were detected between the varieties from the same experimental year according to Duncan's multiple range test at P<0.05

In 2022, the number of attacked leaves increased by 24% and 41% respectively in July, when 95% and 99% of 'Regina' and 'Kordia' leaves contained between 1-5 mines/leaf. It has been observed that, as the number of mines/leaf increased the number of attacked leaves decreased. Thus, the maximum number of mines/leaf was 6 and 7 on 'Kordia' and 'Regina' leaves (Figure 8).

The analyses of the second set of damaged leaves harvested on the 1st of September revealed a slight decrease (2%) in the number of leaves with mines in the 'Kordia' variety (Figure 8).

The shape of the mines developed on the attacked leaves can denote certain characteristics depending on the host plant. On most of the attacked plants, the mines of this pest have a circular form. However, the current research suggests that in sweet cherry trees, the shape of the mines is rather elongated and oval-shaped reaching up to 25 mm in length and 5 mm in width (Figure 3).

These results can be explained by the efficiency of the AtraSCIT pheromone traps in the sweet cherry orchard.

These results are similar to previous results reported by Tălmaciu et al. (2008) indicating a more intense attack of *L. malifoliella* during the 3rd generation of the pest.

The intensity of the attack caused by *L. malifoliella* was also analyzed during this research followed by the determination of the intensity of the attack. The results showed that the intensity of the attack in 2021 year of *L. malifoliella* in the urban sweet cherry plantation was very high, ranging between 80-90% in sweet cherry varieties under study.

Table 2. Average frequency, intensity and damage level of *L. malifoliella* attack in two sweet cherry varieties

Cvs.	Date of capture count	Frequency (%)	Intensity (%)	Damage level (%)
Kordia	23.07.2021	15.02 b	80 b	12.01 b
	23.07.2022	4.51 a	60 a	2.70 a
	01.09.2021	23.64 b	90 b	21.27 b
	01.09.2022	4.13 a	70 a	2.89 a
Regina	23.07.2021	13.64 b	80 b	10.91 b
	23.07.2022	4.10 a	60 a	2.46 a
	01.09.2021	19.04 b	90 b	17.13 b
	01.09.2022	4.66 a	70 a	3.26 a

*Note: different lowercase letters indicate significant differences between the years within the same sweet cherry varieties. No statistically significant differences were detected between the cultivars within the same experimental year according to Duncan's multiple range test at p<0.05.

These high percentages of intensity indicate that both the 'Regina' and 'Kordia' varieties were extremely vulnerable in front of this pest. The occurrence of this pest was also reported by Ulusoy et al. (1999) in Turkey in sweet cherry and confirmed by Tezcan and Gülperçin (2024) but its frequency was much lower. In Romania, previous research results reveal the occurrence of this pest only in apple orchards. However, the frequency of the attack varied between 9.26-35.22%, it is worth mentioning that the highest frequencies were generated by the 3rd generation of the pest and only in urban areas (Tălmaciu et al., 2008). These results are in accordance with our findings that high frequencies of attack are facilitated by urban areas where the number of natural enemies is reduced. Biological control of various pests is provided by the natural enemies from a certain area, which are controlling the phytophagous pest population below the economic threshold. In cities and in the surrounding areas where green areas are more and more replaced by built-up areas, the natural habitats of natural enemies are diminished and no natural pest control can take place (Korányi et al, 2022).

In order to create an overall image upon pest attack abundance and the vulnerability of 'Regina' and 'Kordia' sweet cherry cultivars, the mean number of mines/leaf was calculated and compared. The results of the statistical analyses show that significant differences between the means existed only in the damage caused during the summer (4.13 mines in 'Regina' and 5.49 mines in 'Kordia') suggesting a slightly higher vulnerability of 'Kordia' variety in front of *L. malifoliella* attack. However, as the number of pest generation increased the damage caused in both sweet cherry varieties increased as well, exhibiting no statistically significant differences between the means at $p<0.05$ (7.36 mines in 'Regina' and 7.46 mines in 'Kordia' as presented in Figure 9. Thus, it has been observed that the intensity of the attack increased by 8.62% in 'Kordia' and 5.40% in 'Regina' by the end of the vegetation period. The level of damage followed a similar pattern; in 'Kordia', an increase of 9.26% was observed, while in 'Regina' the level of damage increased by 6.22% (Table 2). These results indicate that 'Regina' is slightly more resistant to the attack from the beginning until the end of the vegetation period. During the year 2021, the frequency of attacked leaves was 30% caused by the first generation of the pest and reached 90% by the end of the biological cycle.

In 2022, a significant decrease in the number of mines/leaf has been detected in both periods (July and September) compared to the attack that occurred during the experimental year of 2021.

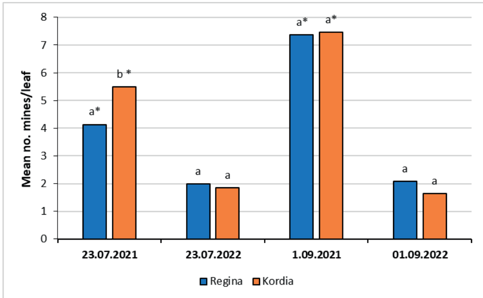


Figure 9. The mean number of mines/leaf recorded in the two sweet cherry varieties under study during 2021 and 2022. The data shown are means. The asterisks indicate significant differences between the means according to Duncan's multiple range test at $P<0.05$

CONCLUSIONS

In summary, *L. malifoliella* can cause severe damage in sweet cherry orchards by destroying up to 70% of the leaf area which leads to a significant decrease in yield. Urban areas seem to be more favorable for pest outbreaks facilitating the development of 2 or 3 generations of the pest. The use of sex pheromone traps proved to be efficient, exhibiting high attractivity and selectivity when placed at the exact time and can be recommended for pest monitoring and mass trapping of male adults for pest management strategies.

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MOLECULAR SCREENING OF APPLE CULTIVARS FOR TWO SCAB RESISTANCE GENES IN ROMANIA

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Abstract

Apple scab, caused by Venturia inaequalis, is considered the most damage disease of the apple growing in Romania. Pyramiding multiple sources of quantitative resistance could be the best way to control the attack of this fungi. A set of forty apple cultivars from the apple gene bank in Pitesti, Romania was evaluated for the presence of two scab resistance genes: Rvi8 and Rvi11. Using marker OPB18, it was made the difference between 2 genes, Rvi2 and Rvi8, located in the same locus (Vr gene). For identification of Rvi11, the SCAR marker, K08, was used and seventeen cultivars amplified the 743 bp fragment that is linked with Rvi11 gene. The results on resistance of apple Romanian cultivars towards economically important disease is necessary for future breeding work and for establishing of new commercial orchards.

Key words: *breeding; genotypes; Rvi2; Rvi8; Rvi11.*

INTRODUCTION

SCAR markers were first developed and initially applied to study on resistance genes for downy mildew in lettuce by Paran and Michelmore (1993). A SCAR marker is a technique mediated by PCR, which identifies the genomic DNA fragment at a single locus, using a pair of specific oligonucleotides, of 15-30 bp, projected from nucleotide sequences derived from dominant polymorphic RAPD fragments.

To highlight some apple resistance genes, the most used markers associated were: AL-07, AM19, Vfc, OPL19, OPB12, OPB18, AD13, OPB12, K08, T06, Z13, 41A24T7, ARGH17 and for resistance to powdery mildew, the most used markers were: OPU02, EM M01, AT20, EMDM01, EM 02. Most of them (Al07, AM19, Vfc, OPL19, AD13, OPB12, AT20, EM M01) were used in the studies initiated at the Research Institute for Fruit Growing Piteşti-Mărăcineni (Militaru et al., 2020; Iancu et al., 2022; Militaru et al., 2022; Iancu et al., 2023).

$OPL19_{433}$ was developed by cloning the 550 bp fragment from the RAPD marker $OPL19_{550}$, located near the *Rvi2* gene, at a distance of 2.5 cM (Gardiner et al. 1999a; Bus et al., 1999). The SCAR marker was also mapped to 1 cM (Bus et al., 2005b) close to *Rvi2* (*Vh2*) in differential host 2 ('TSR34T15'), but also close to the *Rvi8* gene (*Vh8*) in differential host 8 (*M. sieversii W193B*), being considered very useful for identifying varieties carrying the *Vr* gene (*Rvi2* or *Rvi8*).

To differentiate the carrier varieties of one of the genes *Rvi2* or *Rvi8*, Bus et al. (2005a), developed the OPB18 SCAR marker, which amplified in a population 'Royal Gala × *M. sieversii W193B*' only those hybrids carrying of the *Rvi8* gene.

The *Rvi11* gene was identified in *Malus baccata* by Dayton and Williams in 1968 and was mapped to LG-2 by Gessles et al. in 2006. Gygax et al. (2004) showed the presence of the *Rvi11* gene (old name *Vbj*) using the SCAR markers $K08_{743}$, $Z13_{773}$, $T06_{410}$ which were projected from the decamer predecessors $OPK08_{848}$, $OPZ13_{869}$ and $OPT06_{801}$.

Galli et al. (2010) revealed three transcribed putative resistance gene analogues (Resistance Gene Analogs) for resistance to *Venturia inaequalis* (*Vr2-A*, *Vr2-B* and *Vr2-C*) after isolation of Bacterial Artificial Chromosome

(BAC) spanning the *Rvi15* (*Vr2*) region and it sequenced, and Schouten et al. (2014) concluded that Vr2-C is the *Rvi15* (*Vr2*) gene that provides the resistance. Markers 41A24T7 and 43M10RP have cosegregated on the TIR-NBS-LRR domain of the *Vr2-B*. The Vr2-C region was flanked by the markers: 77G20RP (SSR), 21K14T7 (SNP), 8K11RP (SCAR) and GmTNL1 (SNP). Two other flanking markers ARGH17 (CAPS) and 48K16T7 (SCAR) cover the region of the *Vr2-A* gene. ARGH17 and GmTNL1 were mapped closest to both regions of the resistance locus, *Vr2-A* (0.3 cM) and *Vr2-C* (0.2 cM), respectively (Galli et al., 2010).

MATERIALS AND METHODS

Biological material studied

Forty apple cultivars ('Alex', 'Aura', 'Auriu de Cluj', 'Bistriţean', 'Cezar', 'Ciprian', 'Colmar', 'Colonade', 'Dacian', 'Dany', 'Delicios de Voineşti', 'Discoprim', 'Doina', 'Estival', 'Frumos de Voineti', 'Generos', 'Inedit', 'Iris', 'Irisem', 'Ionaprim', 'Luca', 'Nicol', 'Pomona', 'Precoce de Ardeal', 'Productiv de Cluj', 'Real', 'Rebra', 'Redix', 'Revidar', 'Remar', 'Remus', 'Romus 3', 'Romus 4', 'Romus 5', 'Rustic', 'Salva', 'Starkprim', 'Voinicel', 'Voinea', 'Valery'), which belong to the gene bank located at Research Institute for Fruit Growing Pitesti, Romania were used in the study.

Three leaf samples from each three trees per cultivar were collected. For the identification of the scab resistance genes, *Rvi8* and *Rvi11*, the following SCAR markers were applied: OPB18 and K08, respectively (Table 1).

'*Malus baccata jackii*' was used as a positive control for the K08 marker associated with the scab resistance gene *Rvi11*.

Chemical material

The DNA was extracted according to the recommended working method of the "Isolate II Plant DNA Kit" protocol. For the migration of the amplified fragments, a 3% agarose gel was prepared in TBE 1X buffer and subsequently stained with "RedSafe Staining" Nucleic Acid. The amplification of the reactions was performed using the "2x PCRBIO Taq Mix Red" kit from Biosystems.

PCR amplification

PCR amplification was performed using the "FastGene" analyzer. The PCR reaction was performed separately for each of the primer pairs, in a reaction volume of 15 µl, of which: 12 µl "2x PCRBIO Taq Mix Red"; 0.1 µl from every marker and 3 µl DNA (75 ng/µl) for both markers. The reaction conditions were: initial denaturation at 94°C for 3 min; 40 cycles of 1 min at 94°C, 1 min at Tm and 2 min at 72°C; final extension of 10 min at 72°C (65°C for K08, 55°C for OPB18).

Table 1. SCAR molecular markers for *Rvi8* and *Rvi11* genes

Gene	Name primer	Primer sequences	Fragment size (bp)	References
Rvi8 (Vh8)	OPB18	F: CCACAGCAGTCATTGGGA R: CCACAGCAGTGCATAAAC	628; **799**	Bus et al, 2005a
Rvi11(Vbj)	K08	F: GAACACTGGGCAAAGGAAAC R: TAAAAGCCACGTTCTCTCGC	**743;** 900	Gzgax et al., 2004

Evaluation of results

The fragments amplified following the PCR reaction were loaded in a volume of 10 µl for each sample, in the agarose gel and read with a high-quality "Uvitec Cambridge Essential" imaging system using UVITec1D analysis software. The duration of the sample migration was 4 hours at a voltage of 50 volts for gels with a concentration of 3% and a horizontal electrophoresis system "Wide Midi Horizontal Electrophoresis System" from Cleaver Scientific was performed.

Statistical analysis

Statistical analysis has been used to order varieties with polygenic characteristics into groups and subgroups. This grouping was made with the GeneAlex v software. 6.51b2. The genotype-phenotype correlation was calculated with the Pearson correlation coefficient, using Minitab18 software.

The statistical analysis of allelic polymorphism, taking into account only the dominant allele of the genes of interest, was expressed using the PIC index (Content of polymorphic information), which takes into account the allelic frequency, being calculated using mathematical expression: 2f (1-f). Two statistical indices were used to quantify genetic diversity: the Shannon index and the Simpson index. The Shannon index was calculated with the mathematical formula: - $\sum_{i=1}^{n} n/_{N} * ln\, n/N$ and the Simpson index with the formula: ($\frac{\sum_{i=1}^{n} n*(n-1)}{N*(n-1)}$), where: n represents the allele at the monolocus level, and N - the total number of alleles (Shannon et al., 1948; Simpson, 1960).

RESULTS AND DISCUSSIONS

Rvi8. The SCAR markers OPB18 and OPB19 are indicators for *Rvi8* and *Rvi2* genes. Iancu et al. (2022, 2023) using marker OPL19 reported an allele size of 433 bp for *Vr* gene (*Rvi2* or *Rvi8*) for the for twenty cultivars: 'Alex', 'Bistrițean', 'Cezar', 'Ciprian', 'Dany', 'Discoprim', 'Delicios de Voinești', 'Estival', 'Jonaprim', 'Luca', 'Pomona', 'Romus 3', 'Romus 4', 'Romus 5', 'Redix', 'Remar', 'Salva', 'Starkprim', 'Voinicel', 'Voinea'.

The segregation of the OPB18 marker with the gene locus made the difference between the *Rvi8* and *Rvi2* genes, producing the amplification of the 799 bp fragment associated with the dominant allele of the *Rvi8* gene, only. This gene did not segregate into the apple cultivars studied, and, in order to confirm our results, other two cultivars 'Verzişoare' and 'Pionier' were, supplementary, introduced, as control. The OPL18 marker amplified the 799 bp fragment corresponding to the dominant allele of the *Rvi8* gene for both control cultivars (Figure 1).

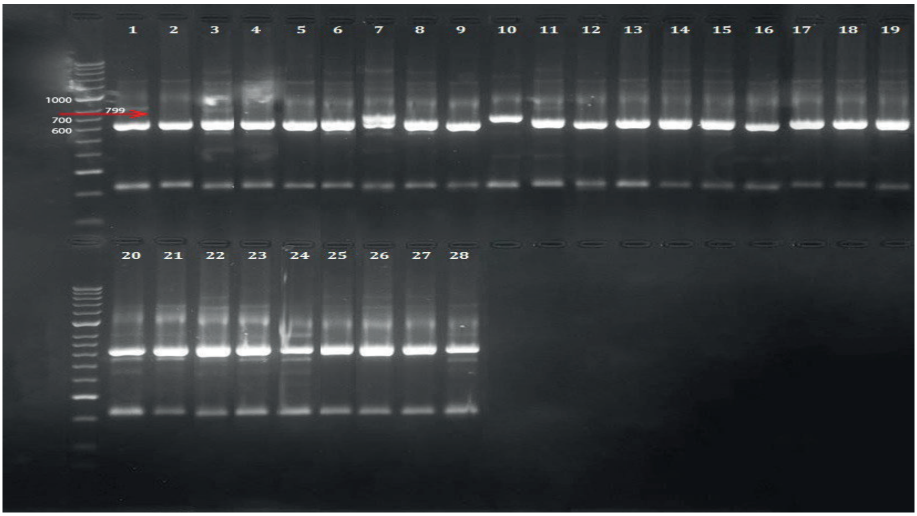

Figure 1. Electrophoretic profile performed with the OPB18 marker for genotypes in which specific amplifications of the Vr locus were obtained with the OPL19 marker:
1. 'Romus 2', 2. 'Romus 3', 3. 'Romus 4', 4. 'Romus 5', 5. 'Florina', 6. 'Prima', 7. 'Pionier', 8. 'Starkrimson', 9. 'Creţesc', 10. 'Verzişoare', 11. 'Parmen d'or', 12. 'Estival', 13. 'Bistriţean', 14. 'Dany', 15. 'Luca', 16. 'Ciprian', 17. 'Salva', 18. 'Ionaprim', 19. 'Starkprim', 20. 'Delicios de Voineşti', 21. 'Redix', 22. 'Alex', 23. 'Voinicel', 24. 'Voinea', 25. 'Discoprim', 26. 'Cezar', 27. 'Pomona', 28. 'Remar'

Rvi11. The codominant marker K08 was segregated into 19 cultivars ('Alex', 'Bistrițean', 'Colonade', 'Ciprian', 'Colmar', 'Dany', 'Dacian', 'Estival', 'Generos', 'Irisem', 'Nicol', 'Pomona', 'Precoce de Ardeal', 'Productiv de Cluj', 'Rebra', 'Romus 3', 'Remus', 'Redix', 'Remar') and *'Malus baccata jackii',* as positive control. This marker made the difference between heterozygous and homozygous genotypes by amplifying the 743 bp and 900 bp, respectively: 11 were heterozygous with both dominant and recessive alleles, and 8 were homozygous with dominant alleles (Figure 2).

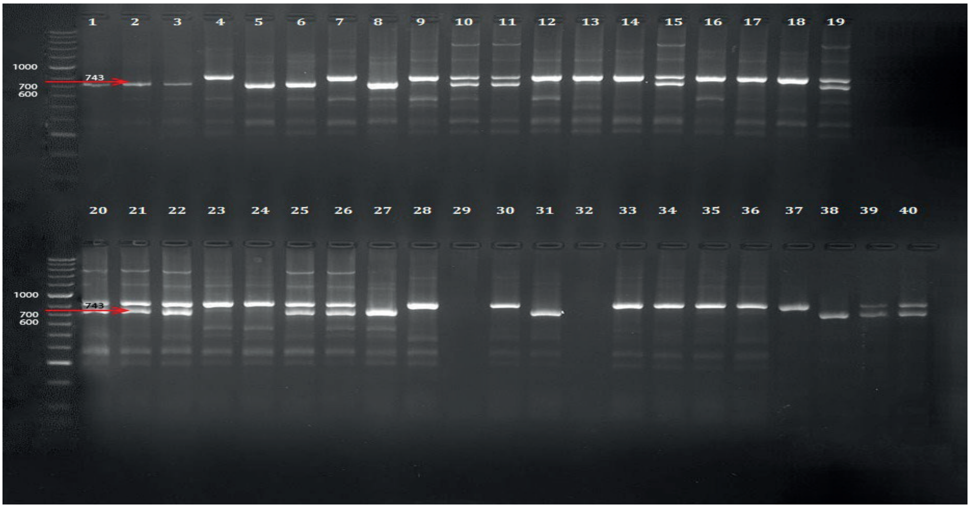

Figure 2. Electrophoretic profile released with the K08 marker:
1. 'Estival', 2. 'Rebra', 3. 'Bistriţean', 4. 'Aura', 5. 'Romus 3', 6. 'Dany', 7. 'Romus 4', 8. 'Productiv de Cluj', 9. 'Luca', 10. 'Ciprian', 11. 'Irisem', 12. 'Slava', 13. 'Ionaprim', 14. 'Rustic', 15. 'Precoce de Ardeal', 16. 'Iris', 17. 'Starkprim', 18. 'Auriu de Cluj', 19. 'Generos', 20. 'Colonade', 21.'Nicol', 22. 'Colmar', 23. 'Delicios de Voineşti', 24. 'Romus 5', 25. 'Remus', 26. Redix'', 27. 'Alex', 28. 'Doina', 29. 'Voinicel', 30. 'Inedit', 31. 'Dacian', 32. 'Voinea', 33. 'Valery', 34. 'Real', 35. 'Discoprim', 36. 'Cezar', 37. 'Frumos de Voineşti', 38. 'Pomona', 39. 'Revidar', 40. 'Remar'

By cumulating the present with previous results, we obtained a scab resistance genetic profile for all forty apple cultivars (Table 2). The data showed different scab gene accumulations, such as: tetragenic *(Rvi2+Rvi4+Rvi6+Rvi11)*, trygenic *(Rvi2+Rvi4+Rvi6*; *Rvi2+Rvi4+Rvi11*; *Rvi2+Rvi6+Rvi11)*, digenic *(Rvi2+Rvi6*; *Rvi6+Rvi11; Rvi5+Rvi11)*, monogenic (*Rvi2*; *Rvi6*; *Rvi11)*. For two cultivars ('Frumos de Voinesti' and 'Auriu de Cluj') which did not show any scab resistance genes.

Table 2. Scab resistance genetic profile

Cultivar	***Rvi₈***[a]	***Rvi11***[a]	***Rvi2***[b]	**Genetic profile**[a,b]
	OPB18	**K08**	**OPL19**	
Estival	.-	+	+	*Rvi2+Rvi4 +Rvi11*
Rebra	-	+	-	*Rvi6+Rvi11*
Bistriţean	.-	+	+	*Rvi2+Rvi6 +Rvi11*
Aura	-	-	-	*Rvi6*
Romus 3	.	+	+	*Rvi2+Rvi6+Rvi4+Rvi11*
Dany	.	+	+	*Rvi2+Rvi6 +Rvi11*
Romus 5	.	-	+	*Rvi2+Rvi6+Rvi4*
Productiv de Cluj	-	+	-	*Rvi11*
Luca	-	-	+	*Rvi2+Rvi6*
Ciprian	-	+	+	*Rvi2+Rvi6 +Rvi11*
Irisem	-	+	-	*Rvi5+ Rvi11*
Salva	-	-	+	*Rvi2+Rvi6*
Ionaprim	-	-	+	*Rvi2+Rvi6*
Rustic	-	-	-	*Rvi6*
Precoce de Ardeal	-	+	-	*Rvi11*
Iris	-	-	-	*Rvi6*
Starkprim	-	-	+	*Rvi2+Rvi6*
Auriu de Cluj	-	-	-	-
Generos	-	+	-	*Rvi5+ Rvi11*
Colonade	-	+	-	*Rvi6+Rvi11*
Nicol	-	+	-	*Rvi5+ Rvi11*
Colmar	-	+	-	*Rvi6+Rvi11*
Delicios de Voineşti	-	-	+	*Rvi2*
Romus 4	.	-	+	*Rvi2+Rvi6*

Remus	-	+	-	*Rvi11*
Redix	-	+	+	*Rvi2+Rvi6+Rvi4+Rvi11*
Alex	-	+	+	*Rvi2+Rvi6 +Rvi11*
Doina	-	-	-	*Rvi6*
Voinicel	-	-	+	*Rvi2+Rvi6+Rvi4*
Inedit	-	-	-	*Rvi6*
Dacian	-	+	-	*Rvi6+Rvi11*
Voinea	+		-	*Rvi2+Rvi6*
Valery	-	-	-	*Rvi6*
Real	-	-	-	*Rvi6*
Discoprim	-	-	+	*Rvi2+Rvi6+Rvi4*
Cezar	-	-	+	*Rvi2+Rvi6+Rvi4*
Frumos de Voineşti	-	-	-	-
Pomona	-	+	+	*Rvi2+Rvi6+Rvi4+Rvi11*
Revidar	-	-	-	*Rvi6+Rvi11*
Remar	-	+	+	*Rvi2+Rvi6+Rvi4+Rvi11*

[a]Molecular screening conducted in this study
[b]Iancu et al., 2023

In figure 3, using the statistical method PCoA analysis (standard covariance) with the help GeneAlex v software. 6.51b2, it can observe the distribution of cultivars which carried dominant alleles for scab resistance in groups and subgroups.

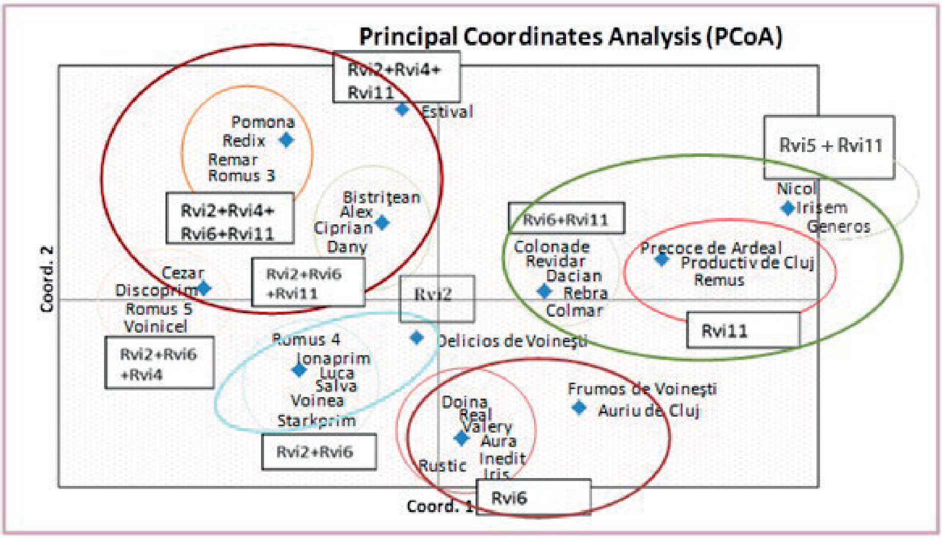

Figure 3. Distribution of cultivars based on R genes screening

Correlating phenotypic data presenting by Militaru et al. (2022) with our genotype results, we conclude that five of the monogenic varieties (‘Aura’, ‘Inedit’, ‘Iris’, ‘Valery, ‘Real’) have a scab resistant response, while the varieties ‘Doina’ and ‘Rustic’, with the same monogenic characteristic (*Rvi6* gene), show a moderate response.
Some varieties with trigenic characteristics from the subgroup “*Rvi2+Rvi6+Rvi11*” (’Bistritean’ and ’Ciprian’) are scab resistant, while ’Dany’ and ’Alex’ varieties, for same subgroup, manifest a phenotypic response of moderate resistance.
’Colmar’, ’Colonade’, ’Revidar’ and ’Dacian’ of the subgroup “*Rvi6+Rvi11*” are resistant to apple scab, while the variety ‘Rebra’ of the same subgroup shows a “moderate resistance”.
Also, varieties with digenic characteristics (’Ionaprim’, ’Starkprim’, ’Luca’, ’Voinea’ and ’Romus 4') from the subgroup "*Rvi2+Rvi6*" are scab resistant, instead, the ‘Salva’ variety, from the same subgroup, shows a phenotypic response as ”moderate resistance”.

The varieties with monogenic characteristics ‘Precoce de Ardeal’ and ’Productiv de Cluj’ (*Rvi11*) have a moderate resistant to apple scab, while ’Remus’ (*Rvi11*) and ’Delicious de Voinesti’ (*Rvi2*) are scab susceptible.
The disease susceptibility response positions the ‘Redix’ cultivar in the category of plants known as GPI (Genotype-Phenotype Incongruence) as the genotyping result is unexpected, this having a polygenic character: *Rvi2+Rvi4+Rvi6+Rvi11*. The gene combination “*Rvi2+Rvi11*” is not enough to produce an immune response to the attack of the pathogen, *Venturia inaequalis*. So, the varieties ‘Nicol’, ’Generos’ and ’Irisem’ are susceptible to this disease. Using as statistical method the correlation, Pearson coefficient correlated with Minitab18 software, the combination of genes “*Rvi2+Rvi4+Rvi11*” is in perfect correlation with the phenotypic expression manifested by ‘Estival’ cultivar.
Significant results were also obtained for the combinations of genes “*Rvi2+Rvi6+Rvi11*” and “*Rvi2+Rvi4+Rvi6+Rvi11*” (Figure 4).

	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
C13	0.031	-0.093	-0.217	0.450	0.015	-0.221	-0.190	-0.202	0.372	0.238	0.277
	0.850	**0.568**	0.179	0.004	**0.927**	0.170	0.241	0.212	0.018	0.139	0.083

Figure 4. Pearson correlation between genotypes and phenotypes:
C2 (Rvi2+Rvi4+Rvi6+Rvi11: ’Pomona’, ‘Redix’, ‘Remar’, ‘Romus 3’); C3 (Rvi2+Rvi6+Rvi11: ‘Alex’, ‘Bistrițean’, ‘Ciprian’, ‘Dany’); C4 (Rvi2+Rvi4+Rvi6: ‘Cezar’, ‘Discoprim’, ‘Romus 5’, ‘Voinicel’); C5 (Rvi5+Rvi11: ‘Generos’, ‘Irisem’, ‘Nicol’); C6 (Rvi2+Rvi4+Rvi11: ‘Estival’); C7 (Rvi2+Rvi6: ‘Ionaprim’, ‘Luca’, ‘Romus 4’, ‘Salva’, ‘Starkprim’, ‘Voinea’); C8 (Rvi6+Rvi11: ‘Colonade’, ‘Colmar’, ‘Dacian’, ‘Rebra’, ‘Revidar’); C9 (Rvi6: ‘Aura’, ‘Doina’, ‘Inedit’, ‘Iris’, ‘Real’, ‘Rustic’, ‘Valery’); C10 (Rvi2: ‘Delicios de Voinești’); C11 (Rvi11:’ Precoce de Ardeal’, ‘Productiv de Cluj’, ‘Remus’); C12 (-); C13 (phenotipic expression)

Genetic diversity and allelic polymorphism were calculated only for the dominant allele of interest genes. The results showed moderately informative polymorphism and low genetic diversity (Table 3). The small value for genetic diversity is explained by the fact that the same gene has been highlighted in several varieties.

Table 3. Statistical analysis of allelic polymorphism and genetic diversity

PIC value>0.25 (moderately informative)	**Shannon Index H > 3 (high genetic diversity)**	**Simson Index (D) D=0 (infinite diversity) D=1 (lack of diversity)**	**Simpson diversity index (1-D) value Є [0.1]**
0.333542	1.294129	0.2494129	0.759944

CONCLUSIONS

In Romania, the major problem of apple production is scab caused by fungal pathogen, *Venturia inaequalis*. In order to solve this problem, the most Romanian breeding programs included releasing of new cultivars with scab resistance, as major objective. So, the present data complete the information about using of Romanian bred cultivars as a source of diverse functional alleles involved in quantitative resistance against *V. inaequalis* strains. Using K08 marker, it has been established that 19 apple cultivars carriers of *Rvi11* gene and, using OPB18 marker, is not obtained amplified for *Rvi8*. Genetic diversity of the dominant characteristic for the gene of interest is reduced and allelic polymorphism has a moderately informative value. Segregation of the genes of interest into descendants allowed for validation of the identity of the genitors.

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PROPAGATION METHODS OF SOME PAWPAW GENOTYPES [*ASIMINA TRILOBA* (L.) DUNAL]

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Abstract

The paper presents the effectiveness of various propagation methods applied to several pawpaw (Asimina triloba (L.) Dunal) genotypes. Generative propagation, propagation by grafting, and by cutting have been studied. Various generative propagation methods as seeds exposure to polarized light and different magnetic fields before sowing and to LED light, throughout the germination period have been tested. The grafting was performed at different times of the year (spring and autumn) on one-year-old rootstock. Following the conducted research, it was concluded that the most effective propagation methods were seed propagation and grafting, while propagation through cuttings was inefficient. By subjecting the seeds to magnetic fields and LED light before sowing, the germination period was significantly reduced, and their germination capacity increased. The most optimal period for grafting was early spring, after the rootstocks started the vegetative growth.

***Key words**: generative propagation, grafting, LED light, magnetic field.*

INTRODUCTION

Asimina triloba (L.) Dunal has been known by various names over the years, such as Indian banana, poor man's banana, northern banana, but the most commonly used name has been pawpaw (Moore, 2015). Originated from the eastern region of the United States (Layne, 1996), pawpaw was introduced in Romania in 1926 by the Suciu family. They brought from USA in Pianu de Sus, Alba County few pawpaw fruits (Stănică, 2012). In 1996-1997, the first pawpaw plants were planted in the didactic-experimental field of the Faculty of Horticulture within University of Agronomic Sciences and Veterinary Medicine of Bucharest (Cepoiu et al., 2004). From a botanical standpoint, it is classified in the *Annonaceae* family, one of the ten families included in the *Magnoliales* order (Kral, 1960; Callaway, 1992).

The fruit is characterized by two rows of sizable, brown, bean-shaped seeds that are laterally compressed, reaching lengths of up to 3 cm in length and 1.5 cm in width (Layne, 1996; Geneve, 2003). Alkaloids with emetic properties are present in the seeds endosperm (Vines, 1960). Chewing the seeds could potentially disrupt mammalian digestion due to the presence of toxic substances. However, if swallowed whole, the seeds can safely traverse the digestive tract without harm (Layne, 1996).

Once the flesh becomes soft, the fruit can be harvest for seed extraction. Following the maceration of the fruit in water, the seeds can be easily extracted as the pulp floats away (Layne, 1996). Mature pawpaw seeds, immediately extracted from fleshy fruits, contain approximately 37% moisture. This value remains relatively stable even if seeds are imbibed. Viability decreases by 50% when seeds are dried from their initial 37% to 25% moisture, with total loss occurring between 15% and 5% moisture (Geneve et al., 2003). For long-term storage and viability retention, pawpaw seeds must be stored moist at chilling temperatures [5°C (41.0°F)] (Finneseth et al., 1998). Finneseth et al. (1998) established that approximately seven weeks of chilling stratification at 5°C were necessary to achieve 50% germination. The highest germination percentages, ranging from 84% to 90%, were observed after approximately 100 days of stratification (Geneve et al., 2003; Pomper et al., 2003).

Figure 1. Pawpaw seeds

The seeds need to planted at approximately 2.5 cm deep. Once seedlings reach a height of 10 to 20 cm, they can be transplanted into tall pots (10 x 10 x 36 cm) with partially open bottoms, positioned on greenhouse benches to allow taproots to be "air-pruned" (Callaway, 1993).
Grafting is a surgical operation that involves joining two partners, one represented by a bud or graft branch, and the other represented by a rooted cutting, a seedling, or a *in vitro* multiplied plant (Ghena, 2004).
The most dependable and widely employed technique for vegetative propagation is chip-budding (Callaway, 1993; Stănică, 2004). The potential to propagate pawpaw cultivars through cuttings offers several benefits compared to seed-based or grafting approaches. Plants derived from cuttings would bloom earlier than those from seeds, and cuttings would address issues related to understock suckering observed in grafted plants (Geneve, 2003).

MATERIALS AND MOTHODS

The experiment location was the didactic-experimental field, the greenhouse and the vegetation house of the Faculty of Horticulture, University of Agronomic Science and Veterinary Medicine of Bucharest.
The biological material was collected from the pawpaw trees grown in the experimental field of the Faculty of Horticulture and it consisted of branches and seeds sampled from:

- 16 cultivars – Simina, Allegheny, Artemis (MJ), Shenandoah, Wabash, Asteria (250-30), Rebecca S.G., Sweet Alice, Sibley, Saa Zim, Sunglo, Prima 1216, Overleese, Ithaca, Sunflower, Davis;
- 17 hybrid genotypes – R1P1, R1P2, R1P3, R1P4, R1P5, R1P6, R1P7, R1P8, R1P9, R1P10, R2P1, R2P4, R2P6, R2P7, R2P8, R2P9, R2P11;
- 16 new hybrids – R0P11, R0P13, R0P14, R0P17, R0P18, R0P21, R0P22, R0P24, R0P25, R0P26, R0P33, R0P35, R0P37, R0P39, R0P42, R0P64.

Generative propagation, propagation by grafting, and by cutting have been attempted.
Generative propagation – three different methods were tried to stimulate the seeds germination rate:

- exposure to LED light;
- treatment with polarized light;
- treatment with magnetic field.

All seeds were sowing at the end of February in multicell trays, in a substrate composed of peat (70%) and perlite (30%) at a constant temperature of 21°C in the greenhouse.
For control and the LED light variant, all the cultivars, genotypes and hybrids were used. The seeds were exposed to LED light throughout their germination period.
In the second experiment, seeds collected from six genotypes (R1P10, R2P1, R2P4, R2P6, R2P9, R2P11) and two cultivars (Simina and Allegheny) were exposed before being sown for 5, 15 and 30 minutes to polarized light. The equipment used for conducting this research was a Bioptron PRO 1 biolamp with a fullerene filter.
For the third experiment, two genotypes (R2P9, R2P11) and two cultivars (Simina and Allegheny) were exposed before being sown for 5, 15 and 30 minutes to magnetic field with positive pole and negative pole.
Propagation by grafting – was done by chip-budding on one year old seedlings, in two different periods: spring – April and autumn – September.
Propagation by cutting – the cuttings were placed for rooting in perlite in June.

RESULTS AND DISCUSSIONS

Generative propagation is widely used because pawpaw seeds have a good or even very good germination capacity depending on the cultivar.

To determine the germination capacity of pawpaw seeds according to the cultivar, the entire evolution period of the seeds was monitored, from the time of sowing until the end of germination. The following results were observed (Figure 2):

- All studied cultivars or genotypes had a germination capacity of over 70%;
- The highest seed germination capacity of, with a percentage of 100%, was recorded in the cultivars Rebecca S.G., Sweet Alice, Sibley, and Davis, as well as in R0P18, R0P33, R0P37, R0P42, and R0P64 hybrids;
- The lowest seed germination capacity was found in the cultivars Shenandoah and Sunglo, with a percentage of 70%.

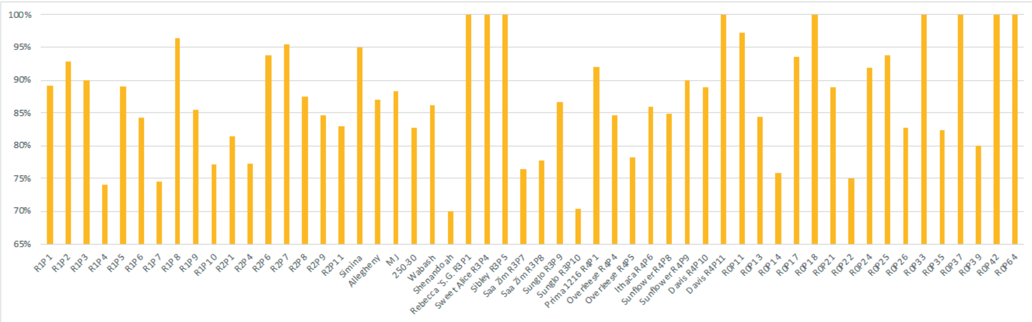

Figure 2. Germination percentage of pawpaw seeds

To observe if LED light influences the seed germination process, several aspects were monitored.

The first, was the tracking of germination and seedling emergence period both in the normal system and under the influence of LED light. Following this experiment, it was observed that LED light helps expedite the seed germination process and simultaneously

reduces the germination spread period. Seeds sown in the normal system generally require a germination period of approximately 6-7 weeks, which can be even longer. For instance, cultivars like Sweet Alice and Saa Zim started to sprout after a period of 9 weeks, while it can be shorter, for R2P1 and 250-30 genotypes (4 weeks). Seeds exposed to LED light generally require a germination period of about 4-5 weeks. For example, R0P33 and R0P35 hybrids sprouted after 6 weeks, Wabash and 250-30 cultivars, sprouted after only 4 weeks.

The second studied aspect was the monitoring the duration period of seedling emergence. It was also observed a reduction of this period by approximately one or even two weeks in seeds exposed to LED light compared to the control seeds. The greatest difference recorded was in genotypes R1P1, R1P5, and R1P9, and cultivar Saa Zim, with a two-week reduction, while the smallest difference was in genotype 250-30, where control seeds sprouted 5 days faster compared to those under LED light.

Figure 3. LED light exposure

Furthermore, the percentage of seed germination was also monitored for those developed under LED light compared to those developed in the normal system. Following this study, it was observed that LED light does not affect the germination percentage, with the number of sprouted plants being similar on both sides or, in many cases, even higher for plants grown in the normal system.

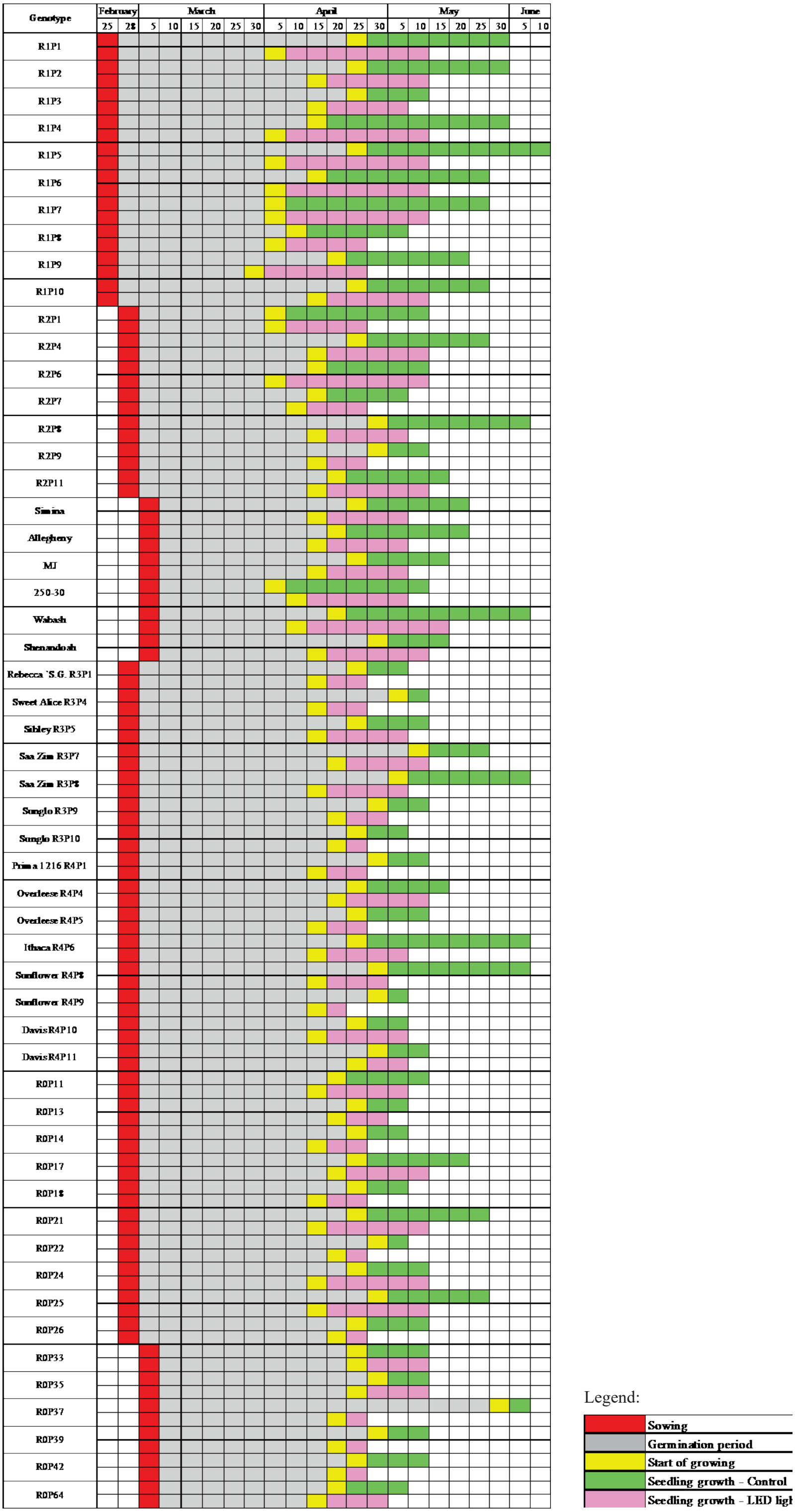

Figure 4. Influence of LED light on pawpaw seeds germination

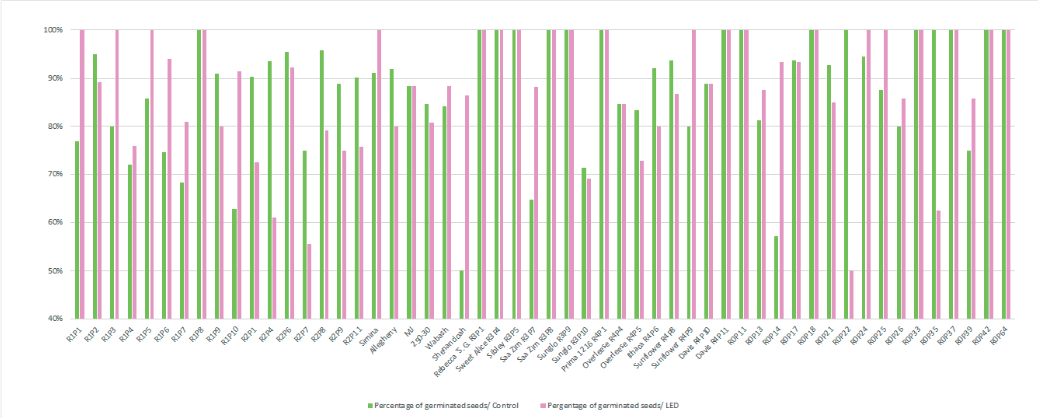

Figure 5. Influence of LED light on pawpaw seeds germination percentage (%)

To observe whether polarized light influences the seed germination process, a study was conducted on 6 genotypes (R1P10, R2P1, R2P4, R2P6, R2P9, R2P11) and 2 varieties (Simina and Allegheny) for which various aspects were monitored.

The first aspect was the monitoring of germination and seedling emergence period. Following this experiment, it was observed, that polarized light does not influence the acceleration of the seed germination process or the reduction of the germination spread period with an exposure of only 5-30 minutes.

The percentage of seed germination was also monitored for seeds that were previously exposed to polarized light. Following this study, it was observed that polarized light does not affect the percentage of seed germination, which is moderate or even low.

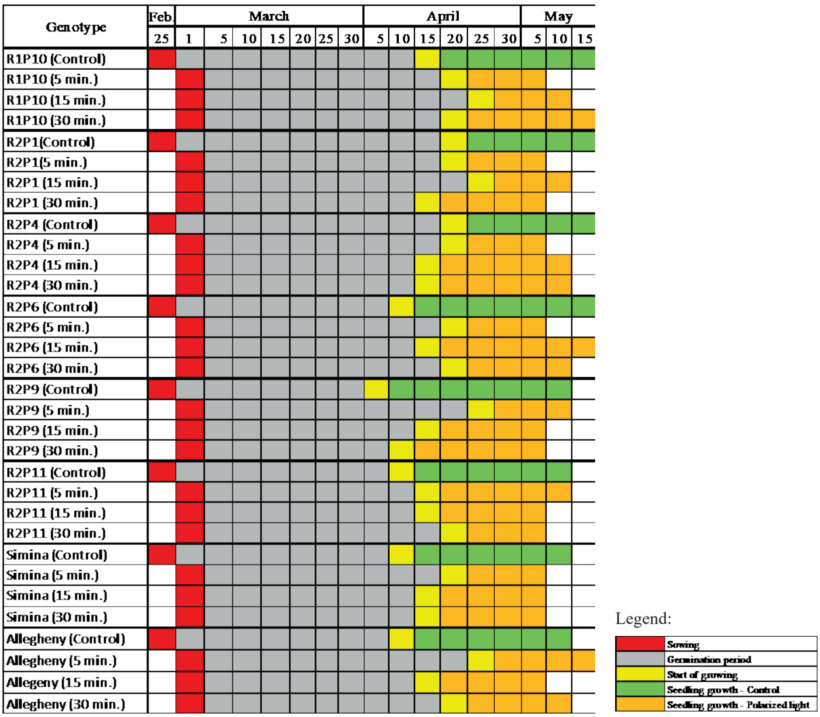

Figure 6. Influence of polarized light on pawpaw seeds germination duration

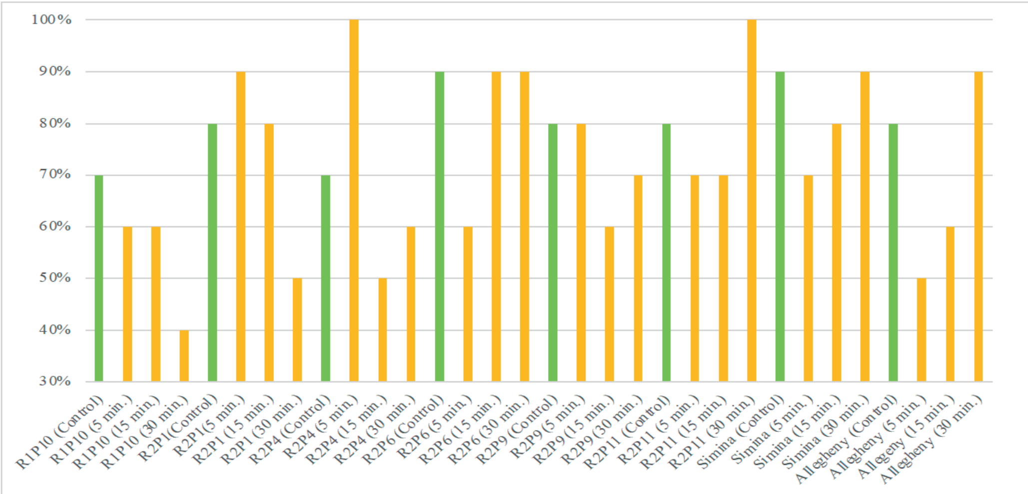

Figure 7. Influence of polarized light on pawpaw seeds germination percentage (%)

To observe if the magnetic field influences the seed germination process, a study was conducted on 2 genotypes (R2P9, R2P11) and 2 varieties (Simina and Allegheny), for which multiple aspects were monitored.

The first involved aspect was monitoring the seeds germination period and the emergence of seedlings. It was observed that the pre-exposure to magnetic fields helps to accelerate the seed germination process and also reduces the germination duration. Shortening of the germination period was recorded for all genotypes, but the best results were obtained with genotype R2P11, where the germination period was reduced by 2 weeks.

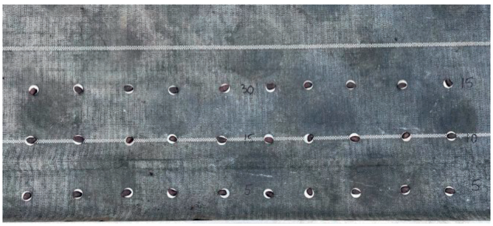

Figure 8. Seeds subjected to magnetic fields

The germination percentage of seeds exposed to magnetic fields was also monitored. Following this study, it was observed that the seed germination rate is positively influenced by exposure to magnetic fields, reaching over 80%.

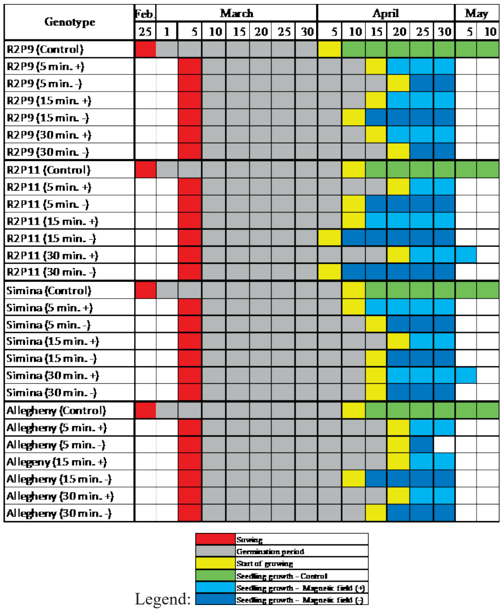

Figure 9. Influence of polarized light on pawpaw seeds germination

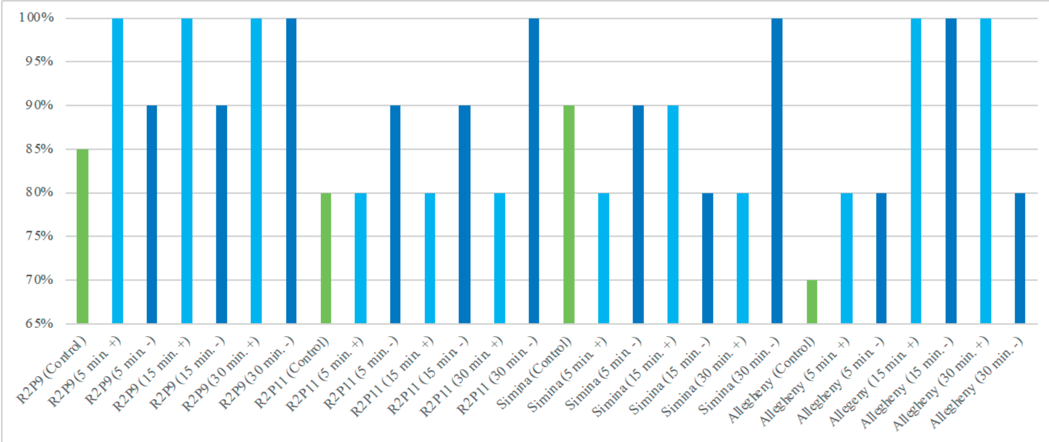

Figure 10. Influence of magnetic field on pawpaw seeds germination percentage (%)

Propagation by chip budding was attempted in two different periods of the year – spring and autumn.

Chip budding in autumn was carried out in September 2022, and the grafts' success rate was very low, below 10%.

The grafting was attempted again in the spring of 2023, on the same rootstocks. This time a good success rate was obtained.

The lowest chip budding success rate was encountered in the Rebecca 'S.G.' variety, at 65%, while the highest success rate of 100% was found in R2P1 and R2P11 genotypes, as well as in Saa Zim, PA Golden, and Sweet Alice varieties.

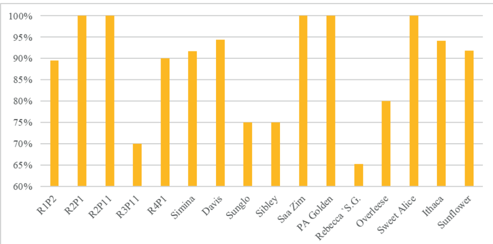

Figure 11. Chip budding success (%) in spring 2023

Propagating by cuttings was inefficient in the case of Simina. The substrate used was perlite and peat with sand, and bio-rooting hormones were used to stimulate root formation. No rooting was registered.

CONCLUSIONS

Following the research conducted on *Asimina triloba* (L.) Dunal propagation methods, several conclusions were drawn:

From the various propagation methods tested, it was concluded that the most efficient methods are seed propagation and chip budding, with few mentions.

Seed propagation can be stimulated through various methods, but from those addressed in this research, the highest score was recorded when seeds were pre-exposed to magnetic fields before sowing and to LED light throughout the germination period. It is noteworthy that LED light stimulates the speed of seed germination and reduces the duration period of seedling emergence but does not influence germination capacity. Pre-exposure to a magnetic field, on the other hand, stimulates both the speed of seed germination and reduces the duration period of seedling emergence and positively influences the seeds germination capacity. Pre-exposure to polarized light does not affects their productivity.

Based on the conducted study, it is evident that grafting/chip budding represents the best vegetative propagation method for pawpaw due to the good success rate. It is worth mentioning that chip budding done in the fall was not effective.

Regarding cutting propagation through, it was concluded that pawpaw does not exhibit rooting capacity.

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DETERMINATIONS REGARDING THE PERCENTAGE OF KERNELS OBTAINED THROUGH DIRECTED HYBRIDIZATION OF THE APRICOT SPECIES AT SCDP CONSTANȚA

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Abstract

As climate changes are more and more evident, obtaining new elites in order to improve the assortment of apricots, with new varieties, having high agrobiological potential, requires a better selection of parental lines used for directed hybridization. In order to obtain hybrids with a high potential, the apricot varieties Elmar, De Valu, Augustin and Amiral were taken into consideration. The combinations used were the following: Elmar x De Valu, Elmar x Augustin, Admiral x Elmar. From the total of 2,500 hybridized flowers, a number of 150 viable kernels were obtained, resulting in a percentage of 6%, which means that there are factors that influence the hybridization process. High numbers of viable kernels were observed to be correlated with favorable weather conditions for the crop in that year. The research took place in the experimental fields of SCDP Constanta. They were planted in 2011, the planting scheme is 4/4 m, in an irrigated system. The trees are at full maturity, benefiting from an agricultural technique specific to the crop.

Key words*: Prunus armeniaca, cultivars, yields, kernels.*

INTRODUCTION

Propagation of fruit plants by grafting is an objective necessity once fruit growing has passed to a status with a crop of great economic and food interest. Sexual hybridization is the main method of obtaining varieties with the complex of characteristics necessary for the fruit economy. There are no particular difficulties regarding the technique of hybridization between varieties and species of apricots and no limits of genetic combination (Balan et al., 2008). From a methodical point of view, it should be remembered that hybridization within the limits of the apricot species ensures a much lower variability, compared to other species, regardless of its character, the acquisition pursued through the breeding program (Cociu, 1989). This fact is explained by the reduced degree of heterozygosity of the apricot, an effect of long self-fertilization. The autogamy of most varieties of common apricot *P. armeniaca* L. contributes to the dominance of traits and basic characters (Cociu et al., 1999). The studies performed and published by Viorica Bălan et all. in Romania, Couranjou and Audergon in France, Bassi et al. in Italy, Egea and Bourgos in Spain include the known data regarding apricot genetics. Couranjou emphasizes high correlation between descendants and parents, heritability four surface colour af fruit, taste, firmness, production, pulp colour, aroma, juiciness, fruit size and maturation, flowering time, genetic transgression at maturation time, early fruit maturation.

Guerriero, Monteleone and Marroco pointed out the genetic transgression for late maturation, genetic correlations between parents and descendant regarding fruit size, long fruit viability, fruit firmness.

Bourgos showed a monofactorial system and multi-allelic series controlling self-compatibility in the apricot tree.

D. Bassy and J.M. Audergon emphasized apricot susceptibility to genetic improvement from the viewpoint of adaptability, resistance to disease, and fruit quality.

Viorica Balan et al. evidenced through investigation the genetic mechanism and heredity of certain features and characteristics of the hybrids resulting from controlled hybridization, crossbreeding and mutagenesis.

MATERIALS AND METHODS

The study was carried out in apricot demonstrative lots at Research Station for Fruit Growing Constanta, located in south-eastern Romania, near the Black Sea.

The site is located at 44°10’ Northern latitude and 28°29’ Eastern longitude, and 70 m above sea level. Climate is continental with warm and droughty summers, frequent dry winds all the year round and temperate winter generally without snow. The mean annual temperature is 12.0°C and the total active temperature is 3988°C, out of which 3170°C during the growing season; the annual precipitation amount is 400 mm, out of which during the growing season (April 1 to September 30), 240.7 mm.

The lowest winter temperatures below -20°C are not very often: 1 out of 10-15 years and so are the spring frosts susceptible to cause apricot yield damage.

The climatic water deficit reaches as much as 400 mm/year, so irrigation application is needed for apricot.

The zonal soil type is a calcaro-calcic chernozem formed on loess, with loam texture and a proper capacity of water preserving, holding and circulation. The humus content ranges between 2.5 and 4%; pH of the soil is poor alkaline (7.0-8.1).

In order to obtain hybrids with a high potential, the apricot varieties Elmar, De Valu, Augustin and Amiral were taken into consideration. The apricot trees are grafted on Constanta 14 described by Indreias et al. (2010). The combinations used were the following: Elmar x De Valu, Elmar x Augustin, Admiral x Elmar. From the total of 2,500 hybridized flowers, a number of 150 viable kernels were obtained (Photo 1 and Photo 2). During the study period, there were deviations from normal climatic parameters, in the sense that winters are warm and without precipitation and springs are long, cold and with a high frequency of late frosts. Observations and determinations were observed between 2021 and 2023 in the demonstration lots and nursery in Field I and II at RSGF fruit tree nursery.

RESULTS AND DISCUSSIONS

The varieties Elmar, De Valu, Augustin, Amiral, used as parents, have a complex heredity.

The apricot variety Elmar, an early ripening variety, slightly sensitive to frost, was used as a basic parent for uniformity in fruiting, earlyness, relatively late flowering.

The De Valu apricot variety, with a medium ripening period, as a parent of character for large, strongly aromatic fruits, good tolerance to the main diseases specific to the apricot culture.

The Augustin apricot variety, with a late ripening period, as a parent of character for frost resistance, productivity, late ripening period.

Within the mentioned combinations, 2,493 flowers were pollinated, from which 1,733 kernels were obtained (Table 1).

Table 1. Pollinated flowers and seeds obtained

COMBINATION	Pollinated flowers		Obtained kernels	
	buc.	%	buc.	%
Elmar x De Valu	825	100	489	59.27
Elmar x Augustin	861	100	654	75.96
Amiral x Elmar	807	100	590	73.11
TOTAL	2.493	100	1733	69.51

Photo 1. Aspects from the hybridization

Photo 2. Aspects from the hybridization

The flower buds are activated 7-10 days before the vegetative buds. Buds begin to swell when the average daily air temperature remains high above 10-12ºC and 140-160ºC active temperatures have accumulated. From the moment of fertilization, the flowers begin the phase of growth and fruit ripening. To go through these phases, 900-1200ºC are needed, i.e. 65-85 days from flowering to ripening for extra-early and early varieties and 1800-2200ºC, i.e. 80-130 days for medium and late ripening varieties. The apricot varieties used for hybridization, Elmar, De Valu, Amiral and Augustin are apricot varieties with staggered ripening, starting from extra-early fruits to late fruits.

From the Elmar x De Valu hybrid combination, 489 kernels were obtained by pollinating 825 flowers, resulting in a 59.27% success rate.
From the Elmar x Augustin hybrid combination, 654 kernels were obtained by pollinating 861 flowers, resulting in a 75.96% success rate.
From the Amiral x Elmar hybrid combination, 590 kernels were obtained by pollinating 807 flowers, resulting in a 73.11% success rate.

From the stated data, the Elmar x Augustin hybrid combination with a percentage of 75.96 was in first place, the Amiral x Elmar hybrid combination with a percentage of 73.11 in second place and the Elmar x De Valu hybrid combination with a percentage of 59.27 in third place.

Photo 3. Hybrid fruits

From the data (Table 2), the Elmar x Augustin hybrid combination had a success rate of 10.25% sprouted kernels out of a total of 489, ranking first, the Admiral x Elmar hybrid combination in second place with a percentage of 8.47 sprouted kernels and the hybrid combination of Elmar x De Valu in third place with a success rate of 6.75%.

Table 2. Seeds planted/sprouted and hybrids obtained

COMBI NATION	Seeds planted		Sprout kernels		Obtained hybrids	
	buc.	%	buc.	%	buc.	%
Elmar x De Valu	489	100	33	6.75	25	100
Elmar x Augustin	654	100	67	10.25	48	100
Amiral x Elmar	590	100	50	8.47	32	100
TOTAL	1733	100	150	8.65	105	100

Photo 4. Hybrid fruits

Photo 5. b)

Aspects from seed planting in the SCDP Constanța nursery

Photo 5. a)

CONCLUSIONS

Among the three hybrid combinations, the Elmar x Augustin hybrid combination achieved a better success rate (10.25%) compared to the other two hybrid combinations.

Instead, overall we observe that all hybrid combinations have less than 10% success.

The unfavorable weather conditions during the year, among which we list: prolonged pedological drought, atmospheric drought, low temperatures during flowering, lack of sunlight and heat necessary for pollen maturation, lack of insects, lead to a low percentage of viable kernels and implicitly to a number lack of hybrids necessary for the improvement processes of the apricot species.

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INFLUENCE OF GENOTYPES AND THE STORAGE TIME ON PEARS QUALITY INDICATORS

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Abstract

In order to study the changes in the quality of the fruits during the storage time, the late ripening seven Romanian genotypes and two international cultivars used as control. Genotypes were refrigerated for different periods of time under conditions of 4°C and 75% humidity. Fruit quality indicators: flesh firmness, skin color, pH, total dry weight, total soluble solids content, titratable acidity, total polyphenols content and vitamin C were evaluated starting at the time of fruit harvesting and continuing in the interval 60, 90 and 120 days after harvesting. All tested pear genotypes retained a hight level of quality after 60 days. The degradation of organic acids and vitamin C, the increase followed by decrease in the level of sugars and total dry weight per mass unit in fruits was influenced by the duration of storage. In the Romcor cultivar, firmness and the average weight decreased significantly compared to the other genotypes. The obtained results were significantly influenced by the genetic characteristics and the time of storage.

***Key words**: biochemical characteristics, pear quality, postharvest storage.*

INTRODUCTION

Pear trees are a tree species of interest for fruit cultivation that belongs to the genus *Pyrus*, family *Rosaceae* and are native to Europe, North Africa and Asia (Kaviani et al., 2023). In Europe, Africa, Asia, North America, South America and Australia, due to the appreciation of nutritional value, *Pyrus communis* L. (European pear) is the main species found in the marketing chain (Thakur & Dalal, 2008). In 2021, the reported world production of European pear (*Pyrus communis* L.) was about 23 million (Prange & Wright, 2023).
The storage, quality, biochemical value, taste and aroma of fruits are affected by various factors, including genetic factors (Nafiye et al., 2023), ecological conditions, cultural systems and harvesting periods (Kader, 2002). Improper handling during pre-harvest, harvest, sorting, packaging, storage, transport or marketing operations has significant repercussions on the appearance and commercial quality of fruit producing mechanical damage, water loss, deformation, fungal decay and fermentation (Sánchez et al., 2012). Summer pears cannot be stored. Autumn pears can be stored for a shorter period of time compared to winter pears (Nótári & Ferencz, 2014). Fruit harvesting and storage operations constitute an important stage of fruit growing. The fruit of the pear is harvested by hand, requiring competence and skill (Soltész, 2007). Another important feature of storage is that the fruit can be kept fresh for a longer period of time, so the market and consumption period can be extended.
The time of harvesting is a particularly significant factor in the formation of the taste of pears. In immature fruits, although the storage capacity is longer, the possibilities to develop the appropriate organoleptic characteristics are generally reduced, while the storage time of overripe fruits is usually very short because the susceptibility to decay increases (García, 2001). Although the dynamics of fruit pulp firmness and refractive index values are particularly important for consumer acceptance (Kader, 2002), in pear, both characteristics are not considered factors to depict the stage of ripeness before pear harvest in terms of harvest date and postharvest treatment (Gamrasni et al., 2015). The biochemical value of fruits can fluctuate

significantly, creating differences in taste (Jeppsson & Johansson, 2000; Kader, 2002).

The total soluble solids content of fruits has an increasing dynamic during storage (Engin & Mert, 2020). The ideal time to harvest the fruit is in the early hours of the morning, just after the dew has cleared from the fruit, or in the evening when the temperatures are a little cooler. The procedure of storage at the minimum acceptable temperature has a crucial role in extending the shelf life of pears (Porritt, 1964). If pears are stored at a higher temperature, fruit senescence and losses due to fungal activities will shorten the storage period, and if stored at a lower temperature, the fruit may develop certain chilling disorders or freeze (Kidd et al., 1927). Preference for lower temperatures varies with cultivar genetic background, early pear cultivars have a shorter requirement, 0-20 days, while later ripening cultivars require longer durations (>40 days) (Gerasopoulos & Richardson, 1999). Chilling is the main method used to maintain the commercial value of fresh fruit after harvest. The range of average storage temperatures for European pear cultivars varies between -1.0 and +0.25°C. In contrast, recommendations for Asian pear varieties range from 0 to +0.5°C, so slightly higher than European pear storage temperatures (Prangeand Wright, 2023). According to Wiseman et al. (2016), pears can be stored at temperatures between -0.5 to 4°C under controlled atmospheric conditions (1 to 2% O_2 and 0.5% CO_2) or in air where they can be stored depending on the cultivar up to 8 months. Higher temperature ranges can accelerate pear metabolism and pathogen activities and accelerate moisture loss, causing greater decay, softening and weight loss during storage. Thus the respiration rate of climacteric fruits such as pears is accentuated (Biale, 1964 cited by Brandes & Zude-Sasse, 2019). The higher the fruit respiration rate, the shorter the shelf life (Brash et al., 1995). Low post-harvest temperatures can slow respiration rate, water loss, ripening and senescence processes and fungal decay of horticultural products (Oliveira et al., 2013).

The indications regarding the recommended temperature for the storage period of the pears, in the vast majority, refer to controlled atmosphere conditions. Controlled atmosphere storage is based on changing and maintaining a gas composition of the air different from that in the natural state (78 kPa N_2, 21 kPa O_2 and 0.03 kPa CO_2). The main benefit of controlled atmosphere is caused by reducing the concentration of O_2 as much as possible without producing undesirable anaerobic metabolism. The decrease in the level of O_2 can inhibit the respiratory metabolism of the fruits and if it is low enough, it can even inhibit the biosynthesis of ethylene during ripening (Prange, 2022). According to Prange and Wright (2023), storage recommendations differ for each variety and each pear-growing region. For example, for 17 varieties of European pears, there are 34 recommendations. The expansion of the varietal pipeline of cultivars in the database is a reflection of changes in the pear industry.

The aim of the present study was to evaluate the biochemical composition of pears refrigerated at 4°C and 75% humidity for 120 days.

MATERIALS AND METHODS

The experiment location and plant material

The research was conducted in 2023 year, in an experimental plot of the Research Institute for Fruit Growing Piteşti-Mărăcineni, in randomized blocks with three repetitions (5 tree per each replicate) and were studied the late ripening seven Romanian genotypes: \`Isadora\`, \`Romcor\`, \`R39P51xParamis\`, \`Aniversare\`, \`P20R41P30\`, \`SP06C2P5\`, \`Monica\`, and two international cultivars \`Packham's Triumph\` and \`Socrovisce\` used as control. The fruit samples were harvested at the tehnical harvest maturity and were sorted, classified and packed directly in the containers. At harvest, fruit are placed into either wooden or plastic binsand then transported immediately to storage were cooled 4°C and 75% humidity for 60, 90 and 120 days.

Soil Description

The experimental scheme was located on a brown-clay soil, flat with a loamy and loamy texture in the first 60-70 cm, and in depth the texture becomes sandy. Soil samples were collected at two depths (0- 20 cm and 20-40 cm), with an agrochemical probe. The soil thus harvested was air-dried and analyzed in the agrochemical laboratory. The soil is characterized by a moderately acidic reaction, a low humus content and a low supply of nitrogen and phosphorus.

Biochemical analyzes and laboratory determinations

The indicators studied were recorded at the optimal time for harvesting the fruits and continuing in the interval 60, 90 and 120 days after harvesting, on a sample of 10 pear fruits.

The *average weight* of the fruit was determined by weighing using the Kern EW digital balance.

Weight loss. Pear weight was measured with a balance (±0.01 g) before treatment and after storage, respectively, and the mass loss was calculated as % weight loss = (AB)/B × 100 (Hosseini et al., 2017).

The *firmness* of the fruit was determined for each sample with a Bareiss HPE II Fff penetrometer, a non-destructive test. The measurement was performed on the two parts of the fruits according to ECPGR, Pear (*Pyrus communis*) recommendations, 2022.

The external *fruit colour* was measured with a colorimeter Konica Minolta CR 400, based on system Hunter L, a*, b* on both sides of the fruit (L corresponds to brightness, a* and b* chromaticity coordinates from green to red and from blue to yellow, respectively).

The biochemical characteristics of the fruit were determined in a sample of approximately 10 pears per repetition.

Total soluble solids (TSS) content was measured with Atago Palette PR32 digital refractometer (0-32°Brix).

Total dry matter was determined by the gravimetric method by measuring water loss to constant weight according to AOAC International (2002).

The *content of organic acids* (TA), expressed as % malic acid, was analysed by the titrimetric method using a 0.1 N NaOH solution in the presence of phenolphthalein as an indicator (AOAC, 2000).

The *content of ascorbic acid* (*vitamin C*) expressed in mg/100 g of fresh fruit was determined by the titrimetric method (PN-A-04019: 1998).

The determination of *total polyphenols* was carried out spectrophotocolorimetrically, with the Folin-Ciocâlteu reagent (Singleton et al., 1999) and the results were expressed as milligrams per kilogram of gallic acid equivalent (mg GAE/kg FW). For the extraction of polyphenols, methanol (70%) was used as a solvent, according to Singleton and Rossi (1965 cited by Ereifej et al., 2016).

Statistical analysis

Statistical analysis was performed using an IBM SPSS 14 program (SPSS Inc., Chicago, IL, USA). All results were analyzed by Anova and using the Duncan Multiple Range test. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

Biochemical quality and commercial value under storage conditions or for fresh consumption are particularly important in cultivar selection for fresh fruit. The period of ripening significantly influences the period of preservation of pears in a fresh state. Also, shelf life and susceptibility to decay differ between pear varieties. The fruits of the hair genotypes were stored at 4°C and 75% humidity and qualitatively analysed starting at the time of harvest and continuing at 60, 90, 120 days after harvest. Fruit decline was low (<2%) in the first 60 days. Continued storage at 4°C for up to 120 days resulted in highly accelerated degradation, fresh

Table 1. Fruit characteristic - weight (g), firmness (HPE units), pH, TA (%), dry weight (%), water (%) - of the pear genotypes

Genotypes	Storage time (days)	Weight (g)	Firmness (HPE units)	pH	TA (%)	Dry weight. (%)	Water (%)
Aniversare	At harvest	187.25±45.59[a]	80.48±3.66[a]	3.78±0.04[b]	0.42±0.06[a]	13.07±0.28[c]	86,93±0.28[a]
	60 days	186.44±45.39[a]	76.85±4.61[b]	3.86±0.08[b]	0.39±0.03[ab]	13.62±0.74[c]	86,38±0.74[b]
	90 days	185.45±45.3[a]	68.56±8.31[b]	4.14±0.06[a]	0.32±0.02[b]	14.68±0.06[b]	85,32±0.06[c]
	120 days	182.33±44.53[a]	67.43±0.64[b]	4.22±0.12[a]	0.16±0.01[c]	16.87±0.58[a]	83,13±0.58[d]
Romcor	At harvest	155.82±26.29[a]	79.18±2.00[a]	3.73±0.26[a]	0.73±0.00[a]	14.61±0.52[b]	85.39±0.52[a]
	60 days	154.13±26.01[a]	38.80±5.69[b]	3.93±0.29[a]	0.50±0.02[b]	15.25±0.33[b]	84.75±0.33[a]
	90 days	152.39±25.32[a]	31.12±1.39[c]	4.25±0.28[a]	0.11±0.01[c]	17.57±0.21[a]	82.43±0.21[b]
	120 days	-	-	-	-	-	-
Isadora	At harvest	212.25±26.75[a]	80.60±2.03[a]	4.29±0.06[b]	0.18±0.01[a]	15.69±0.23[d]	84.31±0.23[a]
	60 days	211.23±26.62[a]	76.41±2.47[b]	4.52±0.22[ab]	0.13±0.02[b]	16.72±0.40[c]	83.28±0.40[b]
	90 days	210.19±26.34[a]	75.85±3.66[b]	4.65±0.21[a]	0.11±0.01[c]	18.77±0.31[b]	81.23±0.31[c]
	120 days	208.77±25.96[a]	71.86±2.32[c]	4.78±0.13[a]	0.10±0.00[c]	19.58±0.61[a]	80.42±0.61[d]
Monica	At harvest	238,09±53.25[a]	79.83±1.94[a]	3.90±0.03[c]	0.32±0.02[a]	16.52±0.40[c]	83.48±0.40[a]
	60 days	236.49±52.89[a]	76.14±2.06[b]	4.13±0.10[b]	0.24±0.01[b]	17.07±0.38[bc]	82.93±0.38[ab]
	90 days	235.00±53.31[a]	73.93±3.54[b]	4.22±0.09[b]	0.21±0.02[b]	17.61±0.18[ab]	82.39±0.18[bc]
	120 days	232.96±53.33[a]	70.46±4.39[c]	4.43±0.14[a]	0.11±0.01[c]	18.34±0.91[a]	81.66±0.91[c]
Packham's Triumph	At harvest	169.02±15.31[a]	65.53±3.01[a]	4.61±0.17[b]	0.13±0.02[a]	15.57±0.60[b]	84.43±0.60[a]
	60 days	167.93±15.21[a]	43.59±12.97[b]	4.75±0.08[ab]	0.11±0.01[b]	16.11±0.60[ab]	83.89±0.6a[b]
	90 days	167.07±15.35[a]	40.37±9.10[b]	5.03±0.21[a]	0.10±0.00[c]	16.93±0.35[a]	83.07±0.35[b]
	120 days	-	-	-	-	-	-
P20R41P30	At harvest	137.93±28.54[a]	69.54±1.00[a]	4.76±0.42[b]	0.21±0.02[a]	14.24±0.36[b]	85.76±0.36[a]
	60 days	136.69±28.28[a]	68.41±5.73[a]	5.02±0.03[ab]	0.18±0.01[b]	14.26±0.94[b]	85.74±0.94[a]
	90 days	135.10±28.05[a]	58.41±7.29[b]	5.36±0.13[a]	0.11±0.01[c]	17.06±0.51[a]	82.94±0.51[b]
	120 days	-	-	-	-	-	-
R39P51x Paramis	At harvest	122.98±34.96[a]	82.75±7.64[a]	4.21±0.03[a]	0.32±0.02[a]	13.79±0.14[c]	86.21±0.14[a]
	60 days	122.38±34.79[a]	80.62±4.67[a]	4.32±0.07[a]	0.27±0.04[b]	16.17±1.29[b]	83.83±1.29[b]
	90 days	121.37±34.50[a]	72.89±4.58[b]	4.41±0.19[a]	0.18±0.01[c]	16.30±0.53[b]	83.70±0.53[b]
	120 days	119.09±33.76[a]	70.08±3.53[b]	4.39±0.21[a]	0.14±0.02[d]	20.20±0.48[a]	79.80±0.48[c]
SP06C2P5	At harvest	151.97±10.40[a]	81.45±5.45[a]	3.74±0.17[a]	0.51±0.01[a]	15.06±0.31[c]	84.94±0.31[a]
	60 days	150.34±10.29[a]	57.79±9.71[b]	3.88±0.13[a]	0.49±0.03[a]	15.77±0.26[b]	84.23±0.26[b]
	90 days	148.32±10.01[a]	42.39±9.05[c]	4.07±0.19[a]	0.35±0.04[b]	16.59±0.33[a]	83.41±0.33[c]
	120 days	-	-	-	-	-	-
Socrovisce	At harvest	165.54±32.61[a]	79.03±3.17[a]	3.63±0.29[b]	0.73±0.00[a]	14.98±0.29[c]	85.02±0.29[a]
	60 days	164.56±32.42[a]	74.13±4.23[b]	4.08±0.16[a]	0.51±0.02[b]	16.56±0.25[b]	83.44±0.25[b]
	90 days	163.81±32.52[a]	71.52±4.91[b]	4.19±0.34[a]	0.48±0.01[b]	16.84±0.12[b]	83.16±0.12[b]
	120 days	162.7±32.30[a]	70.20±0.62[b]	4.31±0.11[a]	0.24±0.06[c]	20.64±0.21[a]	79.36±0.21[c]

*Duncan test. Mean values with the same letter do not present significant differences ($p \leq 0.05$) ($n=3$).

weight loss and pH increase, as well as accelerated decreases in bioactive compounds beneficial to human health.

Sensory characteristics of pears are reported in Table 1.

Average weight and Weight loss

Fresh weights decreased during storage in all cultivars studied, although no statistically significant differences were noted (Table 1). However, the percentage of weight loss increases significantly with the increase of storage time in the genotypes (Figure 1). According to the analysis of variance test, the weight loss was significantly influenced by the genetic background of the cultivar. Significant differences in terms of weight loss percentage were shown by `Aniversare` `R39P51xParamis` and `Socrovisce` genotypes. The percentage of weight loss was different from one cultivar to another. Thus, a variation of this indicator between 1.16% (`Packham's Triumph`) and 3.14% (`R39P51xParamis`) was obtained. The `SP06C2P5' genotype achieved the highest weight loss up to 60 days and was followed by the 'Romcor' genotype, the difference between the two genotypes being low. In genotype `R39P51xParamis`, the difference between the percentage of weight loss at 60 days and 120 days was higher compared to the other varieties that were kept until this date (from 0.49% to 1.86%). Ochoa-Velasco and Guerrero-Beltrán (2014) noted a superior weight loss in pears with red fiber compared to pears with white fiber probably due to the fact that the red fiber is juicier and more fragile in texture. Among the genotypes analyzed, the fruits of the `Romcor` variety deteriorated the fastest in terms of quality up to 90 days of storage (2.18% up to 90 days).

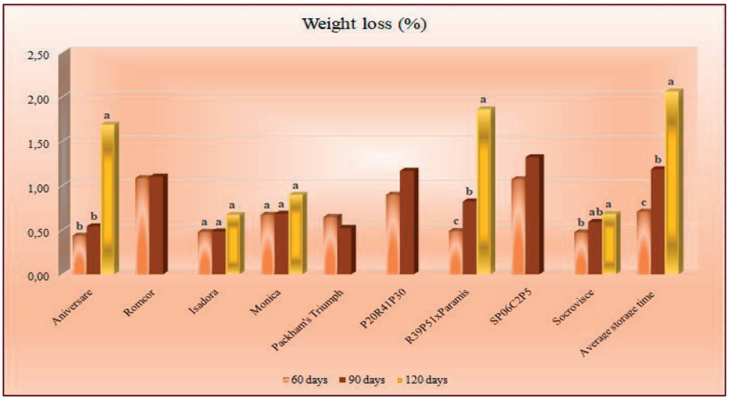

Figure 1. Influence of storage period on weight loss (%) of pears by cultivar (n=3)

Firmness Pears (*Pyrus communis* L.) are eaten in a firm and crisp stage immediately after harvest or storage. Fruit pulp firmness is an indicator used in agriculture or commerce to predict the optimal time to harvest pears (Wang & Sugar, 2015). Control of earlier and later ripening pears revealed a whole range of changes in this quality indicator, from crisp texture at fruit harvest to butter pulp at the end of shelf life (Brandes & Zude-Sasse, 2019). The fruit firmness at harvest was maximum 82.75 HPE units (R39P51xParamis) and it gradually declined as the period of storage advanced (Table 1). The lowest values were recorded for the genotypes 'Romcor' (31.12 HPE units), 'Packham's Triumph' (40.37 HPE units) and SP06C2P5 (42.31 HPE units) at 90 days of storage (the maximum period for these genotypes). The change in flesh firmness may be due to modification of the chemical structure of the cell wall (Sakurai & Nevins, 1997)

Colour: Traditionally, the pear cultivars have green, yellow, or russet-brown skins. Red coloration of pear skin is known to depend mainly on the composition and concentration of anthocyanin (Steyn et al., 2004). In recent years, red pear cultivars have rapidly become more popular for their attractive skin colour and potential nutritional value (Zhang et al., 2012). At European pears (*P. communis* L.), red coloration decreases toward harvest and anthocyanin accumulation reaches maximum about midway between anthesis and harvest (Steyn et al., 2004). A large variation in colour changes was observed between genotypes and storage time (Table 2). The limits of variation of the chromatic coordinate L were 67.90 for the 'R39P51xParamis' genotype and 43.52 for the 'Isadora' genotype. The values of the chromatic coordinate a* were between -14.74 (for 'Packham's Triumph' - 60 days of storage) and 14.28 (for the 'R39P51xParamis' genotype - 90 days of storage). Hunter's L, a*, b* values increased during the first 60 or 90 days of storage followed by a decrease in some genotypes. The effect of storage time on skin colour changes has been reported in several studies (Arzani et al., 2008; Hosseini et al., 2017).

Table 2. Fruit colour characteristic of the pear genotypes

Genotypes	Storage time (days)	*L*	a*	b*
Aniversare	At harvest	49.67±4.54[ab]	-3.88±2.23[b]	22.93±2.28[ab]
	60 days	46.12±3.83[b]	-2.22±1.28[b]	21.17±1.58[b]
	90 days	51.64±4.40[a]	-4.84±3.89[a]	22.75±1.90[ab]
	120 days	51.97±4.98[a]	3.43±4.88[a]	24.55±1.26[a]
Romcor	At harvest	60.15±7.30[a]	-6.60±5.51[a]	26.77±2.55[a]
	60 days	58.47±5.99[a]	-9.13±4.59[a]	27.59±3.17[a]
	90 days	61.09±2.27[a]	-3.87±2.11[a]	28.72±1.90[a]
	120 days	-		
Isadora	At harvest	60.37±4.38[b]	-12.51±3.35[d]	29.46±3.13[b]
	60 days	65.40±4.77[a]	-8.82±1.36[c]	30.53±1.34[ab]
	90 days	68.30±4.22[a]	-5.56±1.32[b]	31.99±0.98[a]
	120 days	67.90±3.38[a]	-2.41±1.59[a]	32.33±0.96[a]
Monica	At harvest	60.66±10.60[a]	17.10±9.49[a]	23.63±5.37[a]
	60 days	60.34±9.97[a]	6.51±12.87[a]	24.90±4.21[a]
	90 days	58.92±9.87[a]	10.02±12.91[a]	24.30±3.99[a]
	120 days	57.87±9.42[a]	8.44±11.91[a]	23.30±4.17[a]
Packham's Triumph	At harvest	55.65±1.40[a]	1.85±3.13[a]	27.11±1.77[a]
	60 days	61.61±4.02[a]	-14.74±46.85[a]	28.37±2.64[a]
	90 days	56.59±8.54[a]	2.35±2.15[a]	25.90±4.88[a]
	120 days	-		
P20R41P30	At harvest	56.89±1.97[a]	2.19±2.09[a]	27.41±1.84[a]
	60 days	61.61±6.02[a]	2.27±2.21[a]	25.46±1.53[b]
	90 days	59.64±5.98[a]	2.97±2.48[a]	25.03±1.87[b]
	120 days	-		
R39P51x Paramis	At harvest	43.52±5.04[a]	4.56±7.84[b]	18.14±3.29[a]
	60 days	49.15±8.13[a]	10.88±6.73[ab]	22.86±5.38[a]
	90 days	48.02±9.30[a]	14.28±8.76[a]	21.51±5.41[a]
	120 days	48.11±8.25[a]	13.0±10.72[ab]	21.03±4.99[a]
SP06C2P5	At harvest	51.49±2.33[a]	-4.18±2.06[b]	24.62±0.85[a]
	60 days	51.15±1.51[a]	8.07±2.00[a]	24.80±1.09[a]
	90 days	48.06±3.44[b]	9.56±1.62[a]	22.74±2.59[b]
	120 days	-		
Socrovisce	At harvest	46.74±1.90[b]	2.60±1.37[b]	22.20±1.40[b]
	60 days	51.58±3.02[a]	3.46±2.08[b]	23.88±1.12[a]
	90 days	51.82±2.44[a]	4.37±2.03[b]	24.73±1.11[a]
	120 days	50.85±1.49[a]	7.39±2.37[a]	23.60±1.54[a]

*Duncan test. Mean values with the same letter do not present significant differences ($p \leq 0.05$) ($n=3$).

TA and pH

pH indicates the acidity or alkalinity of a sample and is a crucial measure for evaluating the quality and ripeness of fruits, including pears (Taghinezhad et al., 2023). It is a quick and simple method used to highlight the level of organic acids in fruits or vegetables. Fruit acidity followed a linear downward trend throughout the storage period. In general, fruit acidity tends to decrease with ripening while a concomitant increase in sugar content occurs and may be due primarily to the use of organic acids in respiration (Raffo et al., 2002; Yaman & Bayoindirli, 2002). During storage, fruits could utilize acids and therefore organic acid content decreases with prolonged storage (Bhattarai & Gautam, 2006). In the Figure 2 a regression of the TA expressed as malic acid is observed in all analysed genotypes influenced by the storage time at the temperature of 4°C and 75% humidity. The content of organic acids in the fruits varied from 0.10% (`Isadora` at 120 days and `Packham's Triumph` at 90 days) and 0.73% (`Romcor` and `Socrovisce` at harvest). In the `Romcor` variety, a massive decrease in the content of organic acids can be observed up to 90 days (0.83%).

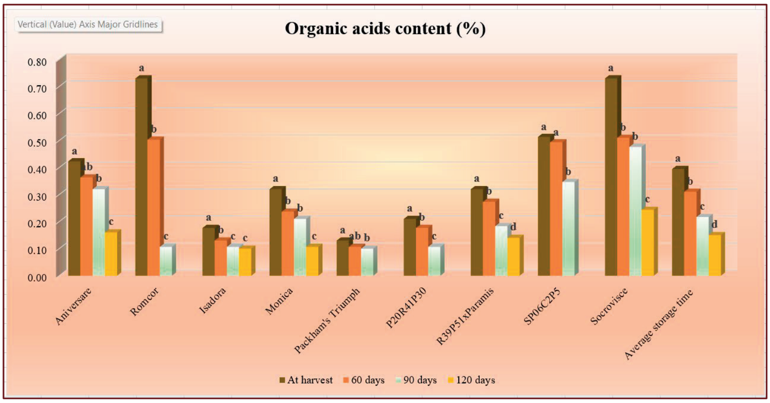

Figure 2. The influence of the storage period on the content of organic acids (%) in pears according to the genotypes (n=3)

TSS

Sugars and organic acids in fruits are substrates that are consumed in fruit respiration during storage (Yaman & Bayoindirli, 2002) by reducing into pyruvic acid, citric acid (Öztürk & Ağlar, 2019). Thus, the change in TSS level is a natural phenomenon encountered during fruit ripening and is correlated with hydrolytic changes in starch content during ripening and storage (Nandane et al., 2017). Previous research has shown that in pears, at the time of harvest, sorbitol was the dominant sugar, followed by fructose, glucose and sucrose. After two months of storage, fructose was dominant, followed by glucose, sorbitol and sucrose. During 2023-2024, stored pears showed an increase in TSS to a maximum value followed by a sharp decrease at the end of shelf life (Figure 3). At genotype `R39P51xParamis` a sudden drop in TSS is observed in the last 30 days from 17.17% Brix to 12.10% Brix. Similar results were obtained by Mahajan and Dhatt (2004). The maximum TSS content was recorded at 90 days of storage for all analysed genotypes. TSS varied between 17.17% Brix (`R39P51xParamis` at 90 days) and 11.3% brix (at `Monica`, 120 days). The progression of TSS during the shelf life is considered to be dependent on respiratory behaviour at the time of collection and may be affected by the storage atmosphere and treatment during the shelf life (Brandes & Zude-Sasse, 2019).

Vitamin C content

Losses of vitamin C during storage were variable from cultivar to cultivar. Among the analysed genotypes, `Monica` and `R39P51xParamis` stood out with a higher content of ascorbic acid (30.31 mg/100 g FW and 9.80 mg/100 g FW, respectively). The vitamin C content of pears showed a gradual decreasing trend during storage in all studied genotypes. If the vitamin C content at harvest recorded the values of 10.31 mg/100 g FW in the `Monica` genotype at the end of the storage period, it decreased to the value of 3.46 mg/100 g FW (Figure 4). The decrease in ascorbic acid level during storage can be attributed to the oxygen released by ascorbic acid, the conversion of dehydroascorbic acid (Sumnu & Bayindirli, 1995). Higher storage temperatures may be responsible for higher oxidation resulting in higher dehydroascorbic acid content (Lee & Kader, 2000).

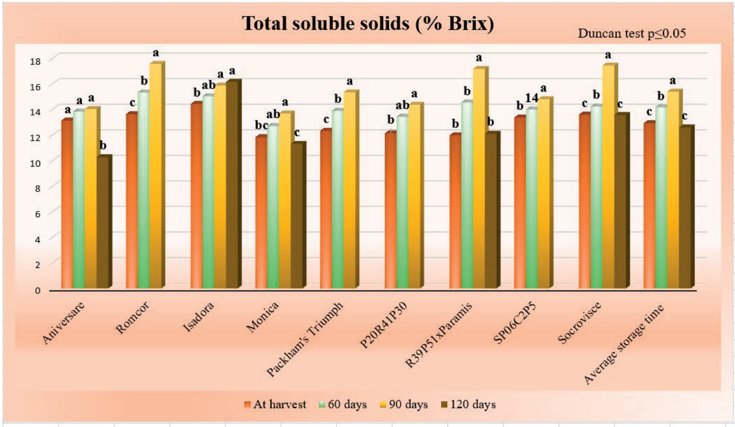

Figure 3. Influence of storage period on total soluble solids content (% Brix) of pears by cultivar (n=3)

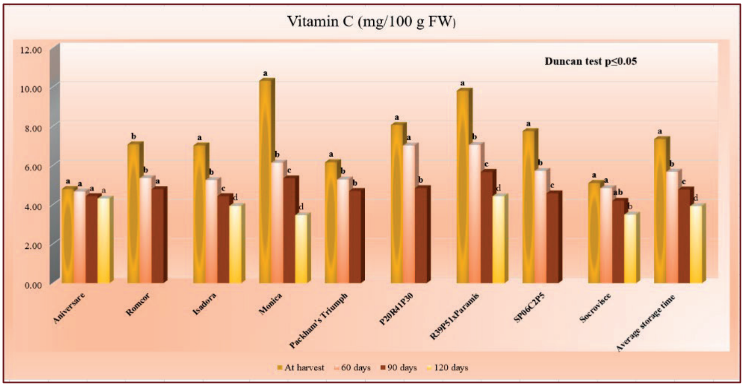

Figure 4. Influence of storage period on vitamin C content (mg/100 g FW) of pears according to cultivar (n=3)

Total polyphenol content (TPH)

The initial content of polyphenolic compounds in pear juice was significantly influenced by the genetic background of the cultivar. From figure 5 it can be seen that the \`Isadora\` and \`Monica\` pear varieties had a content of polyphenolic compounds significantly higher than the other varieties (3894.20 mg GAE/kg FW, 3818.84 mg GAE/kg FW respectively). Compared to the two cultivars used as control (\`Packham's Triumph\` and \`Socrovisce\`), only the genotypes \`P20R41P30\` and \`SP06C2P5\` were characterized by a lower content polyphenolic. Although the fruits of \`Romcor\` cultivar deteriorated the fastest in terms of quality, the content of total polyphenols obtained after 90 days of storage was significantly lower in genotypes \`SP06C2P5\` (720.29 mg GAE/kg FW) and \`P20R41P30\` (1010.15 mg GAE/kg FW) compared to \`Romcor\` (1710.14 mg GAE/kg FW). At the end of the shelf life of the fruit, the total polyphenol content drops sharply. The data are consistent with those in the specialized literature (Arzani et al., 2008).

Following the tests performed on the fruit quality indicators of the nine pear genotypes during the storage period (Table 2), the existence of positive or negative, statistically significant correlations between most of the compounds studied was found (Table 3).

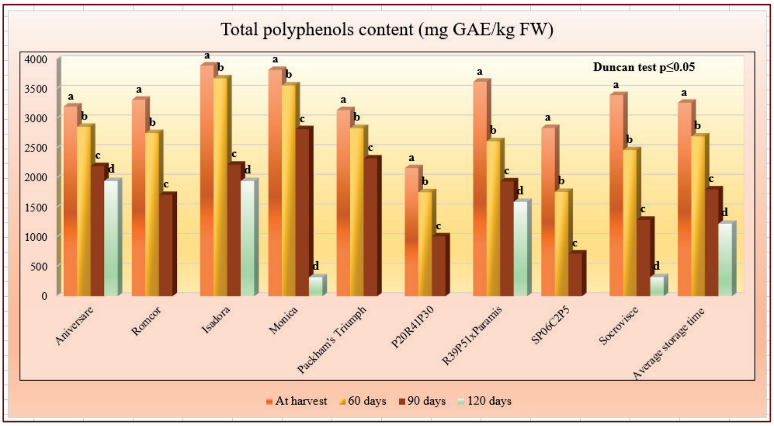

Figure 5. Influence of storage period on total polyphenols content (mg GAE/kg FW) of pears according to cultivar (n=3)

The results obtained indicated a significant negative linear interdependence between fruit weight and weight loss (r=0.330), between fruit weight and firmness (r=0.161). The relationship between fruit weight loss and TSS is negative, distinctly significant (r=-0.471). There was also a distinctly significant negative correlation between fruit pH and polyphenol content (r=-0.315) and a positive linear interdependence between water content and total polyphenols (r=0.313).

Table 3. Correlation between the values of the quality indicators studied in the fruits of the \`Isadora\`, \`Romcor\`, \`Monica\`, \`Aniversare\`, \`P20R41P30\`, \`R39P51xParamis\`, \`SP06C2P5\` \`Packham's Triumph\` and \`Socrovisce\` pear cultivars during the storage period

	Weight (g)	*Weight loss (%)*	*Firmness (HPE units)*	TSS (%Brix)	pH	TA (%)	Water (%)	Dry matter (%)	Vitamin C (mg/100 g FW)	*Total polyphenols (mg GAE/kg FW)*	L	a	b
Weight (g)	1												
Weight loss (%)	-0,330*	1											
Firmness (HPE units)	0,161*	0,196	1										
TSS (%Brix)	-0,088	-0,471*	-0,326**	1									
pH	-0,158	-0,321	-0,177	0,116	1								
TA (%)	-0,220*	0,054	0,158	-0,015	-0,715**	1							
Water (%)	-0,222*	-0,122	0,132	-0,175	-0,286**	0,460**	1						
Dry matter (%)	0,222*	0,122	-0,132	0,175	0,286**	-0,460**	-1,000**	1					
Vitamin C (mg/100 g FW)	0,054	-0,004	0,308**	-0,336**	-0,144	0,182	0,473**	-0,473**	1				
Total polyphenols (mg GAE/kg FW)	0,313**	-0,148	0,378**	-0,160	-0,315**	0,261*	0,503**	-0,503**	0,581**	1			
L	0,189*	-0,283	-0,091	0,122	0,434**	-0,362**	-0,241*	0,241*	-0,156	0,014	1		
A	0,052	0,335*	0,039	-0,103	-0,083	0,021	-0,107	0,107	0,101	-0,133	-0,387**	1	
B	0,094	-0,269	-0,162**	0,194*	0,313**	-0,264**	-0,254*	0,254*	-0,247*	0,017	0,872**	-0,458**	1

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

A positive linear relationship was obtained between water content and vitamin C or total polyphenols (r=-0.473, respectively r=0.503). TA content correlates negatively, distinctly significantly with total dry matter (r=-0.460). The relationship between the pH of the fruits and the acid content is negative, of very high intensity (r=-0.715).

CONCLUSIONS

After 60 days of keeping pears at 4°C and 70% humidity, the quality parameters of fruit indicate a downward trend that is maintained up to 90 and 120 days, respectively. The obtained results were significantly influenced by the genetic characteristics and the time of storage.

At the end of the shelf life of the fruit, the total sugar and polyphenol content drops sharply.

Among the studied genotypes, 'Romcor' collapsed in the range of 60-90 days of storage (fruit firmness after 90 days reached the value of 31.12 HPE units). Under these storage conditions, the genotypes 'Isadora', 'Aniversare' and 'R39P51xParamis' kept the best (up to 120 days).

Prolonged storage of pears at a temperature of 4°C significantly reduces the biochemical quality of the fruits. This storage method is indicated for a maximum period of 60-90 days depending on the genotype.

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FRUIT QUALITY OF NINE BLACKCURRANTS (*RIBES NIGRUM* L.) CULTIVARS SELECTED IN MEADOW ARGEȘ

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Abstract

In Romania, the blackcurrant is an appreciated crop due to the nutritional and therapeutic value of the fruit. Therefore, the study aims to identify the potential parental plants with superior quality indicators that can contribute to the breeding program and stimulate the sustainable development of black currants. Phenological observation, physical and chemical characteristics responsible for fruit quality of nine cultivars 'Abanos', 'Ben Hope', 'Ben More', 'Ben Nevis', 'Bona', 'Geo', 'Joseni17', 'Poli 51' and 'Tiben' were evaluated under the conditions of the Meadow Argeş during 2022-2023. In this sense, the results of biometric determinations (weight, fruit firmness) and biochemical quality indicators (total water content, pH, soluble solids, titratable acidity, total sugar, and vitamin C content) of the fruits are presented. The study found that 'Bona' and 'Ben More' cultivars produce the largest fruits, while 'Geo', 'Poli 51', 'Tiben', 'Ben Nevis', and 'Abanos' cultivars have higher total sugar content. These findings can guide breeders in selecting suitable parent plants for controlled hybridization to improve fruit size and biochemical quality in future generations.

Key words: *biochemical characteristics, fruit quality, phenology, vitamin C.*

INTRODUCTION

Blackcurrants (*Ribes nigrum* L.), a perennial shrub of the Grossulariaceae family (Chiche et al., 2002), is renowned for its cold resilience, thriving in temperate to subpolar regions, characterized by distinct seasons and cold winters (Preedy et al., 2020; Pagter et al., 2022), are primarily cultivated for their berries, which are renowned for their abundance of health-promoting bioactive compounds (Karjalainen et al., 2008). These benefits are attributed to their high levels of ascorbic acid (vitamin C) (Vagiri, 2012; Hummer & Barney, 2002), along with other antioxidants and fatty acids.

Research indicates that the ascorbic acid content in blackcurrant cultivars surpasses that of other fruits and remains more stable, thanks to the presence of anthocyanins and phenols (Hooper & Ayres, 1950; Miller & Rice-Evans, 1997). These compounds provide blackcurrants with exceptional antioxidant properties compared to other fruits and vegetables. They are predominantly found in the fruit but are also present in leaves and buds, attracting attention for their beneficial effects on health (Hummer & Barney, 2002; Raudsepp et al., 2010; Tabart et al., 2011; Del Castillo et al., 2004; Kaldmaë et al., 2013).

Blackcurrants offer versatility beyond their fresh consumption as they can be preserved by freezing, allowing them to be available year-round. Additionally, their rich flavor and nutritional profile make them a sought-after ingredient in the food and confectionery industries. Whether used in jams, jellies, sauces, desserts, or baked goods, blackcurrants add a distinctive tartness and vibrant color, enhancing the taste and visual appeal of various culinary creations (Mattila et al., 2016; Sazonov et al., 2020), since, blackcurrant fruit, like many other purple-colored fruits, contains anthocyanins, compounds responsible for their dark coloration.

Moreover, blackcurrants are rich in important vitamins composition such as vitamins C, A, E, B_3 and K, as well as essential minerals like

potassium, phosphorus, manganese, zinc, chromium, and boron (Paunović et al., 2017).
The consumption of blackcurrants supports the immune system, fortifying the body against infections. In addition to their anti-inflammatory and antioxidant effects, blackcurrants play a role in safeguarding cells from oxidative stress, thereby reducing the risk of chronic diseases (Cortez et al., 2019; Mattila et al., 2016; Ejaz et al., 2023).
Blackcurrant breeding programs are conducted globally to improve crop quality, performance and increase the content of health-beneficial compounds (Brennan et al., 2008a; Pluta et al., 2008; Cortez et al., 2019). These programs focus on enhancing traits such as yield, resistance to key pests and diseases, and fruit quality suitable for processing, freezing, and fresh markets. Additionally, there is an emphasis on developing cultivars adapted to local soil and weather conditions, as well as mechanical fruit harvesting (Markowski & Pluta, 2002).
The biophysical traits, but also the content of bioactive compounds in blackcurrant fruit are predominantly determined by its genotype but can be modified under different climatic and environmental conditions, as noted by Hancock et al., in 2007. Understanding how weather conditions and microclimate affect these traits is also crucial (Kaldmaë et al., 2013). When evaluating cultivars, it's essential to examine characteristics like berry size, soluble solids, acidity, sugar, and ascorbic acid content. Evaluating blackcurrant genotypes is essential in breeding programs. The creation of cultivars requires the analysis of multiple parameters to achieve the formation of a suitable assortment. It is crucial to identify optimal genotypes suitable for use as parental forms in future hybridization programs (Panfilova et al., 2023).
The biochemical composition and environmental influences on fruit quality were studied in nine genotypes of blackcurrants in two consecutive years, grown in Romania, with all plants cultivated under uniform conditions.
The objective is to identify potential parent plants that can contribute to the breeding program and foster the sustainable development of blackcurrants.

MATERIALS AND METHODS

The research was conducted between 2022 and 2023 at the Research Institute for Fruit Growing Pitești-Mărăcineni (RIFG), located at coordinates 44°54'06" north latitude, 24°52'20" east longitude, and an altitude of approximately 280 meters above sea level.
The Pitești area, as per the Köppen-Geiger classification, is characterized by a humid continental climate.
Based on climatological data owned by RIFG Pitești-Mărăcineni, the multiannual air average temperature (1969-2023) stood at 10.1ºC, and the cumulative precipitation was 674.3 mm.
Compared to these values, in the last two agricultural years, 2021-2022 (Figure 1) and 2022-2023 (Figure 2) the average temperature was 1ºC warmer in the 2021-2022 agricultural year (11.1ºC compared to the normal 10.1ºC) and also with 2.1ºC warmer in 2022-2023 (12.2ºC compared to the normal 10.1ºC), following the climatic trends of the last 55 years.

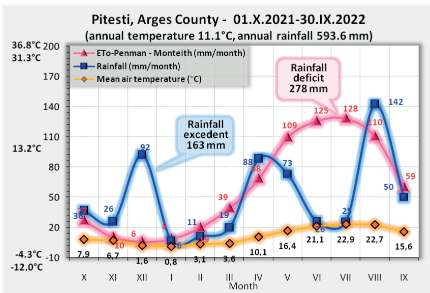

Figure 1. Evolution of monthly values, multiannual averages, of temperature, precipitation and Penman-Monteith potential evapotranspiration in Pitești, Argeș during the 2021-2022 agricultural year (climate diagram)

Even poorer in precipitation, with 80.7 mm in the 2021-2022 agricultural year (593.6 mm compared to 674.3 mm, which represents the normal range of October - September) (Figure 1) and with 103.1 mm in the 2022-2023 agricultural year (571.2 mm compared to 674.3 mm) (Figure 2).
In Romania's current continental-temperate climate, blackcurrant yields can be diminished by occasional late frosts, which harm the flowers.

In their 2009 study, Chițu et al. examined the probability of late frost damage in the Pitești area. They found that the highest risk of frost damage is those that occur between mid-to-late March when 49.7% of the plants were between the stages of green tip and onset of blooming, 0.3% had bloomed, and in 50% of cases, the plants had not yet reached the onset of vegetation.

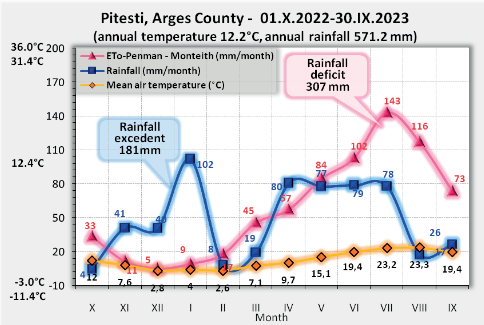

Figure 2. Evolution of monthly values, multiannual averages, of temperature, precipitation and Penman-Monteith potential evapotranspiration in Pitești, Argeș during the 2022-2023 agricultural year (climate diagram)

The research site was specifically situated on a terrace of the Argeș River, characterized by flat terrain and a clay-brown soil type. Agrochemical fertility indicators, including organic matter content, total nitrogen, potassium, and mobile phosphorus, portray the soil as having very low fertility (Table 1). Furthermore, the pH of the soil is moderately acidic (Table 2).

Table 1. Agrochemical soil fertility indicators

Depth (cm)	N_t (%)	P_2O_5 (ppm)	K_2O (ppm)	Total organic carbon (%)	Humus (%)
0-20	0.08	14.3	153	0.8	1.37
20-40	0.05	7.1	148	0.7	1.21

Table 2. Agrochemical indicators of soil acidity

Depth (cm)	pH	Ah (me/100 g soil)	SB (me/100 g soil)	V (%)
0-20	5.94	4.50	19.78	81.41
20-40	5.82	4.64	18.69	80.07

*Ah - Hydrolytic acidity or hard-to-change acidity, is expressed in m.e./100 g soil (determined according to the Kappen method) (Rusu & Mărghitaș, 2010);
**SB - The sum of exchangeable bases, expressed in m.e. (Ca^{2+}, Mg^{2+}, K^+, Na^+, NH_4^+)/100 g soil (determined according to the Kappen method) (Rusu & Mărghitaș, 2010);
***V (%) - Degree of soil saturation with exchange bases, calculated according to the formula V% = [(SB)/(SB+Ah)] x 100 (Rusu & Mărghitaș, 2010).

Observations were conducted on a total of nine blackcurrant cultivars, encompassing both indigenous and foreign types. The blackcurrant cultivars native to Romania were: 'Abanos', 'Geo', 'Joseni 17', and 'Poli 51'. The foreign cultivars under current research comprised followed cultivars 'Ben Hope', 'Ben More', 'Ben Nevis', 'Bona' and 'Tiben'. These cultivars were cultivated under the conditions of the Meadow Argeș. The blackcurrant plantation, where the study was conducted, has a density of 3,333 plants per hectare (3 x 1 m spacing). The crown shape of the blackcurrant plants is bush-like, and the irrigation is carried out through aspersion. All currants were grown in a largely uniform field.
In 2022 and 2023, a series of observations and assessments were conducted to analyse the blooming phenology, harvest maturity, and fruit quality of nine currant cultivars. Specific attention was given to key fruiting phenophases in blackcurrants, such as the onset and conclusion of flowering, as well as the initiation of the harvesting period. This investigation took place within the collection of the Research Institute for Fruit Growing Pitești-Mărăcineni (RIFG), specifically in the Small Fruits and Strawberry Laboratory field.
The average fruit weights (g) were evaluated using a digital balance (model Kern EW 600-2M). One hundred randomly selected fruits were weighed, for each cultivar. The firmness of the fruit (measured in Bareiss HPE II-FFF units) was determined using a non-destructive penetrometer (Qualitest HPE). Total soluble solids (TSS) were measured using a Kern digital refractometer, and the results were reported as °Brix at 20°C, following the AOAC (Association of Official Agricultural Chemists) guidelines from 1999. The pH levels were measured using a mini-Lab pH-meter. Total water content was assessed through a gravimetric method, involving the drying of 10 grams of fruit tissue at 105°C until a constant weight was achieved, following AOAC International guidelines from 2002. The organic acid content (%) in blackcurrant fruit was determined using the titrimetric method. This involved utilizing 25 ml of aqueous fruit extract neutralized with a 0.1N NaOH solution in the presence of phenolphthalein as an indicator, according to AOAC (Association of Official Agricultural Chemists) standards from

2000. Ascorbic acid, expressed in mg/100 g fresh weight (FW), was analyzed using a method based on the oxidation of L-ascorbic acid to dehydroascorbic acid in an acidic medium with a blue dye of 2.6-dichloro-indophenol. This was followed by the reduction of the dye to the colorless form, which turns red at pH 4.2 (PN-A-04019: 1998). The estimation of total sugar content was carried out through the Fehling-Soxhlet method in 1968, as per JAOAC (Journal of the Association of Official Analytical Chemists) standards from the same year. All biochemical determinations were conducted in triplicate.

The statistical analysis was carried out using IBM SPSS 14.0 software. Two-way ANOVA and Duncan Multiple Range Tests at a significance level of *Duncan's test ($p < 0.05$) were employed for all tests.

RESULTS AND DISCUSSIONS

Observations on the phenophases of the fruit organs in the blackcurrant species

During the study years, the flowering period of black currants in Pitești conditions took place between April 11th ('Joseni - 17' and 'Poli 51', year 2023) – April 30th ('Ben More', year 2022). Fruit ripening was observed the earliest on June 21st ('Joseni - 17', year 2023), and the latest record of fruit coloring was on July 9th ('Ben More', year 2022). The examined cultivars were categorized based on their blooming and fruit ripening periods into early ('Joseni - 17', 'Poli51') and half-early ('Abanos', 'Ben Hope', 'Ben More', 'Ben Nevis', 'Bona', 'Geo', 'Tiben') (Table 3).

Table 3. Beginning of flowering and fruit ripening of blackcurrant cultivars evaluated

Cultivars	The beginning of flowering		Fruit ripening	
	2022	2023	2022	2023
Abanos	23.04	18.04	29.06	27.06
Ben Hope	29.04	22.04	07.07	03.07
Ben More	30.04	24.04	09.07	05.07
Ben Nevis	29.04	24.04	08.07	05.07
Bona	24.04	18.04	30.06	28.06
Geo	23.04	17.04	30.06	28.06
Joseni - 17	18.04	11.04	22.06	21.06
Poli51	18.04	11.04	24.06	23.06
Tiben	25.04	22.04	30.06	28.06

Results regarding the physico-chemical indicators of fruit quality

The quality of perishable horticultural products depends on the combination of several indicators: weight (size), texture and firmness, appearance (shape, color, surface uniformity) and taste (sweetness, acidity and aroma) (Fellman et al., 2013).

In both years of study, the average fruit weight of the 'Bona' cultivar was the highest, with a mean value of 197.97 g for the weight of 100 fruits, while the 'Joseni 17' cultivar had the smallest average weight, with a mean value of 109.62 g for the same quantity of fruits (Table 4).

Table 4. Average fruit weight (g) of 100 fruits

Cultivar	2022	2023	2022-2023
Abanos	$124.8±4.61^{c}$	$119.93±3.77^{de}$	$122.37±4.62^{c}$
Ben Hope	$124.5±3.01^{c}$	$130.37±5.85^{cd}$	$127.43±5.26^{c}$
Ben More	$140.13±3.96^{b}$	$149.7±2.46^{b}$	$144.92±6.01^{b}$
Ben Nevis	$125.87±5.59^{c}$	$130.13±2.86^{cd}$	$128±4.61^{c}$
Bona	$188.37±13.29^{a}$	$207.57±2.22^{a}$	$197.97±13.54^{a}$
Geo	$125.9±5.47^{c}$	$129.73±3.94^{cd}$	$127.82±4.75^{c}$
Joseni 17	$105.43±2.8^{d}$	$113.8±8.53^{e}$	$109.62±7.3^{d}$
Poli 51	$128.63±4.65^{c}$	$128.93±5.48^{c}$	$128.78±4.55^{c}$
Tiben	$122.27±1.85^{c}$	$125.87±4.1^{cd}$	$124.07±3.46^{c}$

[a-e]Means followed by different letters differ significantly based on Duncan’s test at $P < 0.05$.

The firmness of the fruits is a particularly important selection criterion, especially of those suitable for fresh consumption. It depends on cell wall metabolism, water level (humidity) and fruit cuticle structure (Paniagua et al., 2013). Determination of firmness is useful for assessing fruit quality in breeding programs and for researchers in variety testing (Sekse et al., 2011).

Table 5.Average fruit firmness (HPE units)

Cultivar	2022	2023	2022-2023
Abanos	$14.37±0.91^{bc}$	$18.63±0.81^{ab}$	$16.5±2.46^{cd}$
Ben Hope	$19.67±2.41^{a}$	$20.67±3.61^{a}$	$20.17±2.8^{ab}$
Ben More	$21.4±2.78^{a}$	$19.53±2.44^{ab}$	$20.47±2.55^{ab}$
Ben Nevis	$17.8±0.82^{ab}$	$17.93±1.7^{abc}$	$17.87±1.2^{bc}$
Bona	$21.97±3.1^{a}$	$20.97±3.06^{a}$	$21.47±2.81^{a}$
Geo	$14.63±0.15^{bc}$	$19.87±0.67^{a}$	$17.25±2.9^{c}$
Joseni 17	$12.77±0.32^{c}$	$15.6±1.23^{bc}$	$14.18±1.75^{d}$
Poli 51	$13.33±0.9^{c}$	$17.6±1.73^{abc}$	$15.47±2.64^{cd}$
Tiben	$13.67±4.89^{bc}$	$14.57±1.91^{c}$	$14.12±3.36^{d}$

[a–d]Means followed by different letters differ significantly based on Duncan’s test at $P < 0.05$.

Fruit pulp firmness serves as a key metric in agriculture and commerce, aiding in the prediction of the ideal harvest time (Wang & Sugar, 2015). In terms of firmness, 'Bona' emerged as the standout variety, exhibiting the highest values over the two-year study period, averaging 21.47. In contrast, 'Tiben' recorded the lowest firmness values at 14.12, followed closely by 'Joseni - 17' at 14.18 (Table 5).

In his study, Vagiri (2012) observed that during the ripening process, berry skin firmness changed. This index depended on the cultivar and the berry ripeness. The firmest skin in all cultivars belonged to berries that were just beginning to ripen.

Throughout both years of the study, 'Bona' and 'Ben Hope' consistently showed the highest water content, averaging 82.58 and 82.22, respectively. Following closely was the 'Poli 51' cultivar with a water content value of 81.35. In contrast, 'Abanos' exhibited the lowest water content, averaging 80.87, indicating a similar concentration of dry matter, as found by Magazin et al. (2012) in a comparative study (Table 6). Knowing the water content and dry matter content of blackcurrant fruits is crucial for selecting appropriate processing and preservation methods.

The dry matter content of blackcurrant fruits is an important quality parameter as it can influence factors such as sweetness, texture and nutritional composition. The water content and dry matter composition significantly impact the processing process, affecting the volume and composition of the final product.

Table 6. Average fruit water content (%)

Cultivar	2022	2023	2022-2023
Abanos	79.12±0.41^{c}	82.61±0.69^{c}	80.87±1.98^{d}
Ben Hope	83.27±0.1^{a}	81.16±0.18^{a}	82.22±1.16^{a}
Ben More	81.44±0.7^{b}	82.11±0.86^{abc}	81.77±0.79^{bcd}
Ben Nevis	81.16±0.33^{b}	81.63±0.4^{abc}	81.39±0.41^{cd}
Bona	82.62±0.11^{a}	82.54±0.85^{a}	82.58±0.54^{ab}
Geo	81.32±0.61^{b}	81.66±0.47^{abc}	81.49±0.52^{acd}
Joseni 17	81.54±0.9^{b}	82.56±0.87^{ab}	82.05±0.97^{bc}
Poli 51	81.73±0.27^{b}	80.97±0.15^{a}	81.35±0.46^{abc}
Tiben	80.83±0.27^{b}	82.22±0.73^{bc}	81.53±0.91^{cd}

$^{a–d}$Means followed by different letters differ significantly based on Duncan's test at P < 0.05.

Fruits with higher water content can be reduced more during processing, while those with higher dry matter provide a denser and more concentrated base. Blackcurrant pomace, a byproduct of fruit processing, can have its shelf life extended and beneficial biological components preserved through specific drying techniques. Given the growing preference for natural ingredients in food manufacturing, the resulting blackcurrant pomace powders, obtained through grinding dried pomace, can serve as a desirable and nutritious addition to various food products (Michalska et al., 2017).

Along with other phytochemical compounds, soluble solids content contributes to fruit flavor.

The total soluble substances made up of soluble sugars and other non-carbohydrate compounds influence the refractive index of the aqueous extract obtained from a horticultural product (Diaconu, 2006). It is a genetic characteristic, specific to each variety (Ciucu-Paraschiv & Hoza, 2021).

During the two-year study period, 'Tiben' consistently showed the highest concentration of soluble solids, averaging 15.78 °Brix, while the cultivars 'Bona' and 'Ben More' exhibited the lowest averages, with values of 13.92 °Brix and 13.87 °Brix, respectively (Table 7).

The obtained values align with the earlier study by Camps et al. (2010), where results for soluble solids were obtained in the range of 12.9 °Brix to 20.8 °Brix.

Table 7. Soluble solids content (°Brix)

Cultivar	2022	2023	2022-2023
Abanos	15.9±0.66^{b}	13.6±0.61^{de}	14.75±1.38^{b}
Ben Hope	13.7±0.85^{e}	15.37±0.38^{a}	14.53±1.09^{bc}
Ben More	14.97±0.76^{bcd}	12.77±0.21^{e}	13.87±1.3^{c}
Ben Nevis	15.2±0.82^{bc}	14.93±0.45^{ab}	15.07±0.61^{b}
Bona	14.1±0.2^{de}	13.73±0.55^{cd}	13.92±0.42^{c}
Geo	15.67±0.25^{bc}	14.33±0.25^{bcd}	15±0.76^{b}
Joseni 17	14.67±0.6^{cde}	14.23±0.21^{bcd}	14.45±0.47^{bc}
Poli 51	14.6±0.26^{cde}	14.67±1.06^{abc}	14.63±0.69^{b}
Tiben	16.9±0^{a}	14.67±0.15^{abc}	15.78±1.23^{a}

a–d Means followed by different letters differ significantly based on Duncan's test at P < 0.05

The sugar content contributes to the determination of the organoleptic quality of the fruit. It can be influenced by the genetic characteristics of the cultivar, environmental conditions, crop maintenance technologies, soil conditions, fruit position in the crown, etc. (Davidescu, 1999; Gündoğdu, 2019). Along with other compounds that contribute to the definition of fruit taste, it is an important indicator in breeding.

The average sugar content was analysed over both study years. During this period, the cultivars 'Geo' and 'Poli 51' displayed the highest averages, with 9.74% and 9.45%, respectively, while 'Ben Hope' and 'Ben More' showed the lowest averages of 8.49% and 8.43%, respectively (Table 8).

Table 8. The average content of total sugar (%)

Cultivar	2022	2023	2022-2023
Abanos	$10.41±0.13^{a}$	$7.82±0.67^{b}$	$9.11±1.48^{abc}$
Ben Hope	$7.32±0.08^{d}$	$9.66±0.78^{a}$	$8.49±1.38^{c}$
Ben More	$9.13±0.52^{bc}$	$7.73±0.39^{b}$	$8.43±0.87^{c}$
Ben Nevis	$9.76±0.08^{abc}$	$8.66±0.41^{ab}$	$9.21±0.66^{abc}$
Bona	$8.84±0.4^{c}$	$8.26±0.67^{b}$	$8.55±0.59^{bc}$
Geo	$9.78±0.25^{abc}$	$9.69±1.1^{a}$	$9.74±0.72^{a}$
Joseni 17	$9.61±1.48^{abc}$	$8.91±0.35^{ab}$	$9.26±1.04^{abc}$
Poli 51	$9.34±0.84^{abc}$	$9.55±0.53^{a}$	$9.45±0.64^{a}$
Tiben	$10.15±0.31^{ab}$	$8.52±0.51^{ab}$	$9.34±0.97^{ab}$

a–c Means followed by different letters differ significantly based on Duncan's test at $P < 0.05$

In their study, Taghinezhad et al. (2023) noted that the pH value reflects the acidity or alkalinity of a sample and serves as a crucial measure for assessing the quality and ripeness of fruits. The content of organic acids in fruits can be influenced by several factors: genotypic differences, fruit ripening stage (Paraschiv & Nicola, 2023), culture and environmental conditions (Iancu & Gavăţ, 2009; Wang et al., 2008; Gündoğdu, 2019), post-harvest handling procedures (Lee & Kader, 2000).

Table 9. Average fruit pH

Cultivar	2022	2023	2022-2023
Abanos	$3.31±0.01^{bc}$	$3.36±0.09^{cde}$	$3.34±0.06^{cd}$
Ben Hope	$3.26±0.03^{cd}$	$3.17±0.05^{e}$	$3.22±0.06^{e}$
Ben More	$3.25±0.02^{d}$	$3.34±0.02^{cde}$	$3.3±0.05^{de}$
Ben Nevis	$3.24±0.02^{d}$	$3.28±0.02^{de}$	$3.26±0.03^{de}$
Bona	$3.45±0.03^{a}$	$3.53±0.15^{abc}$	$3.49±0.11^{a}$
Geo	$3.44±0.01^{a}$	$3.36±0.07^{cde}$	$3.4±0.06^{abc}$
Joseni 17	$3.29±0.04^{bcd}$	$3.63±0.17^{a}$	$3.46±0.22^{ab}$
Poli 51	$3.25±0.06^{d}$	$3.59±0.11^{ab}$	$3.42±0.2^{abc}$
Tiben	$3.34±0.03^{b}$	$3.44±0.1^{bcd}$	$3.39±0.09^{bc}$

a–e Means followed by different letters differ significantly based on Duncan's test at $P <0.05$

The results obtained from the two years of the study indicated that the 'Bona' cultivar had the highest pH level, measuring 3.49, whereas 'Ben Nevis' and 'Ben Hope' showed the lowest values of 3.26 and 3.22, respectively (Table 9). Cultivars display notable statistical variations in organic acid concentrations. Specifically, among these cultivars, 'Abanos' recorded the highest levels of organic acid, averaging 3.32%, while 'Tiben' and 'Bona' exhibited the lowest values, at 2.03% and 1.59%, respectively (Table 10). The study by Raffo et al. (2002) provides information about fruit acidity. It suggests that, in general, as fruits ripen, their acidity tends to decrease while there is a simultaneous increase in sugar content.

Table 10. Titratable acids (%)

Cultivar	2022	2023	2022-2023
Abanos	$3±0.02^{a}$	$3.64±0.08^{a}$	$3.32±0.36^{a}$
Ben Hope	$2.93±0.04^{a}$	$2.34±0.06^{c}$	$2.64±0.32^{c}$
Ben More	$2.95±0.02^{a}$	$3.05±0.09^{b}$	$3±0.08^{b}$
Ben Nevis	$2.9±0.03^{a}$	$3.11±0.12^{b}$	$3.01±0.14^{b}$
Bona	$1.54±0.01^{c}$	$1.63±0.18^{e}$	$1.59±0.12^{f}$
Geo	$3±0.02^{a}$	$2.01±0.04^{d}$	$2.5±0.54^{c}$
Joseni 17	$3±0.04^{a}$	$2.83±0.29^{b}$	$2.92±0.21^{b}$
Poli 51	$1.98±0.03^{b}$	$2.45±0.2^{c}$	$2.22±0.29^{d}$
Tiben	$1.63±0.45^{c}$	$2.42±0.27^{c}$	$2.03±0.54^{e}$

$^{a–f}$Means followed by different letters differ significantly based on Duncan's test at $P < 0.05$

Vitamin C is one of the most abundant antioxidants found in fruits.

Throughout the trial period, significant variation in average ascorbic acid content was observed among cultivars. 'Geo' (193.768 mg/100 g FW), 'Ben More' (191.68 mg/100 g FW), and 'Ben Nevis' (190.52 mg/100 g FW) exhibited the highest ascorbic acid content. In contrast, the 'Bona' cultivar consistently showed lower results over the two-year study period, recording 67.95 mg/100 g FW in 2022 and 82.15 mg/100 g FW in 2023 (Table 11).

In a study conducted in Serbia involving thirteen black currant varieties, the average vitamin C content ranged from 122.4 to 193.2 mg/100 g fresh weight (FW) (Djordjević et al., 2013) with values similar to those observed in this study. The significant levels of ascorbic acid found in black currants, as revealed by the results of the present study, underscore the importance of this parameter. A characteristic feature of black currants is the decrease in ascorbic acid content as the fruit ripens, typically occurring between 1.3 to 2.3 on the ripeness scale, with higher levels observed under less favorable weather conditions throughout the growing season. The challenge lies in understanding the mechanisms governing the accumulation of ascorbic acid in black currant fruits, influenced by both intrinsic and extrinsic factors (Walker et al., 2006; Badejo et al., 2008; Brennan et al., 2008b; Osokina et al., 2020).

Table 11. Ascorbic acid (mg/100 g FW)

Cultivars	2022	2023	2022-2023
Abanos	148.46±6.85[d]	203.61±24.09[ab]	176.04±34.11[ab]
Ben Hope	148.1±4.29[d]	206.82±9.94[ab]	177.46±32.88[ab]
Ben More	140.59±2.36[de]	242.77±4.65[a]	191.68±56.06[a]
Ben Nevis	137.72±4.49[e]	243.32±4.87[a]	190.52±57.99[a]
Bona	67.95±5.16[g]	82.15±25.29[c]	75.05±18.08[c]
Geo	184.86±8.16[b]	202.53±4.31[ab]	193.7±11.3[a]
Joseni 17	196.53±8.11[a]	127.25±7.97[bc]	161.89±38.62[b]
Poli 51	167.05±0.42[c]	177.65±9.84[b]	172.35±8.52[ab]
Tiben	78.32±5.11[f]	108.55±55.77[bc]	93.44±39.1[c]

a–e Means followed by different letters differ significantly based on Duncan's test at $P < 0.05$

CONCLUSIONS

Given the aim of enhancing fruit characteristics, particularly focusing on the commercial aspect of fruits, the study highlighted the 'Bona' and 'Ben More' cultivars as exemplary candidates for parental roles in improving fruit traits. Additionally, considering the preference and benefits of consuming natural vitamins over manufactured ones, cultivars like 'Geo' and 'Ben More', which demonstrate significant levels of vitamin C content, can be employed in breeding programs to elevate vitamin C concentrations. Moreover, to enhance taste qualities, one may consider cultivars such as 'Geo', 'Poli 51', 'Tiben', 'Ben Nevis', and 'Abanos', characterized by high concentrations of total sugar content, making them promising candidates for parental selection in breeding programs.

These findings hold significant implications: they offer guidance to breeders for selecting appropriate cultivars as parent plants for controlled hybridization, aid farmers in making informed choices when setting up new orchards, and offer valuable insights for the fruit market, especially concerning fresh consumption.

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INFLUENCE OF THE BIOLOGICAL CHARACTERISTICS OF THE VARIETY ON THE PRODUCTIVITY OF THE PLANTATION AND THE QUALITY OF THE APRICOT FRUITS DURING THE FRUITING PERIOD

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Abstract

The researches were carried out in the spring of 2015 from the company "Agroparc Management" SRL, located in the southern part of the country. As biological research material, trees from apricot varieties were used: Wonder Cot, Magic Cot, Spring Blush, Lilly Cot, Perle Cot, Pinkcot, Sweet Cot, Orange Red, Big Red, Faralia, Kioto and Farbaly. The Orange Red variety was taken as a control. The varieties were grafted onto Mirobalan 29C rootstock. The trees formed by open vase crown type. Planting distance 5.0 x 3.0 m. The lot is not irrigated. The soil between the intervals, between the rows and between the trees in the row was maintained as black field. The atmospheric precipitation that fell in abundance in the months of April (73.6 mm) and May (46.0 mm) in the southern part of the country had a considerable impact on the quality of the fruits of the early ripening apricot varieties (Wonder Cot, Spring Blush, Magic Cot, Pinkcot). Medium and late ripening cultivars had slower fruit development due to insufficient moisture during the development period.

Key words*: apricot, variety, fruit, average weight, production, quality.*

INTRODUCTION

Apricot culture is a species whose fruits are requested by consumers more and more frequently (Balan et al., 2008; Cociu et al., 1993; Peșteanu et al., 2018a; 2018b).

They are intended for fresh consumption as well as for industrialization. Until recently, the apricot crop was viewed with distrust, even considered risky, due to its lower frost resistance, often affected by late spring temperatures (Balan et al., 2021; Peşteanu & Negru, 2021a; 2021b; Stănică et al., 2010).

Among these disadvantages can be included the more obvious sensitivity to specific diseases, primarily the premature death of plants (Balan et al., 2008; Cociu et al., 1993; Peşteanu et al., 2018b).

When establishing apricot orchards in the Republic of Moldova, cultivar/rootstock associations with high growth vigour are used, in which it is recommended to lead the crown of the trees according to the high-volume pyramid, where the weight of the vegetative macrostructure prevails at the expense of the fruit microstructure (Negru & Peşteanu, 2019b; Peșteanu et al., 2018b; Peşteanu, 2021b).

Worldwide, the species in question has, in the last 20 years, undergone essential transformations regarding varieties with different ripening times, rootstocks with a shorter waist and the shape of the crown used in plantations (Milatovic et al., 2013; Peșteanu, 2021a; Stănică & Eremia, 2014). These changes allow to obtain more obvious performances in the cultivation technology of the given species and to record harvests of 20-25 t/ha of competitive quality (Peșteanu et al., 2021; Stănică et al. 2019).

In order to record an optimization of the structure of the apricot plantation, the cultivation of this species can achieve performance by implementing modern varieties with various ripening periods to have standardized productions during 60-70 days, vegetative rootstocks with balanced vigour to use new forms of crown suitable for the intensification of the given species (Balan et al., 2008; Negru & Peşteanu, 2020; Negru et al., 2021; Peşteanu, 2021b; Stănică et al., 2020). This trend can lead to the increase of trees' resistance to various abiotic and biotic cataclysms, the specialization and concentration within the country's geographic areas of apricot culture, highlighting new cultivation micro zones (Negru & Peşteanu, 2019a; Peşteanu, 2021a; Piagnani et al., 2013)

MATERIALS AND METHODS

The researches were carried out in the commercial orchard of the company SRL "Agroparc Management", town Volcanic. The orchard was established with trees in the form of certified category rods in the spring of 2015. The material was imported from Italy, from the "Vivai Batististini" nursery.

The trees of apricot varieties from the world selection (Cot International, International Plant Selection, Escande, etc.) served as biological research material, which, thanks to a theoretical study, were considered promising for the southern area of the Republic of Moldova: Wonder Cot, Magic Cot, Spring Blush, Lilly Cot, Perle Cot, Pinkcot, Sweet Cot, Orange Red, Big Red, Faralia, Kioto and Farbaly. As a control was the variety Orange Red.

The trees of the studied varieties were grafted onto Mirobalan 29C rootstock. The trees in the experimental group were led according to the crown of the usual vessel. Planting distance 5.0 x 3.0 m. The lot is not irrigated. The soil between the intervals between the rows and between the trees in the row was maintained as black field.

The phenological investigations were investigated by the observation method in all the trees of the variant according to the methodology used in the state trial of the varieties. The ripening period of the apricots was established by the moment when the apricots reached the characteristic color and taste qualities specific to the variety (Baggiolini, 1952).

The total number of fruits within the crown of the trees was determined 2 weeks before harvest. The production obtained within each variety was determined by the fruit weighing method. During the harvest, the number of fruits was determined for each individual tree in the variant. The average weight of an apricot fruit was determined by the calculation method during the harvesting period, i.e. 100 fruits collected in a row within each variety were weighed. After this, the average harvest of a tree and the fruit per surface unit was determined by the calculation method.

Morphological indices such as: the height, the small and the large diameter of one fruit in the studied varieties were determined in the laboratory of the Department of Horticulture and Forestry, by the measurement method. The shape of the fruit was expressed based on the shape index, by the ratio between the height and the large diameter of the fruit.

Fruit quality was determined by measuring the large diameter in the equatorial area of apricots. In accordance with the quality and marketing requirements of fresh fruit, apricots with a diameter smaller than 30 mm cannot be marketed. Those with a fruit diameter of 30-35 mm are assigned to quality category I and II, or are marked with the letter C. Those with a diameter greater than 35 mm are assigned to the extra quality category. Apricots from the extra quality category are divided into the following classes: B – diameter 35-40 mm; A – diameter 40-45 mm; 2A – diameter 45-50 mm; 3A – diameter 50-55 mm and 4A – diameter 55 mm and larger.

The main economic indicators were determined by accounting for the investments implemented in 2023 for fruit production and the value obtained from sales.

The statistical processing of the main indices was carried out by the method of monofactorial dispersion analysis. The experimental results were processed using the ANOVA test and STATGRAPHICS 18 software package.

RESULTS AND DISCUSSIONS

The apricot's requirements for different abiotic factors are very different and therefore each phase of development requires an optimal temperature level and a certain length of time.

In the spring of 2023, the apricot crop in the plantation managed by the company "Agroparc Management" SRL started flowering on 23.03.2023. The data entered in Table 1 highlight that earlier flowering was recorded in the varieties with earlier ripening period Magic Cot and Wonder Cot (23.03). On 25.03, flowering began for the trees of the Perle Cot variety, and on 26.03 for the Pinkcot variety. Flowering one day later was recorded for the Sweet Cot and Spring Blush varieties. Then, in the following sequence, flowering took place in the trees of the Lilly Cot varieties - 28.03, the

Orange Red variety - 30.03, the Big Red variety - 01.04, the Faralia variety - 02.04, the Farbaly variety - 03.04 and the Kioto variety - 04.04. Practically, the flowering of the trees in the studied varieties took place during 12 days.

A degree of flowering of 50% of the flowers in the crown of the apricot trees in the Wonder Cot and Magic Cot varieties was recorded on 25.03, in the Pinkcot and Perle Cot varieties on 29.03, and in the Spring Blush and Sweet Cot varieties on 30.03. Then, in the following sequence, the Lilly Cot variety bloomed - 02.04, the Orange Red, Big Red and Faralia varieties - 04.04, the Farbaly variety - 05.04 and the Kioto variety - 06.04. That is, the period between the beginning of flowering and the 50% flowering phenophase lasted approximately 2-3 days, depending on the biological characteristics of each variety under study and the air temperature during that period.

Table 1. The influence of apricot varieties on the initiation of certain phases of development of the fruit organs of the trees in the southern part of the country, year 2023

Variety	The date of the initiation of the beginning of the flowering phase of the trees				
	Beginning of the flowering	Bloom 50%	Full bloom	Falling petals	Strengthening the endocarp
Wonder Cot	23.03	25.03	29.03	05.04	12.05
Spring Blush	27.03	30.03	02.04	08.04	14.05
Magic Cot	23.03	25.03	29.03	04.04	12.05
Pinkcot	26.03	29.03	01.04	05.04	13.05
Perle Cot	25.03	29.03	01.04	07.04	15.03
Orange Red (m)	30.03	04.04	06.04	09.04	14.05
Sweet Cot	27.03	30.03	04.04	10.04	14.05
Lilly Cot	28.03	02.04	05.04	10.04	14.05
Big Red	01.04	04.04	06.04	12.04	15.05
Kioto	04.04	06.04	08.04	13.04	17.05
Faralia	02.04	04.04	06.04	12.04	16.05
Farbaly	03.04	05.04	07.04	13.04	17.05

The end of flowering can be considered when 100% of the flowers on the perennial wood and the annual branches from the first wave of growth have completely bloomed. In the trees of the studied varieties, the final flowering period coincided with 29.03 - 08.04, that is, it lasted 11 days.

The duration between the phenophase of flowering and full flowering in the trees of the studied apricot varieties varied from 4 to 7 days depending on the biological particularities of the variety. In the case of the group of trees from the varieties with early ripening, this period lasted 5-7 days, and among the trees from the varieties with late fruit ripening it was 4 days.

The falling of the petals refers to the period when the fruits have just been formed and it is necessary not to allow their condition to be affected by both abiotic and biotic factors. Apricots are quite sensitive to various biotic and abiotic changes. The obtained results highlight that the petal falls of the apricot varieties studied in the southern part of the country took place from 04.05.2023 and lasted until 04.13.2023. This phenomenon took place with very early-ripening varieties (Wonder Cot, Magic Cot, Spring Blush, Pinkcot Lilly Cot), continued with medium-ripening varieties (Perle Cot, Sweet Cot, Orange Red, Big Red) and ended with those with ripening late (Kyoto, Faralia, Farbaly).

Studying the consecutiveness of fruit ripening in the trees of the apricot varieties studied in the southern part of the Republic of Moldova during the research, we notice that large deviations from the harvest period possessed by each variety were not recorded.

The study carried out on the number of days recorded from the beginning of flowering to the harvesting of the fruits in the apricot varieties taken in the study, we note that this phenophase was 80 days in the Spring Blush variety and was completed after a period of 129 days in the trees of the variety Farbaly. If we compare the ripening period of the apricot fruits with the Orange Red variety, considered as a control, we note that all the varieties studied can be divided into 4 groups (Figure 1).

The varieties with extra early ripening include Spring Blush and Wonder Cot, whose fruit harvesting started 6-7 days earlier than the control variety. Magic Cot, Pinkcot, Sweet Cot, Lilly Cot and Orange Red varieties belong to the group of early ripening varieties, whose difference in the start of harvesting compared to the control variant was 6-8 days. Within the group of varieties with medium ripening are the varieties Perle Cot, Big Red, Sweet Cot and Kioto (6-7 days), and among those with late ripening are the varieties Faralia and Farbaly, in which the beginning of the harvest period compared to the control variant began with a delay of 23 and 37 days, respectively.

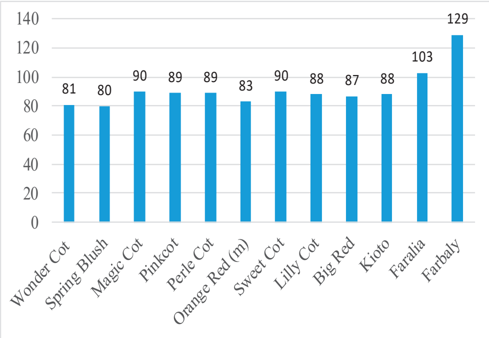

Figure 1. The influence of the biological particularities of apricot varieties on the period from the start of flowering of the trees to the ripening of the fruits, year 2023

The size and shape of the fruits influence the market value and are important indicators in the study of varieties, because they have a direct relationship with the quality of the obtained product, the productivity during sorting and packaging of the fruits.

According to the average weight, the apricot varieties studied can be divided into the following groups. The varieties whose average weight falls within the values up to 40 g, the group of those with small fruits (Wonder Cot, Kioto, Lilly Cot, Big Red, Sweet Cot, Farbaly and Faralia varieties). The Perle Cot and Spring Blush varieties are assigned to the group of varieties with medium fruits, whose average fruit weight was 41-50 g. The Orange Red (m), Pinkcot and Magic Cot varieties, according to the average fruit weight, belong to the group varieties with large fruits (50-60 g) (Table 2).

The difference between the height of the fruits differs from the genetic nature of the variety under study. Lower values of apricot fruit height were recorded in the Lilly Cot (37.0 mm) and Kioto (36.9 mm) varieties, and the highest in the Magic Cot variety (52.0 mm). In general, all apricot varieties studied can be divided according to the height of the fruit into 3 groups. Kioto and Lilly Cot belong to the group of varieties with a fruit height of 35-40 mm, and to those with a fruit height of 40-50 mm the varieties Big Red, Wonder Cot, Sweet Cot, Spring Blush, Pinkcot, Orange Red, Perle Cot, Faralia and Farbaly. Values greater than 50 mm were recorded only in the Magic Cot variety (52.0 mm).

The large and the smallest diameter in the studied varieties were influenced by the biological particularities of the variety. In most apricot varieties the large diameter ranged from 30 to 40 mm (Wonder Cot, Lilly Cot, Sweet Cot, Big Red, Kioto, Faralia and Farbaly), then the varieties Spring Blush, Magic Cot, Pinkcot, Perle Cot and Orange Red recorded values higher than 40.0 mm.

Depending on the values of the small diameter, we record diametrically opposite correlations. In the case of the varieties Lilly Cot, Wonder Cot, Sweet Cot, Big Red and Kioto, the diameter of the apricot fruits was less than 40 mm, and in the varieties Spring Blush, Perle Cot, Faralia, Orange Red (m), Magic Cot, Farbaly, the Pinkcot index in the study recorded values greater than 40.0 mm.

Table 2 Average weight and morphological parameters of apricot fruits according to the biological characteristics of the variety, year 2023

Variety	Average weight, g	Height, mm	Large diameter, mm	Small diameter, mm	Shape index
Wonder Cot	34.4	46.4	36.4	38.1	1.22
Spring Blush	44.6	44.0	40.5	44.4	0.99
Magic Cot	56.2	52.0	43.5	45.3	1.15
Lilly Cot	27.0	37.0	32.0	37.3	0.99
Pinkcot	52.2	46.6	41.4	44.9	1.04
Perle Cot	41.0	42.3	40.0	42.3	1.00
Orange Red (m)	60.3	47.5	43.3	45.7	1.04
Sweet Cot	30.0	40.1	33.6	37.1	1.08
Big Red	30.7	40.6	34.1	38.0	1.07
Kioto	28.5	36.9	33.7	36.3	1.02
Faralia	35.4	46.3	35.8	40.1	1.15
Farbaly	38.2	46.7	35.2	41.1	1.14
LDS 5%	1.98	2.18	1.83	2.05	-

The study carried out on the shape of the fruit by means of the shape index, highlights that in the varieties Lilly Cot, Kioto, Perle Cot, Spring Blush, Orange Red and Pinkcot it was 0.99-1.04, that is, the fruits were round in shape. The varieties Big Red, Magic Cot, Sweet Cot, Faralia and Farbaly, the shape index varied from 1.07 to 1.15, which highlights the spherical shape of the fruit. For the Wonder Cot variety this index registered 1.22, meaning the fruits had an elongated spherical shape.

A smaller number of apricots in the crown of the trees was recorded for the varieties Orange Red - 143 pcs, Magic Cot - 163 pcs, Spring Blush - 223 pcs, Wonder Cot - 311 pcs and Perle Cot - 313 pcs. Further, the varieties Farbaly - 320 pcs., Pinkcot - 325 pcs., Faralia - 396 pcs./tree, Sweet Cot - 447 pcs./tree, Big Red - 464 pcs./tree and Lilly Cot - 479 pcs., and the Kioto variety recorded a higher number of apricots per tree (527 pcs.) (Table 3).

The fruit production within the tree was correlated with the number of fruits obtained after their fall in June and the average weight of an apricot of the respective variety.

The obtained results highlight that the production of fruits within the tree is more essential influenced by the number of remaining fruits and their average weight is secondary.

Lower values of fruit production from an apricot tree were recorded for the varieties Orange Red (m) – 8.62 kg, Magic Cot – 9.17 kg, Spring Blush – 10.26 kg and Wonder Cot – 10, 70 kg. Next, the varieties Farbaly – 12.22 kg, Perle Cot – 12.33 kg, Lilly Cot – 12.33 kg, Sweet Cot – 12.93 kg, Faralia – 14.02 kg and Big Red were placed in growth 14.24 kg. The varieties Kioto and Pinkcot recorded a higher production of apricot fruits per tree, constituting 15.02 and 16.97 kg, respectively.

Table 3. Apricot plantation productivity according to the biological characteristics of the variety, year 2023

Variety	Number of fruits, pcs./tree	Average weight, g	Production	
			kg/tree	t/ha
Wonder Cot	311	34.4	10.70	7.14
Spring Blush	230	44.6	10.26	7.02
Magic Cot	163	56.2	9.17	6.12
Lilly Cot	479	27.0	12.93	8.62
Pinkcot	325	52.2	16.97	11.32
Perle Cot	313	41.0	12.33	8.56
Orange Red (m)	143	60.3	8.62	5.75
Sweet Cot	447	30.0	13.41	8.94
Big Red	464	30.7	14.24	9.50
Kioto	527	28.5	15.02	10.02
Faralia	396	35.4	14.02	9.35
Farbaly	320	38.2	12.22	8.15
LDS 5%	9.51	1.98	0.42	0.36

The study carried out further on the fruit production per surface unit, demonstrates the influence of the production obtained within an apricot tree of the studied varieties.

Higher global production values of apricots were obtained within the variety Kioto (10.02 t/ha) and Pinkcot (11.32 t/ha). Insignificantly lower values were obtained in the trees of the Big Red (9.50 t/ha) and Faralia (10.02 t/ha) varieties. Then, in descending order, the varieties Sweet Cot (8.94 t/ha), Lilly Cot (8.62 t/ha), Perle Cot (8.56 t/ha), Farbaly (8.15 t/ha) were placed ha), Wonder Cot (7.14 t/ha), Spring Blush (7.02 t/ha), Magic Cot (6.12 t/ha) and Orange Red (5.75 t/ha).

The sizes of the fruits are of particular importance because depending on them, they are redistributed to different quality classes, which then depends on the selling price, thus implicitly the economic efficiency.

From the varieties studied, a higher proportion of fruits with a diameter of 35-40 mm were recorded in the Lilly Cot (100.0%), Sweet Cot (92.4%) and Big Red (91.4%) varieties. In quality class A (diameter 40-45 mm), a higher share of fruit returned to the varieties Spring Blush (63.3%), Pinkcot (60.1%), Wonder Cot (92.4%), Perle Cot (60.0%), Faralia (65.7%) and Farbaly (70.2%) (Table 4).

Table 4. Influence of biological characteristics of apricot varieties on fruit quality by diameter, %, year 2023

Variety	By diameter		
	B	A	2A
Wonder Cot	24.3	50.6	25.1
Spring Blush		63.0	37.0
Magic Cot	29.5	30.7	39.8
Lilly Cot	100.0	-	-
Pinkcot	20.0	60.1	199
Perle Cot	18.4	60.0	21.6
Orange Red (m)	10.0	32.2	57.8
Sweet Cot	92.4	76	-
Big Red	91.4	86	-
Kioto	100.0	-	-
Faralia	25.6	50.0	24.4
Farbaly	10.7	70.2	19.1

Apricot fruits with a diameter of 45-50 mm are assigned to class 2A, and the higher share of fruits in that class returned to the Orange Red varieties (57.8%). Lower values in the respective class went to the varieties Wonder Cot (25.1%), Perle Cot (21.6%) and Farbaly (19.1%). The varieties Spring Blush (37.0%) and Magic Cot (39.8%).

The conducted research has highlighted that the income from sales is higher in the variants where the realization price is higher and the harvest of apricot fruits was higher (Tabele 5).

The studied varieties are of different ripening period, have different average fruit weight and specific price for each variety depending on the ripening period. The lowest realized price in 2023 was obtained for the Kioto, Faralia and Farbaly varieties where, due to the low quality of the fruits, the value was 10.0 lei/kg. Next in ascending order were the varieties Lilly Cot, Perle Cot, Sweet Cot and Big Red, with a sales price of 12.0 lei/kg. A higher price was given for the fruits of the varieties Pinkcot (14.0 lei/kg), Orange Red (18.0 lei/kg), Wonder Cot, Spring Blush and Magic Cot (20.0 lei/kg).

Table 5. The economic efficiency of apricot production in the southern part of the country depending on the biological characteristics of the variety, year 2023

Variety	Sales revenue, thousand lei/ha	Production cost, thousands of lei/ha	profit, thousand lei/ha	Profitability level, %
Wonder Cot	142.8	50.7	92.1	181.6
Spring Blush	140.4	50.6	89.8	177.5
Magic Cot	122.4	49.7	72.7	146.3
Lilly Cot	103.4	52.2	51.2	98.1
Pinkcot	158.5	54.9	103.5	188.7
Perle Cot	102.7	52.2	50.5	95.8
Orange Red (m)	103.5	49.4	54.1	109.5
Sweet Cot	107.3	52.5	54.2	104.4
Big Red	114.0	53.1	60.9	114.7
Kioto	100.2	53.0	47.2	89.1
Faralia	93.5	53.0	40.5	76.4
Farbaly	81.5	52.0	29.5	56.7

As part of the investigations undertaken, higher sales income values were obtained for the Pinkcot variety - 158.5 thousand lei/ha. Next, in decreasing order are the varieties Wonder Cot - 142.8 thousand lei/ha, Spring Blush - 140.4 thousand lei/ha, Magic Cot - 122.4 thousand lei/ha, Big Red - 114.0 thousand lei/ ha, Sweet Cot – 107.3 thousand lei/ha, Orange Red (m) – 103.5 thousand lei/ha, Lilly Cot – 103.4 thousand lei/ha, Perle Cot – 102.7 thousand lei/ha, Kyoto – 100.2 thousand lei/ha, Faralia – 93.5 thousand lei/ha and Farbaly – 81.5 thousand lei/ha.

The cost of production, as usual, is an indicator showing expenses that were directed to obtain the respective production of fruits. This indicator correlates directly with the production of apricots obtained per surface unit and those investments directed to obtain this production.

The investigations undertaken have highlighted that the higher value of the production cost was recorded for the Pinkcot variety, where for a production unit it was 54.9 thousand lei/ha, and lower indicators for the Wonder Cot varieties - 50.7 thousand lei/ha ha, Spring Blush – 50.6 thousand lei/ha, Magic Cot – 49.7 thousand lei/ha and Orange Red (m) – 49.7 thousand lei/ha.

The varieties Big Red, Kioto, Faralia, Sweet Cot, Perle Cot, Lilly Cot and Farbaly recorded an average production cost, constituting 53.1; 53.0; 53.0; 52.5; 52.2 and 52.0 thousand lei/ha, respectively.

Another very important economic indicator is the profit, which directly affects the sales revenue of the apricot production and depends a lot on the cost of the registered production. The research carried out highlighted that the most rational link between the cost of apricot production and the income from sales was obtained within the Pinkcot variety, where the profit was 103.5 thousand lei/ha. Average profit values were obtained for Wonder Cot varieties - 92.1 thousand lei/ha, Spring Blush - 89.8 thousand lei/ha, Magic Cot - 72.7 thousand lei/ha Big Red - 60.9 thousand lei /ha, and the lowest for the varieties Sweet Cot – 54.2 thousand lei/ha, Orange Red (m) – 54.1 thousand lei/ha, Lilly Cot – 51.2 thousand lei/ha, Perle Cot – 50, 5 thousand lei/ha, Kyoto – 47.2 thousand lei/ha, Faralia – 40.5 thousand lei/ha and Farbaly – 29.5 thousand lei/ha.

More balanced values between profit and production cost were obtained for trees of the Pinkcot, Wonder Cot, Spring Blush and Magic Cot varieties, where the level of profitability of apricot production was 188.7; 181.6; 177.5 and 146.3%, respectively. Next, lower profit values were obtained for the varieties Big Red - 114.7%, Orange Red - 109.5%, Sweet Cot - 104.4%. Within the varieties Lilly Cot, Perle Cot, Kioto, Faralia and Farbaly, the lowest level of profitability was recorded, constituting 98.1; 95.8; 89.1; 76.4 and 56.7%, respectively.

CONCLUSIONS

The biological peculiarities of the variety influence the initiation of the flowering period of the trees of the studied varieties, as well as their continuation, until the ripening period of the apricot varieties.

The flowering period of the apricot crop in the spring of 2023 in the southern part of the country with different ripening period took place during 7-8 days.

The fruit ripening of the apricot varieties studied in the reference year in the southern part of the country did not show large deviations from the ripening period, which each variety possesses.

The shape index indicates that the Lilly Cot, Kioto, Perle Cot, Spring Blush, Orange Red and Pinkcot varieties have a round fruit shape, the Big Red, Magic Cot, Sweet Cot, Faralia and Farbaly varieties are spherical, and the Wonder Cot variety elongated spherical.

The atmospheric precipitation that fell in abundance in the months of April (73.6 mm) and May (46.0 mm) in the southern part of the country had a considerable impact on the quality of the fruits of the early ripening apricot varieties (Wonder Cot, Spring Blush, Magic Cot, Pinkcot). Medium and late ripening cultivars had slower fruit development due to insufficient moisture during that period.

The results obtained in the southern part of the country, during 2023, highlight the varieties with extra-early ripening (Wonder Cot, Spring Blush) and early (Magic Cot, Pinkcot), which due to the atmospheric precipitation recorded in the spring period, could form a more competitive quality of fruit and a higher level of profitability was obtained. Varieties with later ripening recorded lower fruit quality, which attracted lower economic efficiency.

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QUALITY PARAMETERS AND ASSESSMENT OF HEALTH RISKS IN PLUM ORCHARDS UNDER VARIOUS MANAGEMENT SYSTEMS IN NORTHEAST ROMANIA

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Abstract

The presence of heavy metals in fruits has become a major concern due to their potential impact on human health. This study aimed to assess the concentration of heavy metals (Ni, As, Cd, Cu, Zn) in plum fruits (Prunus domestica L.) varieties (Tuleu Gras and Carpatin), from "Adamachi" Farm district - Iaşi University of Life Sciences (IULS). The samples of ripe plum fruits were collected from the orchards ecological and conventional management systems from August 2023. The concentrations of metals in plum samples were determined using Flame Atomic Absorption Spectroscopy (fAAS) after wet digestion (HNO_3 65%, H_2O_2, > 30% w/v). Specifically, the concentration (mg/kg fresh weight) range of Cd (0.005-0.01), As (nd), Ni (0.13-0.23), Zn (0.41-0.57) and Cu (0.76- 1.23) did not exceed the safe limits. The results indicated that the order of accumulated elements in plum fruits followed Cu>Zn>Ni>Cd>As. The potential health risks associated with consuming these fruits were evaluated DIM and HRI using the USEPA probabilistic risk assessment model. However, the calculated HRI values for heavy metals were all found <1, indicating that consuming plums from this area does not pose health risks to local consumers. Overall, the findings of this study demonstrate that plum fruit consumption in the study area does not present any health hazard associated with any of the selected heavy metals.

Key words: *quality plum fruits, heavy metals, health risk assessment, pollution.*

INTRODUCTION

The current state of food security and assurance is deeply intertwined with the rising incidence of illnesses in people. Plums, with a production of 12.25 million tons, are ranked as the second most produced stone fruit globally (after peaches and nectarines) (Zezulová et al., 2022), play a significant role in human nutrition, offering essential nutrients that promote health and help prevent various diseases (obesity, diabetes, cancer, cardiovascular, and other chronic diseases) (Nistor et al., 2022).
Plum fruits, are rich in phenolic compounds, and boast higher levels of anthocyanins and antioxidants compared to apples, oranges, and strawberries (Leong and Shui, 2002; Kim et al., 2003; Nisar et al., 2015).
These bioactive components, particularly anthocyanins, not only provide the vibrangt hues of plums but also contribute to a positive impact on human health due to phenolic compounds with their antioxidant properties (Kayano et al., 2002; Scutarașu et al., 2024).
However, concerns arise regarding heavy metal contamination in the environment, posing risks to public health due to its adverse effects on organs and the potential for accumulation in the food chain. Sources of heavy metal pollution encompass agricultural and industrial activities, vehicular emissions, and agrochemical usage, necessitating stringent measures to mitigate these risks and safeguard food safety (Jadaa and Mohammed, 2023).
Acknowledging the significance of understanding pollution levels, quality status, and health risks posed by heavy metals in plum fruits, the primary objectives of this study were to (1) assess plum fruit quality parameters in ecological and conventional systems, (2) determine the levels of heavy metals (Ni, As, Cd, Cu, Zn) in plum fruits, and (3) evaluating

potential human health risks in terms of daily intake of metals (DIM) and health risk index (HRI) for adults.
In conclusion, the relationship between heavy metals and the proprieties phytochemical of plum fruit is essential for maintaining human health.

MATERIALS AND METHODS

The study was conducted on plums (*Prunus domestica* L.) orchard at Horticultural "Adamachi" Farm - IULS. Geographically, the city is located in northeastern Romania, at 47°10'24"north latitude and 25°15'3" east longitude. "Adamachi" Farm benefits from a favourable climate and fertile soil classified as aric-cambic chernozem with texture loamy-clay (80%). During Apr.-Sept. 2023, environmental conditions were favourable for the growth of *Prunus domestica* L. Compared to the data of 2022, the average temperature showed a slight increase from 18.54˚C to 18.9˚C. As for precipitation, it decreased significantly from 340.4 mm in 2022 to 246.1 mm in 2023.
The *Prunus domestica* L. orchard, spanning 5500 m^2, was established in autumn 2014 and is separated into buffer strips that are 5 m wide. Plots under study are spaced 200 m apart to prevent interference caused by synthetic inputs.
Methods. In August 2023, soil and mature fruit samples (varieties Tuleu Gras and Carpatin) were collected with precision. Each conventional (Cv) and ecological management system (Eco) was represented by three areas, with five designated points each, with each area comprising 30 trees, creating composite samples that were meticulously stored in polyethylene bags. Soil samples (90) were collected using a shovel and hand drill, and plant matter and stones were removed. Ninety fruit samples were collected, washed with ultrapure water to remove contaminants and dried at room temperature. Fruits were stored at -20°C until analysis. Conventional methods involve the rationale of pesticides and approved fungicides, adhering to regulatory thresholds. Simultaneously, ecological practices, such as manually removing weeds and shallow tillage around tree trunks, enhance soil and plum fruit health. Disease control includes the incorporation of copper-based fungicides in compliance with ecological production guidelines.
The soil's chemical properties were evaluated employing standard techniques. pH measurement was conducted with a pH meter using a glass electrode with a soil-to-water ratio of 1:2.5 w/v, while the total organic carbon (TOC) content was determined utilizing the Walkley-Black method (Calistru et al., 2024). Soil organic matter (SOM) was calculated by multiplying the TOC percentage by the globally recognized Van Bemmelen factor of 1.724 (Heaton et al., 2016). The extraction of available potassium (K) and phosphorus (P) was performed using neutral 1N NH_4OAc, and their quantities were determined following the analytical procedures outlined by ICPA Bucharest (Țopa et al., 2021).
Acid digestion of soil and fruit samples for heavy metals follows defined protocols, such as USEPA method 3052. Soil samples are treated with a blend of nitric acid (HNO_3) and hydrochloric acid (HCl), whereas fruit samples undergo treatment with HNO_3 and hydrogen peroxide (H_2O_2). Post-digestion in the microwave, solutions are filtered to a final volume (50 mL) using ultrapure water. Concentrations of heavy metals are analysed through Atomic Absorption Spectrometry (AAS), adhering to specific parameters in Table 1.

Table 1. Operating parameters AAS for analysis

Parameters	Setting
Elements	Cu, Zn, Ni, Cd, As
Replicates	3
Measurement	Absorbance
Flame	Acetylene (C_2H_2) - Air
Burner height	5-9 (nm)
Fuel Flow (L/h)	25-40 (L/h)

Physical-chemical and phytochemical determinations for plum quality parameters. Fruit weight was ascertained through the weighing of all sampled fruits using an analytical balance (d = 0.1 mg), followed by the calculation of the average fruit weight (g/fruit). Fruit firmness was evaluated employing a Qualitest HPE non-destructive penetrometer. The colour of the samples was measured using a Konica Minolta colorimeter (model CR-410, Tokyo, Japan) following the method described by Pinto et al. (2024). The uniform colour space CIEL*a*b* coordinates were obtained through this device. To measure the pH of the fruits, the pH metre (WTW InoLab, Xylem Analytics GmbH, Weilheim, Germany) was used, which was calibrated before the pH measurement using buffer solutions with pH values of 4 and 7 (Rațu et al., 2023). The acidity was measured using a titrimetric method with phenolphthalein as an indicator. Homogenized fruit mixed with water was filtered, and the filtrate was titrated with NaOH (0.1 mol/L) to determine acidity.

Using BIOBASE BK-FD10T equipment from Jinan, China, plum fruits were freeze-dried for 50 hours at a temperature of -42°C and a pressure of 0.10 mBar to analyse the total content of anthocyanins, flavonoids, polyphenols and the antioxidant activity. After this process the samples were ground using MC 12 equipment (Stephan, Germany) to a fine powder. Before being analysed, these samples were stored in glass jars at room temperature and in the dark. A UV lamp was used to sterilize the finished powder to eliminate contaminants.

Reagents are sourced from Sigma Aldrich Steinheim (Darmstadt, Germany): Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), citric acid (1%), C_2H_5OH, Gallic acid, NaOH, KCl solution, $NaNO_2$, CH_3COONa, $AlCl_3$ and Na_2CO_3.

The method for extracting bioactive compounds from plum powders involves the use of ultrasound, as described by Lima et al. (2017), with minor adjustments. In this process, 1 g of plum powder was dissolved in 9 mL of 70% ethanol, acidified with a 1% citric acid solution (acid: solvent, 1: 8, v/v). The mixture was then subjected to treatment sonication (Elmasonic S 180 H) for 40 minutes at 40 kHz and 25°C. After sonication, samples were centrifuged at 6000 rpm and 4°C for 10 minutes, and the resulting supernatant was analysed for phytochemical content (Lipșa et al., 2024).

The total flavonoid content in plum powder extract was determined using the aluminium chloride method. Briefly, plum powder extract (250 µL) was mixed with sodium nitrite (75 µL) and distilled water (2 mL), followed by the addition of aluminium chloride (150 µL) after 5 minutes and sodium hydroxide (0.5 mL) after 6 minutes. The mixture was then measured at 510 nm using a UV–VIS spectrophotometer (Analytik Jena - Specord 210 Plus, Germany). Catechin was used as a standard for the calibration curve, and the results were reported as milligrams of catechin equivalents per 100 grams of dry weight (mg CE/g d.w.) (Horincar et al., 2019).

The total polyphenolic content of the plum powder extract was measured using the Folin-Ciocalteu method. In summary, 200 µL of the extract was mixed with 1 mL of the Folin-Ciocalteu reagent and 15.8 mL of distilled water. After 10 minutes, 3 mL of Na_2CO_3 20% was added, and the mixture was stored in the dark for 60 minutes at room temperature. The absorbance was measured at 765 nm using a UV-VIS spectrophotometer. Results were calculated in mg of Gallic acid equivalents per gram of dry weight (mg GAE/g d.w.) using a standard curve for Gallic acid (Sadiq et al., 2015).

The total antioxidant activity of the samples was tested using the DPPH method. Diluted extract (100 µL) was mixed with DPPH solution (3.9 mL), shaken for 30 seconds, then left for 30 minutes at room temperature. Absorbance at 515 nm was measured using a UV-Vis spectrophotometer. Methanol (100 µL) was used as a blank with DPPH. Results were expressed as µmol Trolox equivalent per gram of dry weight (µmol TE/g d.w.), determined by a Trolox standard curve (R^2 = 0.993) (Stoica et al., 2024).

The plum extract's anthocyanin content was analyzed using a UV-Vis spectrophotometer with diluted samples (1: 10) following the pH differential method. Plum sample (200 µL) was mixed with buffer solutions at pH 1.0 and 4.5 (800 µL) in a spectrophotometer cuvette. After 15 minutes without light exposure, absorbance was measured at 520 and 700 nm wavelengths. Anthocyanin content was quantified as mg cyanidin 3-glucoside equivalents (C3G)/100 g dry weight (dw) (Meyers et al., 2003).

- The assessment of the potential health hazards posed to humans by the consumption of plum fruits contaminated with metals was conducted using dietary intake of metals (DIM) and health risk index (HRI). If the calculated HRI value is <1, the exposed population faces minimal risk, whereas a value ≥1 signifies a risk to human health (Einolghozati et al., 2023). DIM aids in assessing the daily exposure levels to heavy metals ingested by humans (AL-Huqail et al., 2023).), and is calculated using the formula: $DIM = (IR \times C_{hv} \times C_{factor})/B_w$, where [IR] refers to plum fruit ingestion rate (0.345 kg/person/day), [C_{hv}] represents the concentration of heavy metal in fruit sample (mg/kg) [C_{factor}] signifies the conversion factor (0.085) and [B_w]symbolizes the body mass (male - 67 kg; female - 62.3 kg) (Ezez et al., 2023).

The Health Risk Index (HRI) is determined through the division of the DIM for each specific heavy metal by its corresponding reference dose (R_fD). The RfD values for Cd, Cu, Ni and Zn are 0.001, 0.040, 0.020, and 0.300 mg/kg/day, respectively (USEPA). The calculation of HRI is performed using Equation: $HRI = DIM/R_fD$.

The data presented in this study consist of mean values, obtained from triplicate analyses, along with the standard deviation of the mean. Statistical data processing was done using IBM SPSS v26 software and the Duncan test was used to determine significance at a level of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Chemical soil properties. The results indicate that the soil within the Eco and Cv systems was characterized as possessing neutral pH levels with medium to high fertility and very good availability of macro elements (Table 2). *Prunus domestica* L. demonstrates excellent adaptability to the characteristics of this soil type.

Table 2. Selective chemical soil properties in Aug.

Chemical properties / Depth (cm)	Management system	
	Cv	Eco
	0-20 cm	
pH	7.10±0.21a	7.16±0.11a
C_{org} (%)	2.11±0.25a	2.43±0.19a
Humus (%)	3.64±0.17a	4.19±0.12b
N_t (%)	0.19±0.04a	0.22±0.06a
P (ppm)	97.7±0.34a	82.6±0.41b
K (ppm)	421.5±0.63a	409.2±0.54b
Zn (mg/kg)	65.82±0.39a	49.72±0.41b
Cu (mg/kg)	88.13±0.36a	114.83±0.49b
Ni (mg/kg)	23.21±0.18a	16.42±0.22b
Cd (mg/kg)	0.37±0.07a	0.17±0.05b
As (mg/kg)	1.43±0.09a	1.47±0.10a

[1]Means associated with different letters exhibit significant differences, as determined by Tukey's test at a significance level of $p < 0.05$; [2]Eco-ecological; Cv-conventional;

The rank order of heavy metal concentrations (mg/kg) is: Cu > Zn > Ni > As > Cd, with values 88.13 > 65.82 >23.21 > 1.43 > 0.37 for Cv, 114.83 > 49.72 > 16.42 > 1.47 > 0.17 for Eco (Table 2). The maximum permissible limits according to WHO/FAO, 2023 are: Zn (200 mg/kg), Cu (100 mg/kg), Ni (50 mg/kg), Cd (1 mg/kg), As (5 mg/kg). All values are below the maximum permissible limit except for Cu in the ecological system (114.83 mg/kg), but they did not exceed the intervention limit (200 mg/kg). This higher concentration of Cu in the ecological system results from using antifungal treatments with copper-based products, in recommended doses to maintain productivity and care for the orchard, and to minimize the risk of contaminating the ecosystem with contaminants. The bioavailability of heavy metals is mainly influenced by soil physicochemical properties. The higher content of organic matter (4.19%) at a 0-20 cm depth can essentially impact metal behaviour by binding with toxic metals, increasing metal toxicity. The availability and mobility of heavy metals are influenced by pH. Heavy metals in low-pH soil tend to be more mobile than in soil with higher pH (Sintorini et al., 2021). Numerous studies have investigated the impact of different cultivation practices on the physico-chemical properties of plum fruit, including plant nutrition, soil fertility, and sensory evaluation of the fruits (Walkowiak-Tomczak, 2008; Usenik, 2009; Rop, 2009; Akšić et al., 2023). Ecological orchards have been found to exhibit higher levels of soil organic matter and improved soil biological functions, providing superior support for ecosystem function regulation compared to conventional orchards (Ionica et al., 2013).

Plum quality parameters. Table 3 shows the attributes of two varieties of fruit (fruit mass, pulp firmness, titratable acidity (TA), pH, and colour parameters), as well as the phytochemical characteristics of the "Tuleu Gras" and "Carpatin" plum varieties in Eco and Cv systems. The variety "Carpatin" has larger fruit sizes than the variety "Tuleul Gras", but their weight was more significant in the Cv system for both varieties. According to Mditshwa et al. (2017), Cv-grown plums are larger and have a more uniform appearance. However, there are no significant differences in colour between the two types of management, although eco plums show a more consistent and vigorous colour. "Tuleu Gras" variety in the Eco system management was distinguished by an intense reddish colour (a*=10.27 ± 0.08) which can be attributed to the pigments (anthocyanins) present in the plum pulp.

Table 3. The quality parameters of plum fruits

Parameters		Tuleu Gras		Carpatin	
		Cv	Eco	Cv	Eco
Weight (g)		41.56± 0.49b	38.31 ± 0.55d	47.66±0.58a	43.21±0.51c
Pulp firmness (units HPE) (N/0.10 cm^2)		63.43 ±0.92a	60.12 ± 1.01b	65.71±0.99a	64.24±0.94a
pH		3.65± 0.40a	3.81± 0.41a	4.16±0.54a	4.04±0.56a
Titratable Acidity (%)		1.72± 0.15a	1.27 ± 0.13b	0.87±0.09c	0.81±0.08c
Antioxidant activity	μM Trolox/g dw	21.93 ± 0.40a	22.10 ± 0.33a	20.60 ± 0.25b	21.90 ± 0.28a
	Inhibition %	90.62 ± 1.59a	91.29 ± 0.69a	89.78 ± 0.39b	90.19 ± 0.43a
Total polyphenols (mg GAE/g dw)		3.72 ± 0.09ab	3.89 ± 0.12a	3.37 ± 0.08c	3.59 ± 0.11bc
Total flavonoids (mg CE/g dw)		1.36 ± 0.04b	1.45 ± 0.03a	1.19 ± 0.02d	1.28 ± 0.02c
Total anthocyanins (mg C_3G/100 g dw)		5.03 ± 0.14ab	5.17 ± 0.10a	4.68 ± 0.08c	4.86 ± 0.09bc
Colorimetric parameters	**L***	52.21 ± 0.65b	53.36 ± 0.80a	51.78 ± 0.70b	52.06 ± 0.74b
	a*	9.14 ± 0.16b	10.27 ± 0.08a	8.86 ± 0.06b	9.04 ± 0.07b
	b*	15.33 ± 0.37b	16.47 ± 0.52a	15.07 ± 0.30b	15.21 ± 0.32b

Means associated with different letters exhibit significant differences, as determined by Tukey's test at a significance level of $p < 0.05$.

Also, eco plums have firmer flesh than Cv-grown plums, and smaller sizes may contribute to this increased firmness through reduced water accumulation or a denser mesocarp (Hassan et al., 2021). Studies show that organic plums produce firmer flesh and a slightly different texture than Cv-grown plums. Compared to the variety 'Carpatin' (0.87 ± 0.09%), TA in the variety 'Tuleu Gras' was significantly higher (1.72 ± 0.15%). Regarding the variations between the management systems, the acidity levels were the highest for the "Tuleu Gras" variety in the conventional system and the lowest in the Eco system. Instead, the variety "Carpatin" recorded the lowest level of acidity (0.81 ± 0.08%). The acidity of cultivars *Prunus salicina* L. revealed a wide range of acidity levels, from 0.31 to 0.55% (Rahimi et al., 2005), and even as high as 0.9% (Martínez-Esplá et al., 2014). The fruits of the "Stanley" *Prunus domestica* L. grafted on rootstocks of *Prunus cerasifera* L. showed acidity levels between 0.45% and 0.67% (Saridaş et al., 2016). The total acidity of plums (*Prunus cerasifera* Ehrh.) from the Mediterranean region varies significantly, between 0.72 and 1.81% (Ayanoğlu et al., 2007). These variations in acidity are influenced by factors such as fruit variety, level of maturity, and growing conditions. The preservation of food and products largely depends on titratable acidity and pH values (Da Silva et al., 2016). An inverse relationship was found between TA and pH values for all levels, as indicated in Table 3.

Eco plums displayed higher antioxidant capacity and polyphenol, flavonoid, and anthocyanin content than conventional plums (Table 3). The same results were obtained in a study of 30 Eco vs. Cv plum varieties by Cuevas et al. (2015). Another study is in agreement with our data on anthocyanin and polyphenol content (Cordova and Watson, 2010). Similar results were also found in the research by Arion et al. (2014), although they concluded that autumn compared to summer harvested plums contained higher phenolic content. However, in other fruit species, higher antioxidant contents were found in medium to late-ripening fruits compared to earlier ripening fruits, which were related to higher temperatures and light intensity (Drogoudi et al., 2017; Sarıdaş et al., 2022). Ecological systems of cultivation are linked to a certain level of stress (restricted and limited use of pesticides and fertilizers), which could lead to the accumulation of secondary metabolites responsible for plant defence (Raigón et al., 2010; Oliveira et al., 2013). The most important secondary metabolites in plums are polyphenols (Kim et al., 2003), whose content typically increases under stress conditions, as observed in the analysed samples.

The total polyphenol content of the two plum varieties ranged from 3.37-3.89 mg GAE/g dw, and the anthocyanin content ranged from 4.68-5.0 mg C3G/100 g dw (Table 3). The total polyphenolic content determined in the present study was higher than the range of 174-375 mg GAE 100 g^{-1} fresh weight determined by Kim et al. (2003) for 6 plum varieties grown in New York. This may be due in part to the different extraction protocols used by Kim et al. (2003) (80% MeOH with ultrasound-assisted extraction), which can extract more phenols than stirring-based methods (Aboshora et al., 2014; Musa et al., 2011).

Most of the plum varieties studied in this study had lower levels of anthocyanins compared to the average of 17 mg 100 g^{-1} found by González-Flores et al. (2011), who quantified anthocyanins using HPLC on the Japanese plum variety 'Crimson Globe' (*Prunus salicina*). Compared to common European plums, which were used in this study, Japanese plums typically have higher concentrations of anthocyanins (Fanning et al., 2014), which is the most likely explanation for this difference.

Table 4 presents the results of heavy metal contents: zinc (Zn), arsenic (As), copper (Cu), nickel (Ni), and cadmium (Cd), in plum fruit samples.

Table 4. The influence of plum variety on fruit heavy metals concentrations

Heavy metals	Tuleu Gras		Carpatin		M.A.L
	Cv	Eco	Cv	Eco	
Zn (mg/kg)	0.53± 0.11a	0.41± 0.14a	0.57± 0.10a	0.48± 0.19a	0.60
Cu (mg/kg)	0.76± 0.13b	1.23± 0.36a	0.87± 0.48b	1.32± 0.30a	10
Ni (mg/kg)	0.23± 0.08a	0.19± 0.10a	0.16± 0.11a	0.13± 0.09a	10
Cd (mg/kg)	0.009± 0.01a	0.005± 0.01a	0.01± 0.01a	0.007± 0.01a	0.02
As (mg/kg)	nd	nd	nd	nd	0.50

M.A.L. - Maximum permissible limit; Means associated with different letters exhibit significant differences, as determined by Tukey's test at a significance level of $p < 0.05$.

Variations in metal concentrations are observed between the conventional system (Cv) and the ecological system (Eco) for both varieties. The heavy metals concentrations (mg/kg) in plum fruits collected were in the following range: 0.41-0.57 (Zn), 0.76-1.32 (Cu), 0.13-0.23 (Ni) and 0.005-0.01 (Cd). The specified ranges denote the bioaccumulation of heavy metals in the examined fruits, exhibiting the following descending sequence: copper > zinc > nickel > cadmium > arsenic. Similar to the soil results, higher Cu values are found in the ecological system for both types of plums. Noteworthy, all heavy metal concentrations were higher in the 'Tuleu Gras' cultivar than 'Carpatin', emphasizing its metal adsorption and accumulation capabilities. Cu and Zn were the most frequently detected metals in plum fruits, consistent with findings in soil samples.

Based on these metal concentrations, the potential health risks (DIM, and HRI) associated with the consumption of these fruits were used to evaluate the orchard's management practices. The results of DIM and HRI studies given in Table 5 showed that plum fruit samples collected from Iasi showed no significant health concern related to their dietary consumption. Our study found that the DIM values for the heavy metals we examined suggest that the estimated amount of heavy metals consumed by individuals (R_fD) is below acceptable daily limits, providing a safety net against potential hazardous exposure to heavy metals.

Table 5. Assessment of potential risks health

Variety	Systems	Heavy metals	Males		Females	
			DIM (mg/day)	HRI	DIM (mg/day)	HRI
Tuleu Gras	Cv	Zn	2.32^{-4}	7.73^{-4}	2.49^{-4}	8.31^{-4}
		Cu	3.33^{-4}	8.31^{-3}	3.58^{-4}	8.94^{-3}
		Ni	1.01^{-4}	5.05^{-3}	1.08^{-4}	5.41^{-3}
		Cd	3.93^{-6}	3.93^{-3}	4.24^{-6}	4.24^{-3}
	Eco	Zn	1.79^{-4}	5.96^{-4}	1.92^{-4}	6.43^{-4}
		Cu	5.38^{-4}	1.35^{-2}	5.79^{-4}	1.45^{-2}
		Ni	8.31^{-5}	4.15^{-3}	8.94^{-5}	4.47^{-3}
		Cd	2.19^{-6}	2.19^{-3}	2.35^{-6}	2.35^{-3}
Carpatin	Cv	Zn	2.49^{-4}	8.31^{-4}	2.68^{-4}	8.94^{-4}
		Cu	3.81^{-4}	9.53^{-3}	4.09^{-4}	1.02^{-2}
		Ni	7.00^{-5}	3.50^{-3}	7.53^{-5}	3.77^{-3}
		Cd	4.38^{-6}	4.38^{-3}	4.71^{-6}	4.71^{-3}
	Eco	Zn	2.10^{-4}	7.01^{-4}	2.26^{-4}	7.53^{-4}
		Cu	5.78^{-4}	1.44^{-2}	6.21^{-4}	1.55^{-2}
		Ni	5.69^{-5}	2.84^{-3}	6.11^{-5}	3.06^{-3}
		Cd	3.06^{-6}	3.06^{-3}	3.29^{-6}	3.29^{-3}

Moreover, the calculated HRI values for these heavy metals were consistently below 1, reaffirming that there are no significant health effects from the consumption of plum fruit. This means that in all samples, the level of exposure did not exceed the safe limit, making it highly unlikely to cause any adverse health impact.

CONCLUSIONS

The results of the present study demonstrated that plum fruit samples harvested from orchards in the "Adamachi" Farm - Iaşi University of Life Sciences (IULS), Romania, have benefits for human health due to their phytochemical content. According to the study results, the cultivar 'Tuleu Gras' in Ecological systems of cultivation exhibited elevated levels of total phenolics content, flavonoids, anthocyanins, and antioxidant capacity. The analysis revealed that concentrations of heavy metals (Cd, Cu, Ni and Zn) did not exceed safe limits, according to health risk studies. The decreasing order of heavy metal concentrations in plum fruit was observed as follows: Cu < Zn < Ni < Cd < As. Furthermore, it was found that sampling sites in the ecological system showed higher levels of Cu than those in the conventional system due to the use of ecological-based Cu treatments.

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PEACH RESPONSE TO WATER DEFICIT UNDER THE CLIMATIC CONDITIONS OF SOUTH-EASTERN ROMANIA

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Abstract

In arid and semi-arid regions, the research and application of new irrigation techniques that economize water without altering tree performance and fruit quality is a challenge. In the present work, the impact of water deficit irrigation applied to peach trees was evaluated. In the context of global warming, saving water is a major goal. The studied crop was peach, Catherine sel. 1 cultivar, fourteen years old, grafted on rootstock Tomis 1. The planting distance was 4 m between rows and 3 m between trees in the row. The split-plot experiment described here is monofactorial, with the irrigation strategy having three gradations. The irrigation regime consists of a fully irrigated treatment T1 (100% ETc), a deficit irrigation treatment (T2), irrigated with half the amount of water in T1 (50% ETc), and a control, non-irrigated treatment (T3). The paper describes the quality of fruits for three years of study, 2020, 2021 and 2022, and 2022, respectively, in the semi-arid region of Dobrogea, Romania. The study suggests that moderate water stress can be profitable for enhancing key fruit quality characteristics.

Key words: *Prunus persica (L.) Batsch, irrigation, soil water potential, quality fruit*

INTRODUCTION

Knowing exactly when and how much to irrigate is essential to attaining sustainable and environmentally sound water management since water is a valuable and expensive natural resource. Reduced total precipitation and altered seasonal distribution are predicted by climate change scenarios, which will make the problem of water scarcity for agricultural use worse (Ondrasek, 2014). An irrigation technique known as deficit irrigation (DI) involves irrigating a crop with less water than necessary for the best possible plant growth. It lowers the amount of water needed to irrigate crops, enhances how well plants respond to a certain water shortage, and either lowers irrigation requirements or boosts crop water use efficiency (Chai et al., 2016). The effects of DI on fruit quality depend on the intensity and duration of the water stress period and on the sequence in which the water deficits occur, as well as on the cultivar (Castel & Buj, 1990). Significant water savings with no impact on harvested yield quantity and quality, increased crop water productivity and farm profitability, and enhanced environmental protection are potential benefits of DI approaches (Geerts & Raes, 2009; Ruiz-Sánchez et al., 2010). Deficit irrigation will become more widely used as water resources become increasingly scarce, especially in regions with limited water supplies (Aragues et al., 2014). Although it originated in the Middle East (Persia or China), the peach (*Prunus persica* (L.) Batsch) tree is now grown in every region with a temperate climate (Chavez et al., 2014). Due to the fruits appreciated by the customers, peaches are among the most significant fruit species in the world. Peaches are a rich source of minerals and vitamins and contain a good amount of sugar (Pakbin et al., 2014). Although peaches are very popular in Romania, their climate-related favorability is rather limited. The Dobrogea zone of Romania is more appealing than other parts of Romania because of its favorable environment, with winter temperatures that are not too low. It also has suitable soils (chernozem mostly), and irrigation can solve water deficiency. The purpose of this work was to study and show the effects of moderate water deficit on fruit quality of mature, Catherine sel. 1 cultivar under drip irrigation.

MATERIALS AND METHODS

Climate and soil conditions

The orchard under study is situated in Agigea, Dobrogea, Romania, at latitude 44º05' North and longitude 28º37' East. The experimental plot is located around two kilometers from the Black Sea and has an average elevation of 30 meters. With an average yearly air temperature of 12.0°C, an average yearly precipitation of 498.7 mm, and a climatic water deficit of around -405 mm (Paltineanu et al., 2007), this region can be described as semi-arid (Paltineanu et al., 2016; Septar et al., 2022). An automatic weather station was used to record the climate data (iMetos, IMT 300, Pessl Instruments, Austria) with a 1-h time-step. With an alkaline pH in the topsoil and a loamy texture, the soil is a calcareous chernozem with good soil structure (0-60 cm depth, with 27-32% g/g clay content, 1.6-2.8% g/g humus content, 1.5-6.8% g/g carbonate content), while in the non-structured subsoil, the humus content is lower than 1% g/g and the carbonates from 9 to 14% g/g; land slope is between 2.0 and 2.5% (Paltineanu et al., 2011).
According to Indreias (1997), the average tree rooting depth was 80 cm, and the soil's field capacity and wilting point values were 0.300 and 0.125 cm^3 cm^{-3}, respectively.

Experimental design and Irrigation Application

This split-plot study was mono-factorial and employed three distinct watering treatments. Peach trees (*Prunus persica* (L.) Batsch) were used for this study since they are characteristic of the region. The biological material was Catherine sel.1, a peach cultivar registered in Romania in 2001 at RSFG Constanţa (Figure 1).

Figure 1. Catherine sel. 1 cultivar

The tree is standard, medium vigor, and consistently productive, it is a clingstone cultivar. Its fruit is big, orange with attractive red coloring, with orange, firm, flavourful, and very sweet flesh (Dumitru et al., 2013).
Three seasons in a row (2020, 2021, and 2022) were used for the study. In the spring of 2006, the 4 m × 3 m scheme was used to plant the fruit trees. The plots under study consisted of three consecutive rows of fruit trees, with three trees in the center row designated for measurements and observations. The canopy of the trees was designed like a conventional vase, and clean cultivation was the method of soil management applied both within and between tree rows. As previously reported by Paltineanu et al. (2007) for the area, the irrigation regime consisted of a fully irrigated treatment (T1) watered in accordance with the irrigation needs (100% of ETc = ETo x Kc, Penman-Monteith method, Allen et al., 1998), a deficit irrigation treatment (T2) irrigated with half the amount of water of T1 (50% of ETc), and a control, non-irrigated treatment (T3). Irrigation was provided in T1 when the soil water content (SWC) approached the mid-interval between field capacity (FC) and wilting point (WP). Drip irrigation was employed as the watering technique. There was a 0.6 m dripper spacing and a 2.0 l/h dripper discharge.
The irrigation season ran from May to August in 2020 and from June to August in 2021. Six treatments (20 mm in T1 and 10 mm in T2) were used during the dry period of 2020, with 120 mm in T1 and 60 mm in T2. With 20 mm in T1 and 10 mm in T2, we only used four irrigations in 2021, with a total of 80 mm in T1 and 40 mm in T2. In T3, no water was used. T1 irrigation schedule took the weather forecast into account in addition to ETc and SWC dynamics.
Because only one-fourth of the orchard area received irrigation water, the fruit tree rows displayed a wetted bulb about 1 m wide at the soil surface after irrigation application. The water depth was estimated to be equivalent to 80 mm for the tree rows.

Soil water content measurements

Every week, the soil water potential (SWP) of each fruit tree was measured using Watermark resistance blocks (6450 Watermark Soil Moisture Sensor) positioned at four different depths of 20, 40, 60, and 80 cm, and 150 cm from the tree trunks. The sensors were

positioned at 45° angles below the horizontal on the same vertical line, following the Paltineanu and Howse (1999) methodology.
WatchDog dataloggers (DataLogger, WatchDog Model 1650, Spectrum Technologies, USA) were used to record the data. Previous field data research has established correlations between gravimetrically measured SWC and SWP detected by Watermark sensors (Paltineanu et al., 2011). During the experiment, these relationships were then employed to convert soil water matric potential data into SWC values.

Assessed parameters

An average of fifteen fruits per treatment were evaluated annually. After harvest, fruit height and longitudinal and transversal diameter were measured to track fruit growth. A metric digital caliper (Insize Co., Ltd. China) was used to make the measurements. The average weight of the fruit resulted from weighing ten fruits per treatment and dividing the total weight by the number of weighed fruits. The fruit was weighed by using a precision balance (Kern & Sohn GmbH, Germany). The fruits from the experimental plot were harvested from the 11th to the 12th of August in 2020, the 2nd to the 3rd of August in 2021, and the 3rd to the 4th of August in 2022.

Data analyses

For the analysis of variance and other calculations of fruit quality attributes, SPSS 14.0 software and Microsoft Office Excel were used. The graphs' difference letters indicate significant variations with a probability (P) ≤ 0.05, based on Duncan's multiple comparison test.

RESULTS AND DISCUSSIONS

Climate conditions

During the growing season of the studied period, the average maximum and minimum air temperatures were 26.1 and 12.4°C, respectively, in contrast to the long-term average maximum and minimum air temperatures of 23.1 and 13.8°C. The long-term average air temperature was 18.7°C, while the study period average was 19.0°C (Figure 2). The growing season had a mean annual precipitation of 252.1 mm, slightly lower than the long-term total of 277.5 mm, and a mean annual reference evapotranspiration of 733.2 mm, slightly higher than the long-term total of 722.7 mm. These data indicate a relatively normal period for precipitation and evapotranspiration (Figure 3).

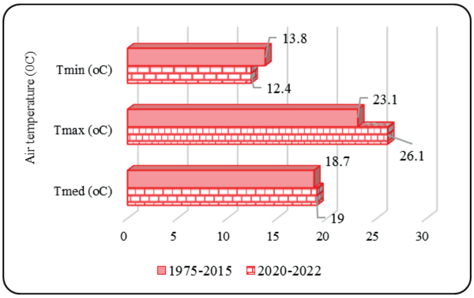

Figure 2. The mean air temperature, maximum and minimum air temperature for the growing season 2020-2022 compared to long-term data, 1975-2015, Agigea, Romania

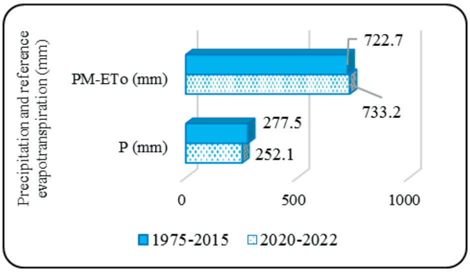

Figure 3. Precipitation and references evapotranspiration for the growing season 2020-2022 compared to long-term data, 1975-2015, Agigea, Romania

Soil water content (SWC)

Following the implementation of the six irrigations, Figure 4 illustrates the dynamics of the soil water content in 2020. Consequently, the values of SWC oscillated between FC and MAD (management permitted deficit, mid-interval between FC and WP), in the irrigated treatments. In the end of vegetation period, in the control treatment (T3) the SWC values approached to WP.
Following the application of the four waterings in 2021, the dynamics of the water content in the soil is illustrated in Figure 5. Without the values from the last watering in the T2 treatment, which were situated in the interval between MAD and WP, with values near to MAD, it was shown that the SWC values in T1 and T2 were between the interval of FC and MAD. In the control treatment (T3), the SWC values are situated in

the interval between MAD and WP due to the dry year.

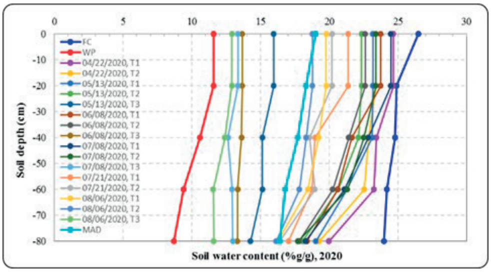

Figure 4. Soil water content in the treatments studied, Agigea village, Romania - 2020

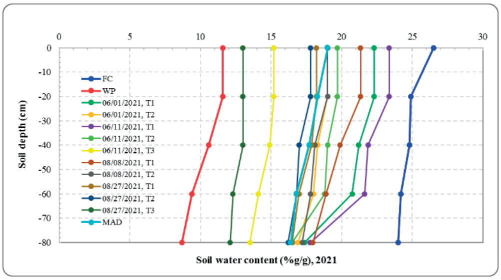

Figure 5. Soil water content in the treatments studied, Agigea village, Romania - 2021

Evaluations of peach fruit quality

Following harvesting, the experiment's fruits underwent laboratory analysis to determine their weight and biometric measures, respectively. The values that are displayed are the three years' worth of study's mean values. Fruit weight measured on the fruits of the under study showed that in the fully irrigated treatment (T1) had the highest value (182.9 g) and the non-irrigated treatment (T3) had the lowest value (112.0 g). Figure 6 illustrates significant differences in fruit weight on the treatments under studied.

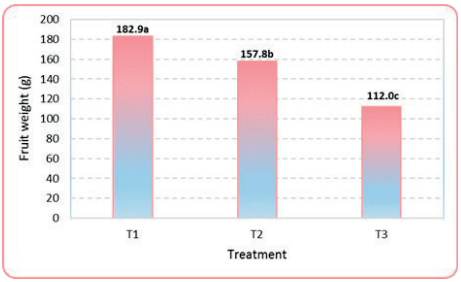

Figure 6. Fruit weight of the fruits to Catherine sel. 1 cultivar, 2020-2022

The longitudinal diameter of the peach fruits to Catherine Sel. I cultivar varied from 59.25 mm to 72.74 mm. The shortest longitudinal diameter can be observed in the control treatment (T3). Figure 7 illustrates significant variations between the researched treatments regarding the longitudinal diameter of the fruit, with probability (P) ≤ 0.05 using Duncan's multiple comparison test. In the case of the transverse diameter of the fruits, applying the watering treatments resulted in a trajectory similar to the longitudinal diameter of the fruits. The highest value regarding the transverse diameter of the fruits was obtained in the fully irrigated treatment (T1) and was 67.9 mm, while in the control treatment (T3), the lowest value of the transverse diameter was 56.1 mm, respectively. The significant differences in terms of transversal diameter of the fruits between the treatments under study are indicated by different letters in Figure 8.

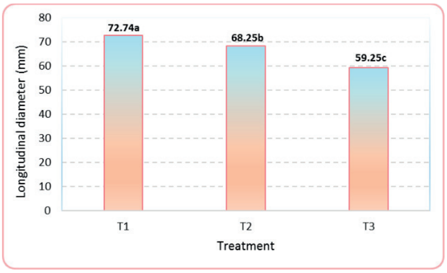

Figure 7. Longitudinal diameter of the fruits to Catherine sel. 1 cultivar, 2020-2022

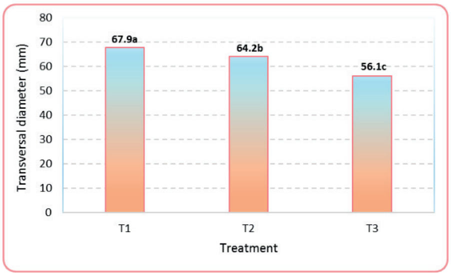

Figure 8. Transversal diameter of the fruits to Catherine sel. 1 cultivar, 2020-2022

The height of the fruits as indicated by the researched treatments showed a similar pattern. The height of the peach fruits oscillated from 52.6 mm to 63.8 mm. The significant variations in fruit height, following the researched treatments, are shown in figure 9.

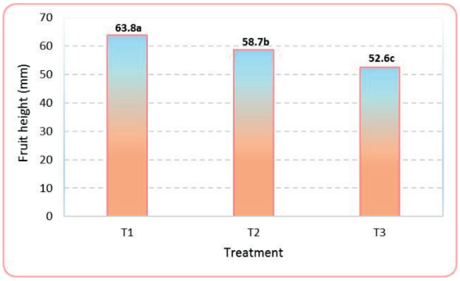

Figure 9. Fruit height of the fruits to Catherine sel. 1 cultivar, 2020-2022

CONCLUSIONS

The fully irrigated treatment (T1) increases fruit quality. However, the use of full irrigation treatment is not a unique option as water use becomes increasingly constrained due to global warming. As a result, deficit irrigation is recommended, taking into account that the soil water content (SWC) does not reach the wilting point (WP) in all horizons, at the same time.

Because drip irrigation delivers water directly to the target, producers may be able to conserve more water.

Effective water management, fruit production protection, and reduced water stress can all be achieved with sustainable practices and the appropriate strategy.

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SOUR CHERRY GERMPLASM RESOURCES AND BREEDING IN ROMANIA

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Abstract

In Romania, after 1970, identification, conservation and evaluation of fruit genetic resources activities were started in order to limit the loss of the biodiversity due to erosion and genetic vulnerability. Regarding the sour cherry germplasm, there is a rich fund, located in Research Institute for Fruit Growing Pitești, Romania with 170 accessions, representing wild species, local population, cultivars and selections. In the breeding work de main objectives are: self fertility, productivity, tolerance / resistance to diseases, fruit quality for fresh market, ripening season extension. Taking into account these objectives, over time were used different genitors from genetic resources fund: 'Timpurii de Cluj', 'Timpurii de Pitești', 'Țarina', 'Bucovina', 'Scuturător', 'Amada', 'Stelar', 'Dropia', 'Ilva' for resistance/tolerance to Monilia and anthracnose; 'Pitic', 'Bucovina', 'Vrâncean', 'De Botoșani', 'Rival', 'Amada' for late blooming; 'Timpurii de Cluj', 'Crișana 2', 'Sătmărean', De Botoșani', 'Rival', 'Amada', 'Stelar' for fruit quality; 'Rival, 'Țarina', 'Stelar' for productivity; Ilva', 'Nana', ' Vrâncean', 'Bucovina', 'Sătmărean', 'Pitic' for self fertility. Using a different methods (selection, crossing, open pollination) 19 cultivars were registered with a very good agrobiological characteristics, many of them are propagated and spread in the Romanian orchards ('Timpurii de Pitești', 'Țarina', 'Ilva', 'Pitic', 'Bucovina', 'Vrâncean', 'De Botoșani', 'Rival').

Key words*: sour cherry, germplasm, breeding, genitors, cultivars.*

INTRODUCTION

In Romania, the area cultivated with sour cherry in 2022 was 2,570 ha, which ensured a production of 28,970 tons (FAOSTAT, 2024). This fruits production ranks Romania on the seven places in Europe, after Russia, Poland, Ukraine, Serbia, Hungary and Belarus. The sour cherry culture is spread in most areas of our country, especially in the hilly area both in industrial plantations and in small areas around households.

Like in the other countries, traditionally, the fruits are used mainly for processing: juices, jam canning, bakery products and spirits (Budan et al., 2005; Schuster, 2019).

The new objectives for sour cherry breeding program, carried out in 11 countries around the world, include excellent fruit characteristics, high production, resistance or tolerance to climatic stress, diseases and pests, mechanical harvesting capacity and the extension of the ripening period (Apostol, 1996; Budan and Gradinariu, 2000; Schuster et al., 2014; Schuster et al., 2017; Schuster, 2019; Zurawicz et al., 2019). Currently, there is a major interest in sour cherry breeding for fresh consumption, with larger fruit size, firmness and good taste (Quero-Garcia et al., 2017; Quero-Garcia, 2019).

Despite the variability, there are no configurations that favorably combine the high frost resistance of the sour cherry and the sugar taste of the sweet cherry. The scientific progress registered in the case of fruit research allows that by using modern biotechnological practices to overcome some limitations of conventional breeding methods, thus achieving the evolution and diversity of the sour cherry variety, improving qualitative and quantitative properties, increasing production and modern technologies (Braniște et al., 2006).

Local and foreign varieties, provide enough genetic material to ensure that the objectives in terms of fruit physical characteristics (color, weight, caliber, the length of the peduncle) and chemical (sugar content and acidity). The sour cherry breeding programs in different countries are related to the performance of the

traditionally varieties grown in the different regions and the new varieties are tested in comparison with the standard ones (Grafe et al., 2009).
Romanian is a country located in South East part of Europe which has good environmental conditions for many fruit species including sour cherry (Butac et al., 2019).
In Romania, after 1970, identification, collection, conservation and evaluation of fruit genetic resources activities were started in order to limit the loss of the biodiversity due to erosion and genetic vulnerability (Butac et al., 2019).
Regarding the sour cherry germplasm, there is a rich fund, located in two centers: Research Institute for Fruit Growing Pitești and Research Station for Fruit Growing Iasi, with over 200 accessions, representing wild species, local population, cultivars and selections.
The genetic resources preserved by *ex situ* methods are very important value and can be use for breeding new cultivars. The success of any breeding program depends on the existence of a rich and valuable germplasm fund (Budan and Gradinariu, 2000).
The aim of this paper is to present a situation of the sour cherry genetic resources from Romania, of their use in the breeding program according to the objectives pursued and of the cultivars registered so far.

MATERIALS AND METHODS

Romanian sour cherry genetic resources have started to be methodically collected since 1967. At present there are a total of 238 sour cherry accessions held in duplicate at the Research Institute for Fruit Growing Pitești-Mărăcineni and Research Station for Fruit Growing Iaşi. Three sour cherry trees per genotype (1 tree = 1 replication) grafted onto 'Mahaleb' seedlings are planted in each location, spaced at 4 x 3 m in Piteşti center and 5 x 4 m in Iaşi center.
Collections contain foreign and autochthonous cultivars, selections, clones, local varieties and landraces. All accessions are evaluated for morphological and biological characteristics as well as agronomic traits according to the IBPGR *Prunus* descriptors updated by the ECP/GR *Prunus* Working Group members (Militaru et al., 2018). At this time, the main objective is to systematize collected data from the two institutions like plant and fruit use, harvest period, blooming time, fruit size, fruit shape, fruit skin color, juice color, fruit taste, fruit cracking susceptibility, susceptibility to diseases and pests.
The main methods used in Romanian breeding program were clonal selection in the landraces 'Crișana', 'Mocănești', 'Oblacinska', control crossing and repeated positive selection in all developing stages of hybrids (Budan et al., 2005; Schuster et al., 2019).

RESULTS AND DISCUSSIONS

Situation of sour cherry genetic resources

In Piteşti and Iaşi centers there are totally 238 accessions, of which: 1 species, 105 autochthonous accessions and 132 foreign cultivars (Table 1). All accessions belong to *Prunus cerasus* L., syn. tart cherry.

Table1. Situation of *ex situ* sour cherry collections

Center	Total accessions	Species	Local accessions	Foreign accessions
RIFG Pitești	145	1	55	89
RSFG Iași	93	0	50	43
Total	**238**	**1**	**105**	**132**

Regarding the genetic resources, one of the Romanian breeder's objectives is to enrich germplasm fund by exchange the biological material with similar institutions, exploring the spontaneous flora and identify valuable genitors for breeding work depending on the objectives pursued.
In Romanian sour cherry breeding program the main objectives are: self-fertility, red fruits (epidermis, pulp, juice) for fresh market, tolerance to specific diseases, upright growth, high productivity, ripening season extension.

Situation of genitors used in the breeding program

Taking into account these objectives, over time in the breeding activity were used different genitors: Nana, Schattenmorelle, Oblacinska, Pitic for high productivity, low vigour, self fertility, early bearing, and frost resistance; Erdi Nogygyumolcsu, Țarina, Timpurii de Osoi, Timpurii de Pitești, Engleze timpurii, Mari timpurii, Meteor Corai for ealy ripening; Scuturător, Crișana, Engleze timpurii, Erdi Nogygyumolcsu, Favorit, Meteor Corai, Țarina, De Botoșani, Granatnaia for fruit quality; Timpurii de Cluj, Spanca, Spaniole, Mari timpurii – for resistance to diseases, etc. (Table 2).

Table 2. Genotypes used in the breeding wotk of sour cherry cultivars

Objectives	Genitors
High productivity	Nana, Schattenmorelle, Oblacinska, Pitic, Meteor, Sumdinka, Breznița, Mocănești 16, Bucovina, Engleze timpurii
Low vigour	Nana, Schattenmorelle, Oblacinska, Pitic, Northstar, Kelleriis 16, Vrâncean
Self-compatibility	Nana, Oblacinska, Schattenmorelle, Bucovina, Meteor, Montmorency, Pitic
Late blooming	Pitic, Schattenmorelle, Vladimirskaia, Sumdinka
Early ripening	Erdi Nogygyumolcsu, Țarina, Timpurii de Osoi, Timpurii de Pitești, Engleze timpurii, Mari timpurii, Meteor Corai
Late ripening	Pitic, Liubskaia, Grossa, Gamba, Pandy 114, De Botoșani, Schattenmorelle, Vladiminskaia
Fruit quality	Scuturător, Crișana, Engleze timpurii, Erdi Nogygyumolcsu, Favorit, Meteor Corai, Țarina, De Botoșani, Granatnaia
Resistance/tolerance to diseases	Timpurii de Cluj, Spanca, Spaniole, Mari timpurii
Early bearing	Oblacinska, Pitic, Northstar, Nana, Schattenmorelle, Țarina
Frost resistance	Schattenmorelle, Oblacinska, Ilva, Pitic, Northstar, Grossa Gamba, Mocănești 16, De Botoșani

Situation of cultivars registered in Romania

In sour cherry breeding program, started 55 years ago, using different methods (controlled hybridization, open pollination, selection of natural clonal populations of Crișana, Mocănești and other landraces), were created 19 new cultivars. Some of these new cultivars have improved characteristics and other did not record positive traits (Table 3). For example, most of cultivars have larger fruits than old cultivars, designated for fresh consumption, early or late ripening and resistance to special diseases: Țarina, Rival, Amada, Stelar, etc.

Similar results were reported by Budan and Butac (2008), Budan et al. (2009), Schuster et al. (2017).

Table 3. Sour cherry cultivars created in Romania between 1967-2023*

No.	Cultivar/year of registration/parents	Genetic gain – trait modified
1	Timpurii de Cluj / 1969 / [(Spaniole x *Pr. fruticosa*) x (Anglaise hative x *Pr. fruticosa*)]	Earliness, large fruit, resistance to diseases
2	Crișana 2 / 1975 / Local selection	Large and dark red fruit, resistance to diseases
3	Mocănești 16 / 1975 / Local selection	Designated for processing
4	Nana / 1977 / Crișana - open pollination	Low vigour
5	Pitic / 1978 / Plodorodnaia Miciurina – open pollination	Lateness, for processing
6	Dropia / 1982 / Vladimirscaia 33/2- open pollination	Resistance to diseases
7	Ilva / 1982 / Local selection	Resistance to diseases
8	Timpurii de Pitești / 1982 / Local selection	Earliness, resistance to diseases
9	Timpurii de Tg. Jiu / 1982 / Local selection	Earliness
10	Țarina / 1984 / Engleze timpurii x Vișin tufă	Earliness, large fruit, for fresh market
11	Bucovina / 1985 / Local selection	Resistance to diseases
12	Scuturător / 1985 / Local selection	Resistance to diseases
13	Vrâncean / 1985 / Local selection	Lateness, large and dark red fruit
14	Timpurii de Osoi / 1990 / Local selection	Earliness, resistance to diseases
15	Sătmărean / 1994 / Engleze timpurii x Vișin tufa	Earliness, large fruit, resistance to diseases
16	De Botoșani / 1994 / Local selection	Lateness, large fruit
17	Rival / 2004 / Griot Moscovski x Nana	Lateness, large and red fruit, for fresh market, resistance to anthracnose
18	Amada / 2005 / Local selection in Suceava area	Lateness, large and dark red fruit, resistance to diseases
19	Stelar / 2008 / Mocănești 16 x Engleze timpurii	Earliness, large fruit, for fresh market, resistance to diseases

Butac et al., 2018

CONCLUSIONS

The success of any breeding program is closely related to the existence of a rich and valuable germplasm fund.

Genetic sour cherry resources preserved *ex situ* offer different and valuable genitors for creating and registering new cultivars.
As a results of breeding activity, 19 new cultivars were registered so far, some of them being widespread in the modern sour cherry orchards from Romania.

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THE INFLUENCE OF INTERANNUAL CLIMATE VARIATION ON THE PHENOLOGY OF SOME WILD FRUIT SPECIES AND THEIR RELATION WITH AVIFAUNA

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Abstract

This paper aimed to investigate the influence of interannual climate variation on the phenology of some wild fruit species and their relationship with avifauna. For this study, wild fruit species such as blackthorn, red and black hawthorn, dog rose, European crab apple, wild pear, elder, dewberry, and some bird species such as white and black stork, common cuckoo, Eurasian hoopoe, barn swallow, and European bee-eater were monitored. The observations were carried out in an ecosystem from the southern part of Oltenia, Romania, during the period 2019-2021. From the three years of study, 2021 stood out with an extension of the spring season, which influenced both plant phenology and the behaviour of bird species. It turns out that the presence of wild fruit species is essential in terms of the provided ecosystem services from an ecological and ecosystem balance point of view.

Key words*: BBCH, birds, phenology, spring, wild fruits.*

INTRODUCTION

Phenology is the time and duration of recurrent biological events under the influence of climatic factors, such as bud breaking, flowering (Yang et al., 2020), fruit ripening. Early bud breaking influences the length of the species vegetation season and their life cycle is under the impact of rising temperatures (Doi & Katano, 2008). There are currently numerous studies on the effects of climate change on ecosystems and implicitly on biodiversity (Pereira et al., 2010; Bellard et al., 2012; Garcia et al., 2014; Urban et al., 2016). The influence of human activities is increasingly evident on the climate (Trenberth, 2018) with repercussions on flora and fauna, reducing or increasing the growth stages of vegetation or birds' migration patterns. The simplest way to observe the influence of climate change on biodiversity is to track the phenology of plant species and the migration of birds (Walther et al., 2002). Phenological growth stages (BBCH) are considered as indicators used in detecting the influence of climate change on ecosystems, offering the possibility to quantify data based on visual observations (Ahas & Aasa, 2006). Spring phenology represents the beginning of the vegetation season of the species and it is the most sensitive to climate change (Yu et al., 2010) being prone to sudden changes in temperature that can have an unfavourable impact on their growth and development (Larue et al., 2021). According to Badeck et al. (2004) and Linkosalo et al. (2006) temperature is the main factor of biological developmental processes. One of the unfavourable consequences that can affect fruit species is a high temperature followed by late frosts and rime (Vitasse et al., 2018), thus affecting buds or flowers (Eccel et al., 2009; Djaman et al., 2021). Another consequence as a result of climate change is the gap of spring phenophases by prolonging some growth stages, respectively budding, flowering, due to much too low temperatures and frequent rainfall. This can be seen in the case of rainy and cold springs that increase the spring growing stages. According to Koleček et al. (2020) in migratory birds, to high temperatures led to their arrival at nesting areas much earlier and thus to the extension of the breeding season. At the same time, the impact of climatic factors on the life cycle of birds also depends on the local and regional characteristics of the area (Zalakevicius et al., 2006), the abundance of food, the presence of nesting sites or how the breeding habitat provides protection against predators. Chuine (2010) mentions phenology as being an adaptive trait specific to each plant species with a high degree of variability. The timing of key life events (phenology) migration, breeding, and nesting are timed every spring to coincide with the peak availability of critical food sources in a delicate synchronization that occurs across large latitudinal gradients and diverse habitats. According to El Yaacoubi et al. (2014), most weather stations in Europe, East Asia and Alaska recorded a significant increase in maximum and minimum annual temperature, especially during winter and spring. As stated by Vitasse et al. (2018) nowadays, the emphasis is more on species resistance to drought than on the relationship between spring phenology and late frosts. These climatic accidents can have undesirable effects on species productivity (Djaman et al., 2021). Species in temperate forests are most vulnerable to frost when the leaves appear (Vitasse et al., 2018). Researches from Estonia suggest that spring has progressed while winter has decreased (Ahas & Aasa, 2006). Chuine et al. (2016) mention a start of the growing season 2.3 days earlier per decade in the last 40 years in the temperate zone of Europe due to climate change. Shifts in phenology are important for conservation actions and the seasonality of species for recreational activities (Badeck et al., 2004). According to Rumeu et al. (2020) understanding how species interact and drive ecological processes across space is crucial given the current scenario of world-wide habitat fragmentation. The simplest method of investigating climate change is *in situ* observations by field trips and records of data corresponding to the onset of vegetation phenophases. The purpose of this paper is to provide additional information on the influence of interannual climate variation on phenology of some wild fruit species and their relation to avifauna, from the southern part of Oltenia region, Romania.

MATERIALS AND METHODS

Materials

In terms of descriptive research, wild fruit species from Bratovoeşti area (44°05'N 23°53'E), Dolj county, southern part of Oltenia region, Romania, were analysed in terms of triggering spring phenophases (on an area of approximately 15 km^2). Eight wild fruit species, blackthorn/sloe - *Prunus spinosa* (L.), hawthorn - *Crataegus monogyna* (L.) Jacq., black hawthorn - *Crataegus pentagyna* (L.) Waldst. et Kit., dog rose - *Rosa canina* (L.), European crab apple - *Malus sylvestris* (L.) Mill., European wild pear - *Pyrus pyraster* (L.) Burgsd., Elder - *Sambucus nigra* (L.) and dewberry - *Rubus caesius* (L.) were observed over 3 years period (2019-2020-2021) for data collection corresponding to the onset of the main spring phenophases. These particularly wild fruit species were chosen because of their importance in terms of ecosystem services that they provide (source of food for birds and animals; shelter; support for several categories of organisms such as insects, butterflies; horticultural importance in terms of breeding programs, medicinal and therapeutic importance due to their content in bioactive compounds useful for human health, cultural services in terms of recreational purposes). Six species of long-distance migratory birds from sub-Saharan Africa (white stork - *Ciconia ciconia*; black stork - *Ciconia nigra*; common cuckoo - *Cuculus canorus*; Eurasian hoopoe - *Upupa epops*; barn swallow - *Hirundo rustica* and European bee-eater - *Merops apiaster*) nesting in the study area, were observed during the three years period in order to mark their date of arrival. Nests of other present species were identified and monitored over the three years of the study to collect data regarding those that chose the studied wild fruit species as a support in building nests.

Climatic data

From the climatic point of view, the analysed ecosystem is situated in the type of continental climate, with annual precipitations of 500 mm (most frequent at the beginning of summer) and a temperature amplitude of over 25˚C (Cojoacă & Niculescu, 2018).

In terms of climatic data (meteocraiova.ro), the average monthly temperature and the total amount of monthly precipitations were used as a comparison between the studied years (2019-2020-2021). The weather station from where the data was acquired is situated at 25 km from the site, being the closest.

Methods

Exploratory research was conducted via field trips made at intervals of 2 to 3 days to observe the development of the principal growth stages and identification keys according to the BBCH scale (bud breaking BBCH 07; full flowering BBCH 65; end of flowering/all petals fallen BBCH 69). The data calendar corresponding to each phenophase was recorded and the number of days between January 1st to the main spring phenophase was calculated. For the species *M. sylvestris*, *P. pyraster*, *C. monogyna*, *C. pentagyna*, *R. caesius* and *P. spinosa* the phenological growth stages and identification keys described by Meier (2001) were used, for *R. canina* the growth stages described by Meier et al. (2009) were applied and for *S. nigra* the growth stages described by Atkinson & Atkinson (2002) were adapted. The same individuals from each wild fruit species were observed each year, so that the obtained data can be conclusive. The studied bird species were observed around their nesting sites in order to register the calendar date of arrival. These particularly species were chosen because of their ease of observation (both visually and after the sounds made, in the case of the common cuckoo and the hoopoe).

Statistical analysis

All data regarding the number of days starting January 1st to the main growth stage (bud break, full flowering, end of flowering) was processed in Microsoft Excel.

RESULTS AND DISCUSSIONS

Tracking phenological landmarks is a simple and easy to apply method, often used in both flora and fauna monitoring. It provides accurate and useful data on how climate affects the development of each organism and thus the ecosystem. Spring phenology is considered to be the most prone to temperature fluctuations but also to precipitation with negative effects on the growth and development of plant species but also on the dynamics of bird migration patterns.

Following the obtained data, the year 2021 was noted as the earliest in terms of bud bursting (Figure 1) for *M. sylvestris*, *P. pyraster*, *C. monogyna*, *R. canina*, *S. nigra*, *P. spinosa* but also the longest in all studied wild fruit species by prolonging the spring phenology (bud break – all petals fallen).

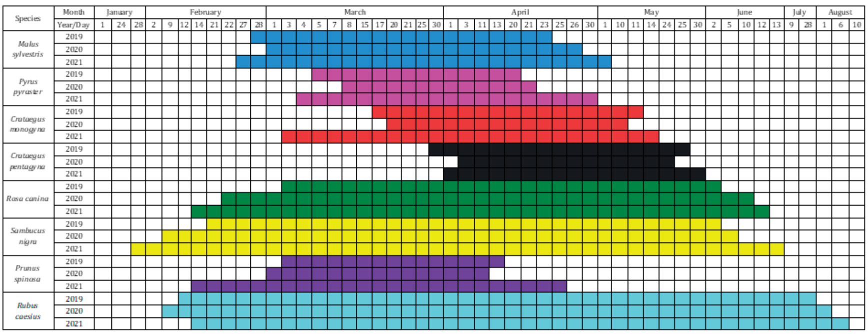

Figure 1. Wild fruit species phenogram of bud breaking - end of flowering (all petals fallen)

Following the analysis of meteorological data (Figure 2), January 2021 was the warmest with an average temperature of 3.77°C compared with 2019 with -3.39°C, and 2020 with 2.96°C. February 2021 was the coldest with a monthly average temperature of 5.2°C, compared with 5.36°C in 2019 and 7.5°C in 2020. These data can explain the phenomenon of faster vegetation entry and at the same time analysing the other data for March and April, 2019 was found to be the warmest with a monthly average of 11.72°C compared to the same month (March) of 2020 (average monthly temperature of 9.61°C) and 2021 (with an average of 7.33°C). April recorded the highest average monthly value in 2020 (14.25°C) followed by 2019 (13.67°C) and 2021 (11.41°C). The last month of spring, May had the highest monthly average temperature in 2021 with 18.61°C, followed by 2020 with 18.58°C, while 2019 had an average monthly temperature of 17.05°C. The monthly average temperature correlated with the monthly total rainfall (Figure 3) offer a better picture on the influence of cold/warm temperatures to the development of spring vegetation season.

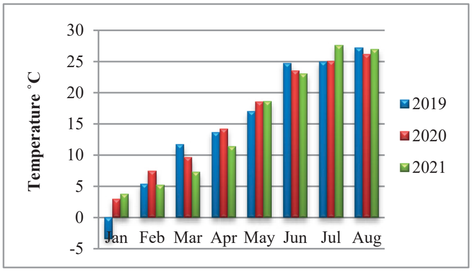

Figure 2. Monthly average temperature over the 3 years of study (˚C)

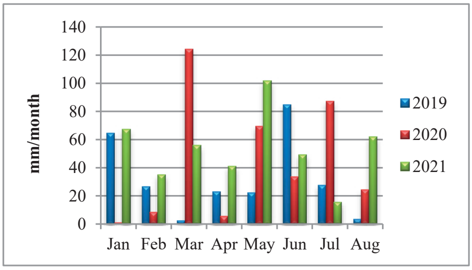

Figure 3. Quantity of monthly total rainfall over the 3 years of study (mm)

The months of January, February, May and June had the highest amount of rainfall in 2021 compared to 2020 and 2019, this could be a possible explanation of the extension of the spring vegetation season. Taking as a landmark the number of days from January 1st to bud breaking, the order in which this phenophase occurred in the identified species was: *S. nigra* > *R. caesius* > *R. canina* > *P. spinosa* > *M. sylvestris* > *P. pyraster* > *C. monogyna* > *C. pentagyna*. According to the study of Cosmulescu et al. (2021) the highest coefficient of variation was obtained at elder followed by blackthorn, dog rose and red hawthorn which indicates a medium to high degree of variability for this phenological growth stage (BBCH 07). Considering the same landmark (number of days from January 1st) to full flowering (BBCH 65), the order in which this phenophase occurred in the identified species was: *P. spinosa* > *P. pyraster* > *M. sylvestris* > *C. monogyna* > *C. pentagyna* > *R. canina* > *S. nigra* > *R. caesius*. Regarding the number of days from January 1st to end of flowering (BBCH 69) the order in which this phenophase occurred in the identified species was: *P. spinosa* > *P. pyraster* > *M. sylvestris* > *C. monogyna* > *C. pentagyna* > *S. nigra* > *R. canina* > *R. caesius*. Based on this data it can be concluded that elder gives the starting of vegetation season, and black hawthorn is the last of the studied species to bud break (about two weeks later after red hawthorn). The timing between the spring phenology and the arrival of studied migratory birds can be found in Table 1. Another important factor for bird species regarding the development of wild fruit species is the plant habit, growing characteristics (presence of thorns, twigs shape and length), which provides shelter from predators, or climatic factors. Species that were observed nesting in studied wild fruit species were: Eurasian collared dove (*Streptopelia decaocto*) in elder, the long-tailed tit (*Aegithalos caudatus*) in dog rose, Red-backed Shrike (*Lanius collurio*) in blackthorn, hawthorn and wild pear, Eurasian Blackbird (*Turdus merula*) in European crab apple. This is mainly due to the growth characteristics of wild fruit species that provide protection against external factors.

According to Vander Mijnsbrugge et al. (2015) the bud breaking phenophase is the one that influences the length of the growing season, flowering and reproduction. Following the obtained data, there are significant differences from one year to another in terms of time of vegetation season starting on wild fruit species with the prolongation of the spring season (bud break – end of flowering/all petals fallen). It is found that the order in which the bud break took place was maintained, but the period of development changed, in connection with environmental factors and location.

Table 1. Date of arrival of long distance migratory bird species and the main BBCH phenophases at the studied wild fruit species

Species	First seen on site	Wild fruit species BBCH phenophases
Ciconia ciconia (White stork)	2019 Mar 17	Pink bud stage at *M. sylvestris;* first leaves fully expanded at *P. pyraster;* bud break at *C. monogyna* and *R. caesius;* full leaves at *S. nigra;*
	2020 Mar 10	First leaves fully expanded at *M. sylvestris*; bud break at *P. pyraster* and *R. caesius*; first leaves fully expanded at *R.canina*; full leaves at *S. nigra;*
	2021 Mar 13	Bud break at *M. sylvestris, P. pyraster, C. monogyna, R, caesius, R. canina;*
Ciconia nigra (Black stork)	2019 Mar 17	Pink bud stage at *M. sylvestris;* first leaves fully expanded at *P. pyraster;* bud break at *C. monogyna;* full leaves at *S. nigra;*
	2020 Mar 14	Visible flower buds at *M. sylvestris*; bud-breaking at *P. pyraster;*
	2021 Mar 28	Visible flower buds at *M.* sylvestris and *C. monogyna*; pink bud stage at *P. pyraster*; bud burst at *P. spinosa*; first leaves at *R.canina;*
Cuculus canorus (Common cuckoo)	2019 Apr 16	Flower buds at *C. pentagyna* and *C. monogyna;* flowers fading at *M. sylvestris* and *P. pyraster*; flower buds at *S. nigra;*
	2020 Apr 14	Flowers fading at *M. sylvestris* and *P. pyraster*; first flowers at *C. monogyna*; flower buds at *C. pentagyna*; all petals fallen at *P. spinosa*; flower buds at *S. nigra;*
	2021 Apr 03	Pink bud stage at *M. sylvestris* and P. *pyraster*; first flowers at *P. spinosa*; flower buds at *S. nigra* and *C. monogyna*; bud break at *C. pentagyna;*
Upupa epops (Eurasian hoopoe)	2019 Mar 24	First flowers at *M. sylvestris*; flower buds at *P. pyraster*; bud break at *C. monogyna*; first flowers at *P. spinosa*; first leaves at *R. canina*; flower buds at *S. nigra;*
	2020 Apr 11	Full flowering at *M. sylvestris*; flowers fading at *P. pyraster*; pink bud stage at *C. monogyna*; leaves unfolding at *C. pentagyna*; end of flowering at *P. spinosa*; flower buds at *S. nigra;*
	2021 Mar 27	Leaves fully expanded to flower buds at *M. sylvestris*; pink bud stage at *P. pyraster*; flower buds at *C. monogyna*; bud burst at *P. spinosa*; first leaves at *R.canina;*
Hirundo rustica (Barn swallow)	2019 Mar 20	Pink bud stage at *M. sylvestris*; leaves fully expanded to flower buds at *P.pyraster*; bud break at *C. monogyna*; bud burst at *P. spinosa*; first leaves at *R. canina* and *R. caesius;*
	2020 Mar 17	Flower buds at *M. sylvestris*; first leaves unfolding at *P. pyraster*; bud break at *C. monogyna*; bud burst at *P. spinosa*; first leaves fully expanded at *R.caesius;*
	2021 Apr 08	Pink bud stage at *M. sylvestris*; pink bud stage to first flowers at *P. pyraster*; flower buds at *C. monogyna*; bud break at *C. pentagyna*; flowering at *P. spinosa*; flower buds at *S. nigra*
Merops apiaster (European bee-eater)	2019 May 05	Full flowering to flowers fading at *C. monogyna*; pink bud stage at *C. pentagyna*; bud burst at *R. canina*; flower buds at *S. nigra;*
	2020 Apr 26	Full flowering at *C. monogyna*; flower buds at *C. pentagyna* and *S. nigra*; end of flowering at *M. sylvestris*; bud burst at *R.canina;*
	2021 May 01	End of flowering at *M. sylvestris* and P. pyraster; first flowers at *C. monogyna*; flower buds at *C. pentagyna* and *R. canina* and *S. nigra.*

Triggering these changes depends on climatic factors (Forrest, 2016) of the area (temperature, photoperiod). According to Grime et al. (1988) bud break of *S. nigra* may occur earlier due to high temperatures in winter, while Atkinson & Atkinson (2002) refer to leaves emergence in February-March and flowers in May-June as reference periods, consistent with the data obtained in this study. The differences between the two species of the same genus (*Crataegus*) are significant in terms of bud breaking in 2019 occurred 13 days apart, in 2020 at 14 days and in 2021 at a difference of 29 days. According to Fichtner & Wissemann (2021), bud break at *C. monogyna* takes place in mid-March to April, depending on altitude and location, while the "end of the flowering" takes place from April to June. Dönmez (2004) mentions that hawthorn has a period of 1-2 weeks with flowers present, and *C. pentagyna* blooms later than *C. monogyna*, which confirms the results obtained in this study. The results obtained within the *P. spinosa* species are consistent with those obtained by Cosmulescu & Căluşaru (2020) in the study from Gura Văii, Oltenia region. Worrell et al. (2019) remarks the flowering of crab apple in mid-late May in Scotland, much later than the data obtained in this paper (April), which supports the influence of environmental conditions and location on phenology. Šebek (2019) analysed *P. pyraster* regarding the moments of onset of the main phenophases (flowering, fruit ripening) in Montenegro, and the results obtained are consistent with those of this paper (the beginning of the flowering period took place between 7th of April and 9th of May). Another relevant study mentions the red hawthorn blooming around 13th of May (SD:8.4 days), the dog rose around 8th of June (SD:6.2 days) and elder around 4th of June (SD:6.7 days) according to the study conducted by Tooke & Battey (2010) in temperate zones. A later starting of the growing season is preferable so that the species are not affected by late frosts or rime which can have serious repercussions on fruit productivity and quality. At the same time wild fruit species should trigger their starting of vegetation season prior to arrival of the migrating birds or at least upon their arrival, this way they can benefit through the interactions established between species. Foliage and flowers attract insects, pollinators, represent a source of food for larvae and these in turn represent the food source for insectivorous birds (hoopoes, swallows, cuckoos, bee-eaters). Storks feed on a wide range of animals (lizards, snakes, amphibians, small birds, insects, fish, invertebrates), including mice (in many cases they tend to have nests at the base of hawthorn or dog rose) and also, they feed on large insects which can be found on leaves or flowers of wild fruits trees. Migratory birds arrive in different decades of spring months, some arrive earlier (storks, barn swallows, Eurasian hoopoes) while others arrive later (common cuckoo and European bee-eater) in accordance with their own breeding strategy (for example the cuckoo returns to the breeding grounds before the host species in order to lay eggs in the nest of a single host species). Upon arrival, the birds must procure their food in order to recover after the long journey. This means that the insects must have already emerged and started their activity, and the wild fruit trees must have started growing season The arrival of migratory species at breeding sites is different, because the distance travelled from one point to another is different, the birds needing rest and food at different points along the crossed routes. The white stork arrived in Lithuania between March 30th and April 8th, 1961-2000 in a study by Vaitkuvienė et al. (2015) who mention temperature as an important factor in this landmark. Ptaszyk et al. (2002) confirm the arrival of white storks between March 11th and April 1st in western Poland, and in Slovakia between March 12th and May 15th (Gordo et al., 2013). The study conducted by Bozó & Csörgő (2020), mentions the arrival in the period 2005 - 2019 in south-eastern Hungary and western Romania of the species *U. epops* between 19th of March to 6th of April, *C. ciconia* between 13th of March to 5th of April, *H. rustica* between 20th of March to 5th of April and *C. canorus* between 3rd to 28th of April, results in accordance with those obtained in this paper for the species mentioned in the south-western part of Romania. Scientists mention the arrival of white storks in Southern Oltenia as being generally between 20-25th of March. The species are insectivorous with different trophic niche. Common cuckoos eat different types of insects, including hairy caterpillars which are not eaten by many other birds. European bee-eaters' diet mostly consists of bees, but also includes dragonflies. Barn swallow is a specialized aerial feeder and the diet of the Eurasian hoopoe is mostly composed of insects, but also of small reptiles, frogs and even plant matter. According to ornitodata (pasaridinromânia.sor.ro) the Red-backed Shrike is the most widespread species of Shrike in Romania, and its diet consists mainly in insects, small vertebrates, but also fruits especially before beginning of migration. The long-tailed tit feeds on small invertebrates, but also seeds, buds and fruits (hawthorn, rosehips especially during winter season) as observed during their monitoring. Other present and abundant species that use among others, also fruits and seeds to their diet are blue tits (*Cyanistes caeruleus*), great tits (*Parus major*), Eurasian bullfinch (*Pyrrhula pyrrhula*), chaffinch (*Fringilla coelebs*), European robin (*Erithacus rubecula*), common starling (*Sturnus vulgaris*), European greenfinch (*Chloris chloris*), European Goldfinch (*Carduelis carduelis*), Eurasian golden oriole (*Oriolus oriolus*), green woodpecker (*Picus viridis*) and corn bunting (*Miliaria calandra*). By consuming wild fruits, they contribute to seed dispersal, and so to genetic variability based on the observations made, each year new individuals are spreading and creating new microhabitats, thus maintaining a constant food availability during autumn migration (Gallinat et al., 2020) and cold winter months (in some cases observations were made to fruit species bearing until April, such as rosehips, hawthorn and in some spare cases blackthorns). Lázaro et al. (2005) studied the wild fruit species seed dispersal in northern Europe, and correlates the bird's diet with the formations of "bird-made fruit orchards" (*Sambucus nigra* and *Crataegus monogyna* being the first two most spread species from the studied ones). Also, Rumeu et al. (2020) mention in their study on frugivore species their trophic and spatial role in seed dispersal networks so that they have the ability to shape vegetation structure and maintain landscape connectivity. Existing research suggests phenological or phenometric determinations as a useful tool for addressing changes due to fluctuations in environmental conditions regarding the time and extent of phenological stages (Romo-Leon et al., 2016). Phenological maps can be used successfully in illustrating and interpreting data on the onset, progress and duration of the species vegetation season (Jochner et al., 2011) taking into account the main growth stages (BBCH). The dynamics of phenological processes is determined by complex interactions between genetic and environmental factors (Ruml & Vulić, 2005). The wild fruit species and avifauna are crucial for the provided ecosystem services maintaining balance, stability and functionality.

CONCLUSIONS

Although the temperature is according to researchers the determining factor in phenology, other abiotic factors can also be correlated with the moments of triggering each phenophase. The observations and results obtained in this research confirm the existence of variations in spring phenology from one year to another at the studied species and also the existence of favourable relationships between wild fruit species and avifauna. The presence of wild fruit species is essential in terms of the provided ecosystem services from an ecological and ecosystems balance point of view. Further studies will be carried out to be able to draw some conclusions over a longer period of time regarding the development of the main spring phenological stages on the analysed wild fruit species.

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INFLUENCE OF THE PROTECTIVE NETS ON FRUIT PRODUCTION IN MODERN ORCHARDS - A REVIEW

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Abstract

The protective nets and screens have become indispensable features in many fruit crops such as apple, cherry, kiwi, apricot, grapevine, and berries. Considering climate changes, the protective nets contribute both to creating a safe environment for plants by controlling hail and reducing wind, but at the same time they have an important role in reducing excessive solar radiation, pests attack, while maintaining a suitable microclimate for the crop. The net color and density influences most of the physiological processes in plant with important effect on growth, yield and fruit quality. The paper presents the main scientific results regarding the influence of protective nets on mentioned parameters.

Key words: *protection system, anti-hail net, solar radiation, yield, fruit quality.*

INTRODUCTION

Climate changes occurred as a result of human activities and are a worldwide problem (Klingelhofer et al., 2020).
Agriculture represents an indispensable sector in the country's economy (Tindeche et al., 2014), which is visibly affected under the conditions of climate changes (Alam, 2023).
New technologies have the role of combating or substantially reducing the factors that cause the crops depreciation and at the same time increase the plants productivity. By implementing them, farmers manage to successfully overcome the challenges that have arisen in recent years using environmentally friendly methods.
An important preventive measure used in the context of climate change is anti-hail/rain protection (Stewart and Ahmed, 2020).
This technology contributes both to the crop protection and the improvement of products quality, as well as to the reduction of solar radiation and the pest's development (Marinka, et al., 2015).
The need for the anti-hail net is primarily given by the plant's requirements as well as the crop area and the frequency of extreme weather phenomena, while the anti-rain screen is indispensable for crops with a high degree of perishability, due to fruit cracking incidence.

IMPORTANCE OF PROTECTIVE NETS. TYPES OF NETS

Anti-hail/anti-rain nets are designed to act as a barrier against hail or heavy rains, thus preventing the depreciation of crops and loss of production. They also have the role of reducing solar radiation, preventing frost, and controlling phytosanitary conditions (Oprea and Stănică, 2021). These nets can be used for a variety of crops, including fruits, vegetables, flowers and vines.
Currently, many types of protection are used depending on the plant's requirements. The material, degree of permeability, colour, or texture are just some of the important factors in choosing the method of covering the crops (Castellano et al., 2008). Depending on the plant's needs, the protection material can be white/transparent (Iglesias and Alegre, 2006), yellow (Moura et al., 2022), green, grey, or black (Cugneto and Masoero, 2021), which influences the amount of light that reaches the plants foliage (Brito et al., 2021).
For such protection, the installation of a support system is indispensable. Anti-hail nets protect crops from hailstorms that can have devastating effects on both production and plants. At the same time, they reduce solar radiation, contribute to the improvement of production quality, control diseases and pests, and ensure a

favourable microclimate, thus increasing the lifespan of plants (Aoun, 2018).
On the other hand, to prevent frost and combat the adverse effects of rain, it is recommended to install protections made of plastic material (HDPE), with different densities and colours depending on the need. This also contributes to humidity control, disease prevention, increased production, and even reduced soil erosion. Being an impermeable material, it is also used when setting up a greenhouse (Ashok and Sujitha, 2021). In the case of species susceptible to fruit cracking or highly perishable species, especially cherries, rain protection is recommended to prevent the production loss (Grandi et al., 2017).
For both types of protection, it is necessary to periodically check the nets for any possible damage and repair the affected areas (https://www.fruitsecurityholland.com/en/hail-rain-protection-collapse/l606c13). Both types of nets are crucial for protecting crops against specific weather-related threats, ensuring better yield quality and quantity by mitigating the risk posed by hail, rain, and frost.

INFLUENCE OF THE PROTECTIVE NETS

The innovation and continuous improvement of anti-hail/anti-rain nets have provided farmers with a reliable means to protect their crops from some of nature's most unpredictable and destructive forces, ensuring more stable yields and contributing to global food security (https://fruitgrowersnews.com/article/orchard-covers-netting-solutions-have-varied-impact).
Studies show that crops such as cherries and berries require protection against rain due to their perishability, the risk of frost during the flowering period, and the risk of fruit cracking, while species such as apples, pears, grapes, and kiwis require protection against hail and wind due to their sensitivity to these elements and solar radiation (Oprea and Stănică, 2021).
Numerous research papers present the beneficial effects of protective nets as well as their influence on fruit quality, which are increasingly common in species such as apples (Bastías and Boini, 2022) and pears (Kiprijanovski et al., 2016). In 2006, a study carried out in an apple plantation demonstrated that the black anti-hail net considerably reduced the solar radiation and positively influences the trees vigour, while the transparent net affected the fruits quality (Iglesias and Alegre, 2006). Another study conducted between 2019-2021 demonstrated the effectiveness of combined anti-hail and anti-rain nets against diseases and pests. The resulted system, called "Keep in Touch", tested in different apple orchards achieved a reduction of phytosanitary treatments between 70-85% (Boutry et al., 2022).
The impact of anti-hail nets was also demonstrated in grapevine plantations in South Brazil, with favourable results on production (Forte et al., 2022). In Romania, Mehedinți County, following a comparative study within a grapevine plantation, the results of using the anti-hail net regarding production safety were positive. However, the installation of the system required the application of a greater number of treatments against diseases in the covered vineyard (Mountain, 2018).
Kiwi is one of the species that requires anti-hail and anti-wind protection. Besides the physical protection of the crop, the net colour can directly influence the development of the plants. Basile (2008) states that the photo selective effect of the nets influences the vigour of kiwi plants as follows: the area covered with the blue net shows a decrease in the plant's vigour, while the red and grey nets stimulate their vigour. In the case of softwoods such as cherries (Børve and Meland, 1998) or apricots, rain protection is recommended (Oprea and Stănică, 2021). In Romania, at the Faculty of Horticulture in Bucharest, several cherry varieties are protected with anti-rain nets with positive effect on orchard protection against late frosts and on fruit quality, including cracking elimination (Oprea and Stănică, 2021).

CONCLUSIONS

Crop protection with anti-hail and anti-rain netting is an important investment for fruit farmers, ensuring their production throughout the growing season. Although the costs of the protection system are quite high, from an economic point of view, the crop is much more advantageous considering the high number of benefits.

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BREEDING OF KIWIFRUIT SPECIES (*ACTINIDIA* SP.) - A REVIEW

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Abstract

The origin of most kiwi plant species is located mainly in Yangtze River Valley in the North Eastern part of China. At the beginning of 20th Century, seeds brought from China have been used by Hayward Right, a nursery man from New Zealand, to obtain the first hybrids plants and respectively, first selected cultivars, including Hayward the most cultivated genotype in the world. Later on, together with the extension of kiwifruit crop on several continents, breeding works started in the other countries. New Zealand remained at the forefront of breeding activity and, at the 90's released the first yellow flesh kiwi Hort 16A - Zespri Gold. Other cultivars were obtained in Italy, USA, China, etc. In countries with a colder climate, kiwiberry (A. arguta) has become popular and breeding programs were carried out in New Zealand, Italy, China, Poland, Belgium, and Romania. In addition to public breeding programs, many private ones are active. The review presents the main kiwifruit breeding achievements.

Key words: *cultivars, yellow flesh, crossings, kiwiberry.*

INTRODUCTION

The kiwi plant comes from the North East of China, mainly from the Yangtze-River valley (Sachin, 2015) and the coast of Zhejiang Province.

After the introduction in New Zealand of *Actinidia deliciosa* seeds brought from China, by a young lady named Isabel Fraser, a local nurseryman, Hayward Wright, obtained the first hybrids and started the selections works. Also there, the new fruit plant received the name of kiwi, after the national bird, symbol of New Zealand (Mishra and Shukla, 2014).

In countries with a colder climate, kiwiberry (*Actinidia arguta* Chev. & Zucch.) has become recently popular. First kiwiberry cultivars were created by Ivan Vladimirovici Miciurin in Russia in the first half of the 20th century. His cultivar Annanasnaja, is known also under the name of 'Michurin's Pineapple' (Strik and Hummer, 2006).

Many cultivars were obtained in Italy, China, Poland, Belgium, New Zealand and recently are in vogue creations with red pulp like Hongyang (Red Heart) in China and Rubyred™ in New Zealand. Important achievements were also obtained for *Actinidia deliciosa* in USA, Italy, China and Romania.

Currently, *Actinidia* species are frequently cultivated in New Zealand, China, Japan, Greece, Chile, Italy (Testolin, 2015) and recently, in Romania (Peticilă et al, 2015).

3rd place in the top of the big kiwi producers, Italy recorded a production of 416,060 tons, after China with 2,380,787.59 tons and New Zealand with 628,496.41 tons. (FAOSTAT).

Recognized for their health benefits (Saliyan et al, 2017), rich Vitamin C content and nutrient intake (Richardson et al, 2018), kiwi fruits can be eaten fresh or in different preparations (https://www.kiwifruit.ir/homegapes/kiwifruit2/kiwi-products.html), the most popular being the fruits of *Actinidia deliciosa* (Drzewiecki et al., 2015).

The *Actinidia* genus includes over 76 species (Hamet et al., 2018), among which, we mention *A. deliciosa, A. chinensis, A. arguta, A. eriantha* and *A. kolomikta* species that are the subject of this review.

The aim of most breeding programs is to obtain new cultivars and selections, with increasing resistance to diseases and pests, with high quality fruits.

Table 1 shows some of the countries with kiwifruits breeding programs and their results.

Breeding programs

Together with the extension of the crop on several continents, the first breeding works also began in the cultivating countries. Kiwifruit knowledge was diffused mainly through ISHS International Symposia, until now, 10 dedicated to *Actinidia* being organized.

The first occurred in 1987, in Padua, Italy, and represented the launching pad for kiwi plants worldwide.

New Zealand was the forefront of breeding programs for many years, which at the beginning of the 90's released the first variety of *Actinidia chinensis* - Hort 16A - Zespri Gold (Ferguson & Stanley, 2003) (Table 1).

Zespri Gold was the first important step for the promotion of kiwifruits with yellow flesh (Martin and Luxton, 2005). New Zealand also release several *Actinidia arguta* cultivars as: 'Hortgem Tahi', 'Hortgem Toru', 'Hortgem Wha', 'Hortgem Rua' (Advances in food and research, volume 52).

Within the breeding programs, **Italy** obtained cultivars such as 'Autari' (*A. deliciosa*), 'Soreli' and 'Belen' (*A. chinensis*).

In 2006, CRA-FRU Centro di Ricerca per la Frutticoltura developed an important breeding program in which the cultivars were studied Hort 16 A, 'Jintao' (*Actinidia chinensis*) and 'Hayward', 'Green Light', 'Summer kiwi' (*Actinidia deliciosa*), as well as selections of male and female plants of these two species (Conte *et al*, 2011). Before the introduction of the 'Jintao' cultivar in Europe, other fewer known selections were brought as well as the 'Lushan' and 'Kuimi' cultivars which unfortunately did not meet the conditions required by the specialists (Cipriani and Testolin, 2006).

Another breeding program took place at in **China** at Wuhan Botanical Garden, Chinese Academy of Sciences starting with 2002. This was aimed at obtaining new cultivars and selections of *Actinidia chinensis*, by crossing male and female plants of the same species. At the end of the program, the 'Jinmei' was obtained, and in 2014 it was approved by National Forestry Cultivar Approval Committee (Zhong, C. et al, 2018). In 2011, the cultivar 'Guichang' (*A. deliciosa*) was registered.

In 2014, **Korea** obtained Goldone which represent an important cultivar belonging to the *A. chinensis* species (Kwack et al., 2017). Another important cultivar is Chiak which belongs the *A. arguta* species (Advances in food and research, volume 52).

Two other lesser-known species of *Actinidia* spp. are *Actinidia eriantha* and *Actinidia kolomikta.*

Actinidia eriantha was introduced from China to Korea through Sungkunwan University (Kiwifruit, 2016). In 1997, a breeding program was also initiated with the obtaining of seedlings through the interspecific crossing of seeds, and a year later they were planted at the Wando Station. In 2003, following the selections, 'Bidan' cultivar was obtained, and was registered by Korea National Seed Management Office. Although the specie is less known, 'Bidan' cultivar is appreciated for its nutraceutical properties (Jo et al, 2007)

A. kolomikta was studied at the University of Agriculture, Kaunas, **Lithuania** since 1988. This is also where the 'Laiba', 'Lanke', 'Lande', 'Paukstes Sakarua' cultivars were obtained (Pranckietis et al, 2009).

In **Turkey**, kiwifruit was brought for the first time in 1988 at Ataturk Horticultural Central Research Institute, for scientific purposes. The first breeding program started in 2008 and had as main objective the study of several cultivars as 'Hort16A',' Hayward', 'Jintao', 'Topstar', as well as other numerous genotypes (Atak et al, 2018).

At the same time, the development of research programs for kiwi continued (Atak, 2015) and in 2018, the cultivar 'Ilkaltin' (*A. chinensis*) was obtained. (https://arastirma.tarimorman.gov.tr/yalovabahce/News/80/The-First-Kiwifruit-Cultivar-Of-Our-Country-Was-Registered-Under-The-Name-Ilkaltin).

The first kiwi crop in **Romania** was established in 1993, in Ostrov, Constanța county, and the first species cultivated was *Actinidia deliciosa* Chev (Stănică, 2009). Since then, a Romanian-Italian breeding program was started and several *A. arguta* cultivars as 'Jumbo', 'Rosana', 'Francesca' were used for crossings (Stănică et al., 2004) (Table 1).

The Faculty of Horticulture in Bucharest, in collaboration with Vitroplant Italia from Cesena, continued in the following years, the selection of kiwiberry hybrids, and in 2017, two elites were patented: R8P23, with green flesh, became the 'Vip Green' cultivar, and R9P20, with red flesh became the 'Vip Red' cultivar.

The two cultivars proved to be resistant to diseases and pests as well as frost and were registered at CPVO, Angers (Stănică et al, 2007).

From the same breeding program, following the crossings of *Actinidia deliciosa* and *Actinidia chinensis*, valuable inter- and intra-specific hybrids were obtained. Four of the selected elites were introduced into culture (Stănică et al, 2022), and on 30 March 2023, they were registered under the name of 'Kisweet' (R0P13), 'Kiball' (R1P9), 'Kigiant' (R1P12) and the male 'Kiflor' (R2P8).

At the same time, two kiwiberry cultivars were also registered: 'Ariana' (R8P1) and 'Andros' (R9P16) (Table 2).

In addition to public breeding programs, have recently been expanded private breeding programs in many countries.

Table 1. Countries with breeding programs and results obtained

Country	Year of registration	Species	Cultivar
New Zealand	1924	*A. deliciosa*	Hayward
New Zealand	1997	*A. deliciosa*	Zespri Green
New Zealand	1997	*A. chinensis*	Zespri Gold
New Zealand	1997	*A. chinensis*	RubyRed
New Zealand	1990	*A. arguta*	Nergi
China	2011	*A. deliciosa*	Guichang
China	2014	*A. chinensis*	Jinmei
China	2007	*A. chinensis*	Jintao
Korea	2003	*A. eriantha*	Bidan
Korea	2014	*A. chinensis*	Goldone
Korea	2014	*A. arguta*	Chiak
Italy	2001	*A. deliciosa*	Autari
Italy	2006	*A. deliciosa*	Green Light
Italy	1999	*A. deliciosa*	Summer Kiwi
Italy	2008	*A. chinensis*	Soreli
Italy	2015	*A. chinensis*	Belen
Italy	2012	*A. chinensis*	Dori
Lithuania	2009	*A. kolomikta*	Laiba
Lithuania	2009	*A. kolomikta*	Lanke
Lithuania	2009	*A. kolomikta*	Lande
Lithuania	2009	*A. kolomikta*	Paukstes
Lithuania	2009	*A. kolomikta*	Sakarua
Romania	2001	*A. chinensis*	Jumbo
Romania	2001	*A. chinensis*	Rosana
Romania	2001	*A. chinensis*	Francesca
Romania	2017	*A. arguta*	Vip Green
Romania	2017	*A. arguta*	Vip Red
Romania	2023	*A. deliciosa*	Kiflor
Romania	2023	*A. deliciosa*	Kisweet
Romania	2023	*A. deliciosa*	Kigiant
Romania	2023	*A. deliciosa*	Kiball
Romania	2023	*A. arguta*	Ariana
Romania	2023	*A. arguta*	Andros
Turkey	2018	*A. chinensis*	Ilkaltin
Poland	2010	*A. arguta*	Bingo

Table 2. Kiwifruit cultivars obtained in Romania

Country	Year of registration	Species	Cultivar
Romania	2001	*A. chinensis*	Jumbo
Romania	2001	*A. chinensis*	Rosana
Romania	2001	*A. chinensis*	Francesca
Romania	2017	*A. arguta*	Vip Green
Romania	2017	*A. arguta*	Vip Red
Romania	2023	*A. deliciosa*	Kiflor
Romania	2023	*A. deliciosa*	Kisweet
Romania	2023	*A. deliciosa*	Kigiant
Romania	2023	*A. deliciosa*	Kiball
Romania	2023	*A. arguta*	Ariana
Romania	2023	*A. arguta*	Andros

CONCLUSIONS

In addition to recognizing the merits of researchers, breeding programs also support farmers by creating new valuable cultivars to be introduced into production.

Thanks to breeding programs, the evolution of *Actinidia* sp. from their origins to the present has become easy to follow.

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NORTHERN BANANA OR PAW-PAW GROWN IN SOUTHERN ROMANIA SANDY SOILS

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Abstract

The Northern banana (Asimina triloba L.) or paw-paw is one of the fruit tree species belonging to the Annonaceae Family, which behaves very well in areas with a temperate climate and is considered here an exotic species. In Romania it was introduced in 1926, but until now there are no commercial orchards. In 2019, seven genotypes were planted at SCDCPN Dăbuleni, which were evaluated by the growth capacity, productive capacity, and fruit quality. Following the study, the Mary Foos genotype stood out with an average length of the annual shoots of 71 cm, an average fruit weight of 182 g/fruit and a fruit yield of 4.6 t/ha.

Key words*: shoots length, fruit quality, yield.*

INTRODUCTION

The Northern banana (*Asimina triloba* L.) is part of the Annonaceae family, the largest family in the *Magnoliales* order, with over 2300 species of trees and shrubs with tropical distribution. The fruits of some species of this family are eaten in the tropical areas of the world. An exception to the predominantly tropical and subtropical distribution of Annonaceae is the genus *Asimina*, the only genus of the family with species adapted to cold climates (Hormaza, 2014). *Asimina* includes at least eight species and several interspecific hybrids, all native to North America (Brannan, 2021) or pawpaw originates from the American continent, it is naturally propagated in an area starting from northern Florida to southern Ontario, and continued to eastern Nebraska. The modern literature provides information that this species dates back to the Eocene and the first ones that clearly resemble *A. triloba* in the Miocene (Brannan, 2012). The fruits of this species were an important source of food for the Native Americans before America was discovered, they were also appreciated by the European colonists, they were also a source of survival for American slaves, and they supplemented the increasingly low food rations of the American Civil War soldiers (Brindza, 2019). There is evidence that pawpaw was considered a delicacy by famous Americans such as the third US president Thomas Jefferson, Daniel Boone and the famous publicist Mark Twain. It was not until the beginning of the 20th century that the pawpaw attracted the interest of members of the American Horticultural Society as a fruit with promising potential, and studies and propagation practices were extended outside the areas where the species grew in wild flora (Ragan, 1888; Reich, 1991). Currently these fruits are consumed, especially in rural areas, as no marketing network has been developed (Brannan & Coyl, 2021).

In Europe, the first information about pawpaw comes from England, being mentioned in 1736, in a register from the Botanical Garden of the University of Cambridge, which even today has a collection of paw-paw with North American germplasm (Cambridge, Botanic Garden, 2021). In Italy, *Asimina triloba* was first planted in the Botanical Garden of Padua in 1801 (Mayer., 1959). More recently, in 1983, commercial paw-paw plantations were initiated in Faenza, and the Department of Horticulture of the University of Florence began to study this plant in 1990, focusing on breeding work (Bellini & Montanari, 1992). Here, in 2000, resulting in the largest orchard and variety collection (Bellini et al., 2003), in Europe, and efforts to marketing paw-paw fruit are still ongoing. Research activity at the University of Turin on paw-paw has been focused on the biochemical composition and antioxidant activity of the fruit at different stages of ripening (Donno, 2014). Many of the studies carried out supported the increased interest in the promotion of paw-paw, which led to the appearance of several nurseries that offer paw-paw trees both for amateurs and for possible commercial plantations.

Research activity at the University of Turin on pawpaw has been focused on the biochemical composition and antioxidant activity of the fruit at different stages of ripening (Donno, 2014). Many of the studies carried out supported the increased interest in the promotion of papaw, which led to the appearance of several nurseries that offer paw-paw trees both for amateurs and for possible commercial plantations. In the 2000 year, Stănică F., has initiated an extensive research activity aimed at pawpaw germplasm, propagation techniques, orchard management, to USAMV Bucharest, in the fields of the Faculty of Horticulture, here the improvement activity led to obtaining the first genotype of Romanian paw-paw named "Asimina" (Stanica F., 2012). Also, a report with information about the accessions that resulted from the breeding activity, following six study year, provided valuable information about the morphology, phenology of the plants, in relation to the climate (Szilagvi et al., 2016b), with a special emphasis on the effects of climate change (Szilagvi et al., 2016a; 2017) in the Romania. The recent research has caracterized the paw-paw as one of the most exotic plants that has been adapted in Romania. As a result of research carried out at USAMV Bucharest, Romania is mentioned alongside Korea in a report carried out by Robert G. Brannan & Maria N. Coyl, in 2021, as future countries where paw-paw has the possibility of expansion in cultivation, in the same report China, Israel, Belgium and Portugal are mentioned as countries with cultivation potential.

In 2019 year, researchers from SCDCPN Dăbuleni initiated research on some pawpaw genotypes in search of fruit tree species that can requers sandy soils for they grow. In this paper, the productivity and quality characteristics of pawpaw genotypes will be analyzed, under the conditions of sandy soils in Southwestern Romania.

MATERIALS AND METHODS

In 2019 year, to SCDCPN Dăbuleni, an experimental lot was established with 7 genotypes of paw-paw, as: Ithaca, Rebecca, Mary Foos, Sunflower, Prima, PT and PTS. The planting distance is 5 m between rows and 2.5 m between plants per row, on raised beds, mulched with agrotextile and irrigated with a drip irrigation system. Until the 3rd year after planting, the plants were shaded with corn plants planted on either side of the paw-paw row at a distance of 0.25 m from the row of paw-paws. To evaluate the growth capacity, the growth dynamics of the shoots (cm) were evaluated monthly; and to evaluate the quality of the fruits was measured: the average weight of the fruits (g); fruit diameter (mm); fruit height (mm); fruit color indices measured in the CIEL* system, where L*= brightness; a*= red shade + or green -; b*= yellow + or blue-. The fruits of the paw-paw genotypes ripen in stages, at each time of fruit harvesting, the fruits were analysed in the laboratory to determine the skin and pulp color. The color parameters were measured using a Minolta CR-400 colorimeter (Osaka, Japan) with mode L*, a*, b* and indices C* (defines color saturation) = (a*x1000)/(L*x b*) (after Minelkis, 2019), and h° (hue) = tan-(b*/a*) (Abbott, 1999), is defined as the hue angle on the colour wheel (0° = red/purple, 90° = yellow, 180° = green and 270° = blue).

The skin colour indices were measured on the diameter of the fruit, then the skin was removed with a knife on a 1 cm^2 portion and the values for the pulp of the fruit were read. The determinations of the biochemistry of the fruit were carried out, such as: soluble solids (% Brix); the total dry matter (%), the water content (%), the C vitamin content (mg %); the glucides content (%).The obtained results were calculated and statistically interpreted with the analysis of variance (ANOVA) Duncan test at $p \leq 0.5$, and the bars in each column of the figures represent the standard deviation, the letters accompanying the values in the figures represent the significance according to the Duncan test.

RESULTS AND DISCUSSIONS

The dynamics of annual shoot growth. The analysis of the data in the Table 1 shows that all the studied varieties grew with a monthly increment of 5 to 20 cm until the second decade of August, after this period the growth increment was much reduced.

Table 1. The dynamics of paw-paw annual shoot growth

Cultivar	Date					Media
	20. V	20. VI	20. VII	20. IX	5. X	
Mary Foos	13.0b	28.30c	51.8a	112.13a	108.43a	69.8a
Rebecca	10.0c	24.3d	43.7 b	69.46c	73.3c	47.3c
Sunflower	13.2b	35.83a	51.7 a	104.13a	108.0a	68.1a
Ithaca	15.26a	24.3d	35.8 c	46.5e	47.8e	35.73e
Prima	10.3c	31.83b	31.83b	86.13b	88.5b	57.2b
PT	7.70d	15.7f	20.66d	29.43f	31.8f	21.73f
PTS	10.0c	21.2e	37.0 c	58.0d	59.8d	42.26d

On average over the entire study period, the Mary Foos variety recorded an average shoot growth length of 69.8 cm, and the lowest average annual shoot length was recorded for the PT genotype, respectively 21.73 cm (Table 1).

Average fruit weight, illustrated in Figure 2, shows that the evaluated genotypes recorded an average fruit weight that varied from one year to another, with differences between the two years of study between 41.8 g in the case of the Mary variety Foos, and only 4.4 g in the case of the Prima variety. On average over the two years of the study, the highest values of the average fruit weight were recorded in the Mary Foos genotype (185.2 g) and the lowest value of the average fruit weight was recorded in the Sunflower genotype (117.9 g).

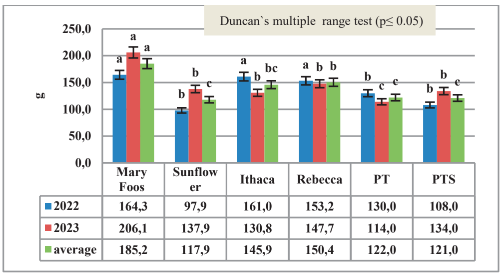

Figure 1. The fruits weight, during the study period

The average length of the fruits is one of the biometric characteristics of the quality of the fruits, which, together with the diameter of the fruits/their thickness, can be the basis of the adjustments of the future machines for conditioning and preserving the fruits of this species, which is why the two characteristics were evaluated annually and represented graphically in Figures 2 and 3.

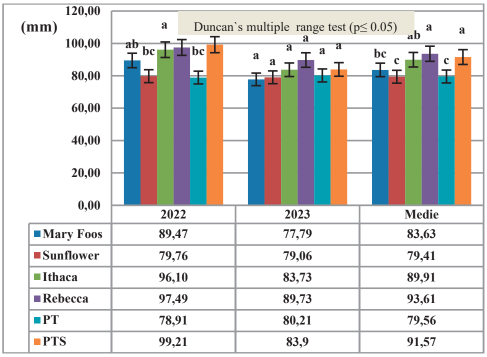

Figure 2. The paw-paw fruits length, during the study period

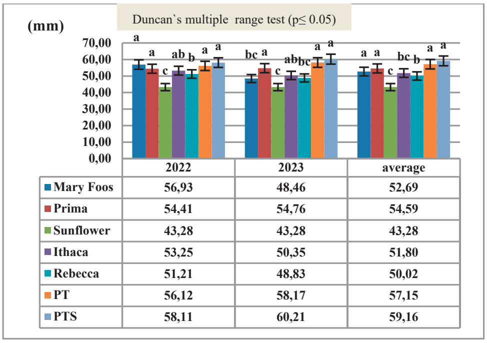

Figure 3. The paw-paw fruits thickness, during the study period

Thus, it can be seen that the Rebecca and PTS genotypes recorded the highest values of the average length of the fruits, it is shows that from the statistically point of view this genotypes versus the other studied genotypes had recorded differences statistically assured (Figure 2) for this quality characteristic. Regarding the average fruit thickness over the two years of the study, the highest values were recorded for the PTS genotype (59.16 mm) which shows statistically assured differences only from the Sunflower genotype which recorded the lowest average fruit thickness value of only 43.28 mm.

The colour of the fruit. The analysis of the brightness of the fruit skin (L*) to the seven northern banana genotypes studied highlighted the Mary Foos genotype with the highest values (69.19), with statistically assured differences compared to the values recorded by the genotypes: PT, Rebecca and PTS (Figure 4). In the case of the brightness of the fruit pulp between the seven evaluated genotypes, no differences statistically assured were recorded, but it should be highlighted that the highest value was recorded for the PT genotype (Figure 5).

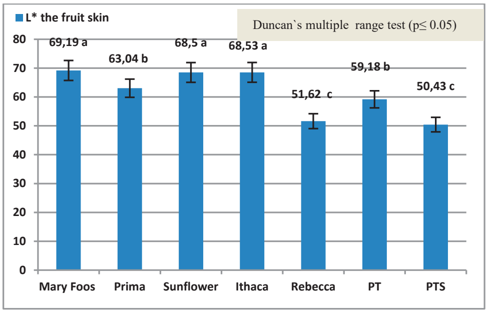

Figure 4. The skin brightness of the paw-paw fruits

In the case of the brightness of the fruit pulp between the seven evaluated genotypes, no differences statistically assured were recorded, but it should be highlighted that the highest value was recorded for the PT genotype.

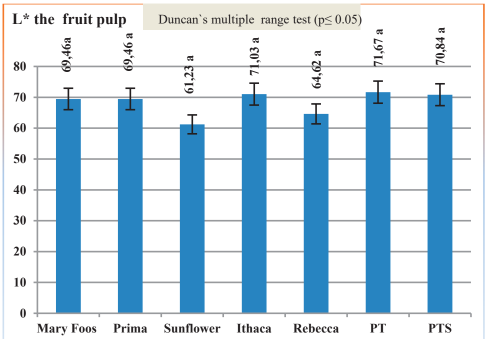

Figure 5. The brightness of the paw-paw fruit pulp

The evaluation of the colour hue angle of the fruits skin and the flesh (h^0) it did not reveal differences statistically assured (Figures 6 and 7), between the seven studied genotypes, but the highest value of the colour hue angle for the skin of the fruit being recorded for the Ithaca genotype, and in the case of the the pulp the highest value being recorded for the Mary Foos genotype.

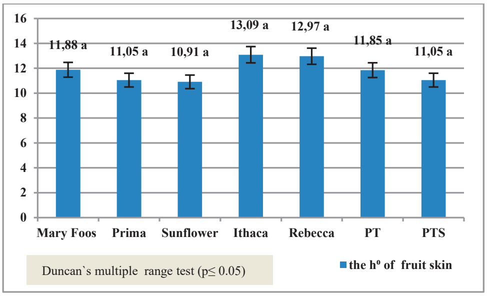

Figure 6. The hue angle of the fruits skin

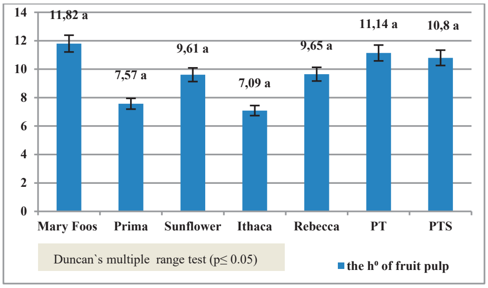

Figure 7. The hue angle of the paw-paw fruits pulp

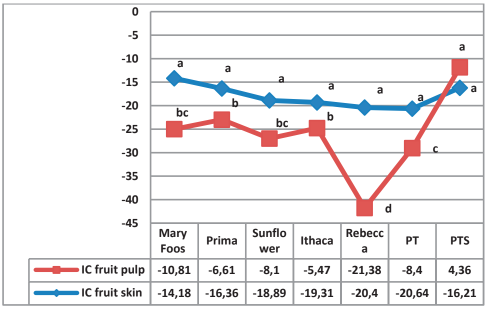

Figure 8. The chroma index to the paw-paw fruits to the studied genotypes

Table 2. Fruit yield, during the study period

The genotype	Yield (kg/tree)			Estimated fruits yield (tons/hectar) (800 trees/ha)
	2022	2023	Average yield	
Mary Foos	5.48	6.14	5,81	4.6
Rebecca	1.08	2.36	1.72	1.3
Sunflower	1.26	1.31	1.28	1.0
Ithaca	1.23	5.66	3.45	2.7
Prima	1.09	1.22	1.16	0.92
PT	0.91	3.22	2.06	1.6
PTS	0.87	3.02	1.94	1.5
Media	1.7	3.27	2.49	1.9

The analysis of the chromatic index (color saturation) of the fruit skin (Figure 9), recorded values between -14.18 (Mary Foos) and -20.64

(PT) with differences that are not statistically ensured between the genotypes studied. In the case of the same index, the values recorded on the pulp for the studied genotypes divide them into three statistical classes, the highest value being recorded by the PTS genotype, and the lowest value by the Rebeka genotype (Figure 8).

The evaluation of the production capacity to the studied genotypes revealed an average production per tree that increased from one year to another, and the average of the two years of the study was between 1.16 kg/tree (Prima) and 5.81 kg/tree (Mary Foos) (Table 2).

CONCLUSIONS

Following the study, it was found that from the point of view of the vegetative growth capacity, the evaluated genotypes developed normally, recording annual increases that varied between 21 and 71 cm average length of the annual shoots.

It is necessary to deepen the research regarding the establishment of the technical moment of harvesting, in this study the optimal moment of harvest could not be established, the fruit were collected only when they downed from the tree branches.

During the entire study period, the studied paw-paw genotypes recorded an average yield in the fourth year from planting of 1.22 to 6.14 kg/tree, which represents an estimated production per hectare of up to 4.0 t/ha.

It is recommended to continue research to determine the suitability of this species in the southern area of Romania.

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EFFECTS OF MODIFIED ATMOSPHERE PACKAGING ON QUALITY FEATURES OF 'FAVORIT' APRICOT CULTIVAR DURING COLD STORAGE

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Abstract

Apricots are stone fruit highly perishable and their limited post-harvest life is a problem for marketing. Low-cost postharvest techniques should be employed to extend the shelf life of fresh fruit and reduce postharvest losses. Apricots were packaged using 2 types of polypropylene films: microperforated and non-perforated and then stored at 1°C to study the effects of modified atmosphere packaging (MAP) on the maintenance of fruits quality and optimum storage time by comparison with non-wrapped fruits. Dry matter, weight, firmness, soluble solids content, titratable acidity, color, respiration rate, and ethylene production were evaluated. Apricots sealed in non-perforated plastic films at low temperature had an extended marketable life of around 15 days. Our results indicate that apricot fruit in non-perforated films showed lower weight loss, firmness and TA, as well as higher sugar content during storage. The respiration rate and ethylene production were lower in MAP package treatments than in control fruits.

Key words: *stone fruit, ethylene production, quality, low temperature, postharvest techniques.*

INTRODUCTION

Apricots are climacteric fruit with a limited postharvest storage to just a few days which is closely related to fruit variety. Their short storage life is often limited by high respiration rate and pronounced ethylene production and so rapid ripening process (Chambroy et al., 1995; Fan et al., 2000). This rapid deterioration usually leads to harvesting unripe fruits and therefore they cannot develop their organoleptic potential. Thus, in order to ensure a superior quality of the fruits with a sufficient storage potential, they must be harvested at the optimal maturity specific to this purpose. The use of cold storage may extend the shelf life of fruits; however, it may not be enough to preserve quality of apricots during storage, shipping and marketing.

Modified atmosphere packaging (MAP) has been found to be a successful method in delaying ripening, reducing water loss, ethylene production and softening, visible shriveling symptoms, and decay of different fruit during cold storage (Pretel et al., 2000; Amoros et al., 2008; Diaz-Mula et al.,2011; Aglar et al., 2017; Moradinezhad & Jahani, 2019; Ozturk et al., 2019; Aslanturk et al., 2022). In recent years, MAP has become widely used for fruit storage and shipping in fruit exporting countries (Phakdee & Chaiprasart, 2019; Palma et al., 2023).

The modified atmosphere increases CO_2 and decreases O_2 concentration around the fruit inside the packaging, thus slowing fruit ripening and stopping the growth of various microorganisms that cause diseases (Peano et al., 2014). Recently, Aslanturk et al. (2022) support that MAP treatments retarded weight loss, flesh softening, respiration rate and increase color of apricot fruit. Also, Moradinezhad and Jahani (2019) reported that the use of passive MAP effectively reduced postharvest losses of apricot fruits, and improved the qualitative and sensory characteristics of the fruit stored at 0.5°C for 28 days. Data of Ezzat (2018) showed the positive effect of MAP on reducing fruit weight loss and fruit firmness; fruit stored in MAP for 13 days showed the lowest fruit weight loss compared to control untreated.

Optimal storage duration of apricots depends on different factors such as the cultivar, the degree of ripeness at harvest, temperature, and air composition in MAP.

Therefore, the objective of this study was to evaluate the suitability of two films packaging and low temperature storage for quality maintaining of 'Favorit' apricot cultivar, originated from Romania and extend the postharvest life.

MATERIALS AND METHODS

The fruits of 'Favorit' apricot cultivar originated from Romania were harvested from the experimental orchard of Research Station for Tree Fruit Growing Băneasa, during two consecutive seasons. After harvesting, the fruits were rapidly transported to the laboratory where they were rinsed with tap water, drained and used for experiment. Fruits were packed on the same day of harvest in 25 μm polypropylene film and placed in 2 kg capacity plastic boxes. Each bag was sealed without gas packaging, so the modified atmosphere was established passively. The samples were stored for twenty days and analyzed at five days intervals.

Storage conditions

The fruits were divided into three groups, one for each storage condition. Three storage conditions of fruits were tested: refrigeration at 1ºC and 90% relative humidity without film (V1); MAP in non perforated films with refrigeration at 1ºC and 90% relative humidity (V2); MAP in microperforated films with refrigeration at 1ºC and 90% relative humidity (V3). Specific methods were used for each of the physical and biochemical parameters analyzed. Each variant was performed in four replicate.

Weight loss was determined by the difference between initial and final weights of each fruit during storage and expressed as percent.

Dry matter was measured by drying some slices from each side of equatorial part of fruits to a constant weight in an oven at 105°C. The results were expressed in percent (w/w).

Soluble solids content(SSC) were measured with a digital refractometer (Atago, Tokyo, Japan) at 20°C from juice extracted from the fruits, and expressed in °Brix.

Titratable acidity (TA) was quantified in juice by titration with 0.1N NaOH up to pH 8.1. TA is expressed as percent of malic acid which is the predominant acid in this species mg acid malic/100 g fresh weight. The ratio between the SSC/TA, which reflects the fruit taste feature, has been determined.

Acoustic firmness of fruits was measured at the equator of the unpeeled fruit in two repetitions using an AWETA Acoustic Firmness Sensor. In this device, an acoustic signal is generated by means of a gentle impact on the equator of the fruit. This signal is processed and transformed to obtain a peak of natural frequency, which is used to calculate the stiffness factor as $f^2 \times m^{2/3}$, where f represents this frequency and m is the fruit mass.

Color characteristics were measured at opposite sides of each fruit with a HunterLab MiniScan XE Plus spectrophotometer. Color was measured on the basis of the CIELAB color system. In this system L*, a* and b*, describe a three dimensional space, where L* is the vertical axis and its value varies from 100 for perfect white, to zero for black. Values a* and b* specify the red-green and blue-yellow axis, respectively ranging from -60 to + 60 or from – a (green) to +a (red) and from –b (blue) to + b (yellow). C* and h° values are calculated based on a* and b* values according to the following equation: $h^o = tg^{-1}\, b^*/a^*$ and $C^* = (a^{*2} + b^{*2})^{1/2}$.

The measurement of physiological parameters (respiratory activity and C_2H_4 concentration) was carried out following the closed-system method at 20°C (Chambroy et al., 1995). Apricots were placed in hermetic glass containers (1500 mL) equipped with rubber sampling ports. Three replicates were prepared from each maturity stage.

Concentration of CO_2 produced in respiration time of apricot was determined using an IR-RIKEN analyzer. Respiratory intensity of fruit was expressed in mg CO_2 kg^{-1} h^{-1}.

Concentration of C_2H_4 was determined using a Fisons GC 9000 series gas chromatograph with a flame ionization detector EL980 and a

Chrompack CP-Carboplot P7 column (inside diameter 0.53 mm, length 10 m). The temperature of the oven was 60°C and the detector temperature was 100°C. The carrier gas used was H_2. The values were expressed in µl C_2H_4 kg^{-1} h^{-1}.

RESULTS AND DISCUSSIONS

Apricot fruit quality after harvest, it damage very quickly, if special precautions are not taken and thus to meet an adequate quality, a combination of suitable storage conditions and a well-defined harvesting period must be met.

The quality of apricots after harvesting deteriorates very quickly if no special measures are taken, so in order to maintain an adequate quality, a combination of storage conditions and duration of storage must be met.

For these reasons, we chose the technology that allows keeping the fruits in an different atmosphere than the ambient one, namely modified atmosphere packaging. Inside the plastic box, fruit metabolism and especially respiration involves a decrease in the concentration of O_2 and an increase in the CO_2 concentration and humidity. Thus, the decrease in the concentration of O_2 is favorable for the induction of the slowing of the respiration rate and ethylene emission and thus the senescence of the horticultural product. The high concentration of CO_2 has an antagonistic effect on ethylene activity and also has fungistatic activity (Chambroy et al., 1995).

The obtained results indicate that weight, acoustic firmness, color, soluble solids and titratable acidity as well as physiological parameters give reliable indications of quality changes during storage. Apricots of the 'Favorit' cultivar without film change their color visibly during the first 5 days of storage. Thus, a decrease in the luminance value (L*) can be observed during the first 10 days of storage. This means a light color of the fruit (Figure 1). Also, studies of Aslanturk et al., (2022) and Muftuoğlu et al. (2012) reported that MAP - treated apricot fruit had a lower L* and chroma values. Peano et al. (2014) supported that after 21 days, apricots wrapped with the perforated film and fruits maintained under the normal atmosphere (control) lost brightness more than those in the polypropilen modified atmosphere packaging treatments, which exhibited lower *L** values of 55.2. At the end of storage, all treatments reduced the h^o values compared with the values observed at harvest. In fact, the highest h^o values (69.5 and 68.8) were maintained by films (V2 treatments), which corresponded to a high water vapor barrier.

Regarding the a* parameter, its values increase progressively from 12.59 at harvest to values of 27.14 after 5 days of storage, which denotes an orange - red color. During the next 5 days of storage, a slight decrease of this parameter was observed up to values of 23.63. The yellow parameter (b*) has increased values since harvesting around 49.06, which denotes an intense yellow degree and a yellow-orange color of the fruit, which is maintained during the first 5 days of storage, after that a slight decrease is observed up to around 42.5 (Figure 1).

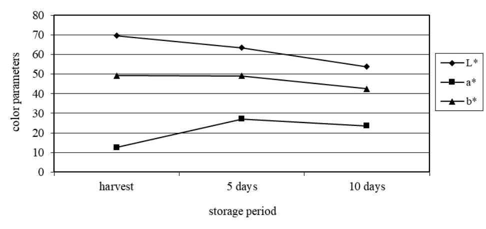

Figure 1. Color parameters variation during the storage apricot fruits without film

For fruits covered with perforated film (Figure 2), the a* parameter increases during storage but more slowly reaching higher values (28.20) compared to the variant without film and therefore the fruits have a more intense degree of red. The parameter b* increases slightly during the first 5 days of storage, after that, it slowly decreases during the last 10 days of storage, which once again supports a pronounced yellow-orange color.

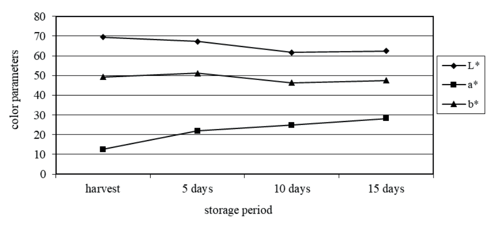

Figure 2. Color parameters modification during the storage apricot fruits with perforated film

The value of the L* parameter decreases slowly during the 15 days of storage, the color becoming increasingly lighter. In the case of the condition with non-perforated film (Figure 3), the same pattern of changes in color parameters can be observed as in the case of the perforated film, with the mention that the a* parameter increases slightly during the first 5 days of storage and remains almost unchanged during the next 10 days.

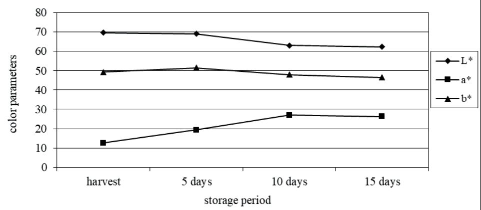

Figure 3. Color parameters modification during the storage apricot fruits with non-perforated film

Among the color components L*, a*, b* of the fruits, the a* parameter shows significant variations among the studied variants. The values of b* change much less during storage and sometimes remain constant. No consistent change in the value of the C* parameter was detected during apricot storage. The values of the h° parameter decrease during apricot storage, these results being in accordance with those obtained by Fan et al. (2000).

The shape of the spectra of the 'Favorit' cultivar is generally similar in the case of the three variants studied, usually having a maximum at 610 nm (at harvest) and one at 640 nm after 5 days of storage) after 10 and 15 days of storage a single maximum at 640 nm remains, which implies that the fruits have reached the maximum color of a ripe fruit (Figure 4, Figure 5).

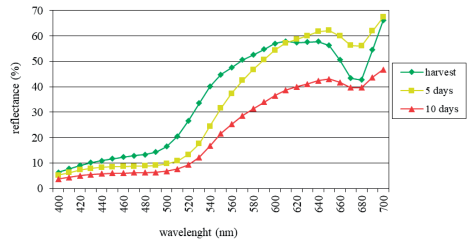

Figure 4. Reflectance spectra's modification during the storage apricot fruits without film

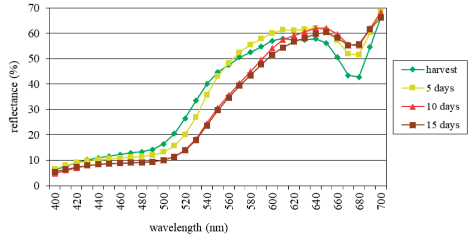

Figure 5. Reflectance spectra's modification during the storage apricot fruits with non-perforated film

Regarding acoustic firmness (Table 1), it decreases strongly for uncovered fruits and slowly for those covered with film. It is observed that the fruits covered with non-perforated film maintain an increased firmness even after 15 days of storage compared to those without film where the firmness drops to values close to zero (0.8) even after 10 days of storage. The effects of the modified atmosphere in maintaining fruit firmness, delaying fruit softening and increasing shelf life have also been reported for other climacteric fruits (Agar and Polat, 1995; Gouble et al., 2006; Jay et al., 2006; Antunes et al., 2007; Infante et al., 2008). Peano et al. (2014) supported that after 21 days, the apricot flesh firmness values in modified atmosphere packaging were similar to those of control fruits.

Table 1. Evolution of some quality parameters of 'Favorit' apricot variety during storage in MAP at 1°C

Variety	Storage period (Days)		Dry matter %	Weight loss %	Acoustic Firmness	SSC °Brix	Titratable acidity mg malic ac. %
'Favorit'	0 (harvest)		17.87	0	4.4	16.2	1.1
	5	V1	15.93	14.1	1.7	15.5	0.98
		V2	12.72	15.5	3.3	12	1
		V3	13.81	18.5	2.5	13.1	0.96
	10	V1	13.45	29.3	0.8	13	0.95
		V2	12.12	16.7	2.8	12.2	0.98
		V3	13.2	19.8	1.2	13.3	0.95
	15	V1	14.2	41.2	0.4	8.2	0.98
		V2	12	23	2.1	11.8	0.87
		V3	12.8	26.2	0.9	12.7	0.82

However, the highest values were observed for fruits stored with multilayer films, which maintained the highest state of hydration and the highest CO_2 concentrations.

The weight of the fruits of the 'Favorit' cultivar covered with film gradually decreased much more slowly than those without film (Table 1). In uncovered fruits, the weight loss was strong in the first 10 days of storage but also linear, and after 15 days of storage they had a wilted and uncommercial appearance. The difference between the weight loss of fruits covered with different films is probably due the speed of water vapor transmission through the film. In the same way as the weight, the total dry matter also varies during fruit storage (Table 1). This has high values at harvest of 17.87% and decreases to around 12% or 12.8% after 15 days of storage for the variants covered with the two types of films.

Post-harvest changes in fruit weight during storage are due to water loss through transpiration, which leads to withering and shriveling of the fruit (taking into account that apricots do not have a waxy skin) which reduces the commercial value and consumer acceptability. Apricots stored in the modified atmosphere show a significantly lower weight loss (three times lower) than the control without film. Non-perforated film and low temperature can prevent excessive fruit weight loss. Our results agree with the results of Agar and Polat (1995) and Antunes et al. (2007) who found that apricots stored in modified atmosphere lost about 3% of their weight after 18 days, while the control without foil lost 8%. Several studies also reported retarded weight loss with MAP treatments (Diaz-Mula et al., 2011; Muftuoğlu et al., 2012; Peano et al., 2014; Ezzat, 2018; Aslanturk et al., 2022). Results of Dorostkar et al. (2022) showed that apricot fruit weight remained stable during storage under active MAP treatments, whereas control samples showed an average weight loss of about 6-fold higher than MAP samples after 28 days.

The soluble solid content (Table 1) decreases slowly during the storage of the fruits both in those covered with film and in those not covered. It decreases linearly from values of 16.2°Brix at the time of harvesting to values between 11.8°Brix and 12.7°Brix after 15 days of storage. These results are consistent with those of Chira et al. (2020) and Jay et al. (2006), but are contradictory to those of Agar and Polat (1995) and Ishaq et al. (2009) who support the increase in soluble dry matter content during apricot ripening. These values as well as the titratable acidity indicate a balanced taste for the fruits of this cultivar covered with film even after 15 days of storage. This may be due to the dehydration of the fruit or the increase in respiration rate and the conversion of sugars into CO_2 and H_2O at the end of storage. It is interesting that after the postharvest ripening, the fruits do not reach the same refractometric index (sugar/acidity ratio) as those harvested at horticultural maturity.

The amount of fruit acids during storage varies similarly for all tested variants. It decreases progressively almost linearly in all cases, although the rate of decrease was higher in the case of the variant without film. These trends are consistent with those reported by Infante et al., (2008) and Jay et al. (2006) for peaches and with those of Agar and Polat (1995) for apricots. Organic acids are substrates for respiration and the modified atmosphere reduces respiration (Ishaq et al., 2009) therefore slowing down the metabolism of organic acids. However, Jay (2006) says that the decrease in acidity during storage was limited. During the storage of 'Favorit' apricots the acidity decreases, but some samples do not reach the acidity of those picked mature.

The concentration of ethylene in the 'Favorit' phenotype increases progressively (Figure 6) in all variants up to 10 days of storage, the most intense in the variant without film. After 15 days of storage for the variants covered with film, the rate of ethylene production increases very slightly, being almost constant.

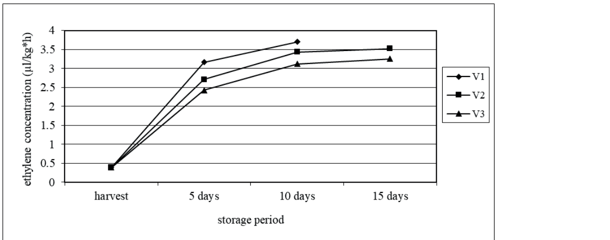

Figure 6. Variation of ethylene concentration during the storage apricot fruit

The evolution of respiration during the storage 'Favorit' fruits is almost similar to the variants covered with film, this having a maximum after 10 days of storage, after which it drops visibly after 15 days of storage. However, in the uncovered variant, the respiratory intensity increases continuously up to 10 days of storage (Figure 7).

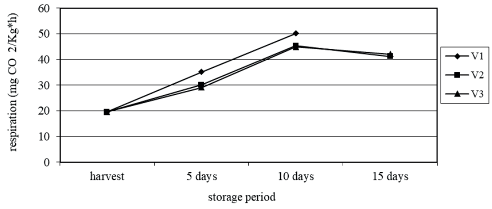

Figure 7. Variation of respiration intensity during the storage apricot fruits

Chira et al. (2020) supported that the optimal duration (14-20 days) of fruit storage varied for every apricot cultivar studied according to the moment of fruit harvesting and storage condition (MAP or low temperature), they highlights that the fruit harvested at the ripening phase had a longer storage period.

CONCLUSIONS

In essence, it is observed at this cultivar that the variant without film storage averages 10 days when the fruits have a color that allows industrial processing rather than fresh marketing due to the very low firmness.

Use of the two types of film makes this cultivar have a longer storage period and even after 15 days it has a pleasant commercial appearance with a yellow-orange color and an adequate firmness, around 2.1, with a balanced taste.

Nonperforated film represent the best packaging materials to store 'Favorit' apricot cultivar in modified atmosphere packaging for limiting weight loss, maintaining the firmness and the color of fruits.

The obtained results highlight the fact that the fruits of the variant with non-perforated film had the longest shelf life, followed by the variant with perforated film and then the non-covered one.

MAP treatments could be used as an efficient tool to prevent or minimize the apricot quality losses throughout the cold storage period

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IMPROVING FRUIT QUALITY AND STORABILITY USING POSTHARVEST TREATMENTS WITH BENEFICIAL MICROBES AND NATURAL COMPOUNDS – AN OVERVIEW

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Abstract

Fresh fruits are very perishable and susceptibile to damage very quickly after harvest during storage with significant losses of quality characteristics and thus of the yield. Chemical fungicides were intensively used to reduce the incidence of post-harvest diseases, maintaining quality and extend shelf life, but they led to the development of resistance to various pathogens and the appearance of residues on the fruit surface, which represents a risk for consumers. To minimize storage losses, a varied range of postharvest treatments have been evaluated to reduce fungal disease and extend the storage period of the fruits while maintaining the quality. The present review provides a brief overview on the use of different postharvest treatments with natural compounds and/or beneficial microorganisms and summarises information about their effect on maintaining quality, antioxidant capacity and reduce fungal diseases during fruit storage.

***Key words**: fungal disease, storage, Trichoderma, chitosan, salicylic acid, essential oils.*

INTRODUCTION

Fruits are an important part of the human diet because they are a source of soluble sugars, minerals, and dietary fibers. Nowadays other properties of fruits are gaining importance, such as their antioxidant potential due to a wide range of bioactive compounds and enzymes with health benefits in decreasing the risk of developing cancer and cardiovascular diseases, among others. Some of these compounds are polyphenols (including anthocyanins), carotenoids, vitamin C, and tocopherols, which are present in varying concentrations in fruits.

Due to their perishable nature, fruits deteriorate very quickly after harvest with significant loss of quality characteristics and thus production. During the storage period, due to various internal and external factors, chemical, physical and physiological changes that occur in fresh fruit can lead to significant losses of nutritional and sensory quality and production.

Various postharvest treatments can be applied to maintain fresh quality with high nutritional value and meet the safety standards of fresh produce. These postharvest treatments are generally combined with appropriate management of storage temperatures (Mahajan et al., 2014).

In this context, in order to maintain the nutritional quality but also the bioactive compounds and the antioxidant capacity during fruit storage, the purpose of this study is to identify new and effective post-harvest treatments with natural compounds or beneficial microorganisms.

Effect of salicylic acid application on fruit quality during storage conditions

In recent years, salicylic acid (SA) has been widely used to maintain postharvest quality, delay fruit ripening, and increase nutritional value during storage (Chen et al., 2023; Supapvanich and Promyou, 2013). Being a natural hormone, salicylic acid is considered a

safe compound as a post-harvest treatment (Asghari and Aghdam, 2010).
As recent research reports show, salicylic acid can improve fruit physical properties such as size, weight (Shafiee et al., 2010) and firmness, soluble sugars, and acidity (Shafiee et al., 2010; Zhang et al., 2003; Srivastava and Dwivedi, 2000). Da Rocha Neto et al. (2016) also support the positive effect of maintaining the quality of apple fruits treated post-harvest with salicylic acid.
Salicylic acid was very effective in delaying fruit ripening and senescence (Mohammadi and Aminifrad, 2013), thus increasing fruit keeping capacity by decreasing weight loss, maintaining firmness (Khademi and Ershadi, 2013) along with improving peach fruit health. Similarly, Shafiee et al. (2010) noted that salicylic acid was able to reduce fruit weight loss while maintaining firmness and color.
Also, salicylic acid treatments applied to apples during storage reduce electrolyte leakage, prevent loss of firmness, and maintain the characteristics of acidity, vitamin C, soluble sugars along with increased carotene and anthocyanin content compared to the untreated control.
Previous research suggests that exogenous application of salicylic acid can decrease metabolic rate, delay ethylene production preventing postharvest diseases, and alleviate physiological disturbances such as frost injury in fresh strawberry or pomegranates fruit during storage (Sayyari et al. 2011; Babalar et al., 2007).
Also, salicylic acid induces an increase of bioactive compounds contents and the activity of antioxidant enzymes including catalase, peroxidase, and superoxide dismutase (Ghasemzadeh and Jaafar, 2013; Dokhanieh et al., 2013). The antioxidant activity of peaches treated with SA is significantly reduced during the storage period (Shokri et al., 2020).
The research of the authors Supapvanich and Promyou (2017) showed that the application of a 2.0 mM salicylic acid solution maintains the post-harvest quality of papaya fruits of the 'Kaek Dam' and 'Holland' varieties and any higher concentration can cause damage to the skin of the fruit and susceptibility to fungal attack. Also, the research of Supapvanich and Promyou (2013) suggest that a concentration of 0.5 mM applied to 'Taamtimjaan' apples maintains postharvest quality characteristics such as fruit firmness and freshness during short-term storage. However, exceeding this concentration leads to the appearance of brown spots on the skin of apples.
Some studies have shown that fruits treated with salicylic acid such as cherries (Giménez et al., 2017), peaches (Tareen et al., 2012), and apples (Delijou et al., 2017) show a reduced production of superoxide free radicals and an increased activity of antioxidant enzymes compared to the untreated control during storage.
However, Imran et al. (2007) demonstrated that salicylic acid improved the antioxidant capacity of pears. But, Adhikary et al. (2020) found that pears treated with salicylic acid showed reduced oxidation of phenolic content by inhibiting the action of polyphenol-oxidase, maintaining an increased phenolic content thus leading to an increased incidence of browning, and also maintaining the content of vitamin C and superoxide dismutase activity, these results contradicting the results of Imran et al. (2007). However, pears treated with 2 mM salicylic acid solution exhibited 15% and 20% higher firmness than untreated fruits after 30 respectively 70 days of storage. Similar results were demonstrated by Razavi et al. (2014) in peaches and by Delijou et al. (2017) who reported that post-harvest treatment of fruits maintains firmness during storage.
Recently, Haider et al. (2020), supporting that the post-harvest application of salicylic acid influences antioxidant activity and antioxidant enzymes, but decreases the effect of this treatment on the content of carotenoids, enzymes involved in fruit softening. So, they argue that a solution of salicylic acid in a concentration of 0.002 µmol/L applied to 'Kinnow' tangerines variety was very effective in reducing the loss of weight, firmness, juice content and also slowed down the activity of the enzymes involved in the fruit softening maintaining the higher content in soluble sugars, carotenoids and vitamin C extending the storage time at low temperatures.
The relationship between the concentration of salicylic acid applied, the degree of fruit ripening and the activity of enzymes involved in cell wall degradation has been well

documented (Zhang et al., 2003). They reported that ethylene production and polygalacturonase, pectinmethylesterase and cellulase activity decreased after postharvest salicylic acid treatment.

Effect of chitosan/chitin application on fruit quality during storage conditions

Chitosan is a natural polysaccharide, derived from chitin found in the cell wall of pathogenic fungi or shell of crustaceans. Chitosan and its derivatives are able to form semi-permeable films on the fruit surface that can act as a mechanical barrier to protect the fruit from pathogen infection and induce host defense response, decreasing the infection during storage period (Meng et al., 2010; Bautista-Banos et al., 2006) and also regulates the gas exchange and reduces transpiration loses and fruit ripening is slowed down (Jiang & Li, 2001; Du et al., 1997).

The combination between the treatment with chitosan and the storage temperature is associated with a reduced production of CO_2 resulting in a decrease in the fruit ethylene production. (Li & Yu, 2000; Du et al., 1997).

The results of studies by Ali et al. (2011) suggest that chitosan treatment not only maintains papaya fruit firmness but also improves fruit quality during cold storage.

Cui et al. (2020) indicate that the application of a combination of chitosan and salicylic acid before harvest has a more pronounced effect in delaying the ripening of apricots than their individual application. Moreover, the treatment with this combination maintains the quality characteristics of the 'Xiaobai' apricot variety during storage, including the delay in the loss of firmness, the decrease in the content of soluble sugars, acidity and color change. It also increases the frost tolerance of apricots. The effects of chitosan treatment are much more evident in terms of reducing the intensity of respiration and ethylene synthesis when it is applied post-harvest to mandarin and guava fruit (Elmenofy et al., 2021; Baswal et al., 2020).

Chitosan was also able to reduce anthocyanin degradation and prevent pomegranate fruit color deterioration during cold storage (Varasteh et al., 2012). In the same trend, Jiang et al. (2018) reported that a new formulation based on chitosan (Kadozan), led to a reduction in the intensity of fruit respiration, delaying the increase in cell membrane permeability, maintaining an increased content of anthocyanins and thus maintaining quality and extending the shelf life of litchi fruits.

Strawberries showed a reduction of flesh browning maintain anthocyanins and polyphenol content and prolonged storage life after treatment with chitosan (Petriccione et al., 2015b). The importance of postharvest chitosan treatment has also been reported for apricots where increased phenols content and antioxidant enzyme activities (Petriccione et al., 2015a).

The effect of chitin and chitosan has been demonstrated in post-harvest diseases control to maintain quality and extend fruit shelf life. Both substances are effective in reducing post-harvest diseases by inhibiting spore germination and mycelial growth of phytopathogenic fungi due to the formation of a film on the fruit surface.

In recent years, many reports have shown that chitin can increase the effectiveness of yeasts such as *Rhodotorula glutinis*, *Rhodosporidium paludigenum,* to control post-harvest fruit diseases (Lu et al., 2014). The results of Fu et al. (2016) showed that 0.1-1% colloidal chitin solution could effectively inhibit blue rot produced by *Penicilium expansum* in pears without adverse effects on quality, because chitin does not present a risk to human health and for the environment and is widely available in nature, its applications individually may be more economical than in combination with other biocontrol agents for postharvest fruit fungal diseases.

Chitosan induces the biochemical defense response in stored fruits, maintains firmness and moisture and the amount of vitamin C. Chitosan applied individually in 0.5 or 1% concentrations was the most effective against pathogens of stored fruits, but in combination with yeasts such as *Cryptococcus laurentii* in a concentration of 0.1% was effective for *Penicilium expansum* in apples stored at 20°C. Perdones et al, (2012) reported the increased efficacy of the combined application of chitosan with lemon oil for the inhibition of postharvest pathogens and extend strawberries shelf-life.

In Romania, the studies of Radu (2012) demonstrated the effectiveness of the application of chitosan film after harvest for maintaining the quality of apples, especially of an increse quantity of soluble sugars in the Generos, Starkrimson and Ionagold varieties and maintaining a high acidity in the Idared and Ionagold varieties.

Effect of essential oils application on fruit quality during storage conditions

Essential oils are natural substances extracted from aromatic and medicinal plants. These compounds play an important role in fruit preservation, contributing to the safety and extension of the shelf life. They are also non-toxic, hypoallergenic, and safe for consumption (Laranjo et al., 2017).

The effectiveness of essential oils is attributed to the presence of phenolic compounds, terpenes and alcohols being an important and healthy alternative to chemical compounds applied to fruits during storage (Laranjo et al., 2017). The extension of the fruit storage period results from their action by reducing the activity of antioxidant enzymes.

Experiments conducted by Rabiei et al. (2011) reported that post-harvest treatment of apples with thyme and sage oil improved fruit quality after 5 months of storage at low temperature 1°C and relative humidity 90%.

The results of Shehata et al. (2020) indicated that strawberry fruits treated with citrus essential oils (lemon, orange, tangerine) have a higher content of antioxidants and phytochemical compounds than untreated fruits, due to the protective effect against molds. It is noteworthy that the application of all citrus oils extended the storage period and delayed the deterioration of strawberry fruits up to 18 days, maintaining the content of soluble sugars, acidity and anthocyanin content.

Recently, Cai et al. (2020) developed starch films impregnated with thyme essential oil that by applying them to mango fruits during storage found that it inhibited fruit weight loss, reduce vitamin C loss, and delayed quality changes related to mango fruit ripening. Essential oils are a source of antimicrobial bioactive compounds, which can be used for plant protection with strong antifungal and antibacterial effects.

Romanian researchers have used essential oils with an antimicrobial effect especially as antifungal agents for cereals during storage, and also to stimulate germination in different cereals (Dudoiu et al., 2017; Fatu et al., 2017).

A wide variety of plant volatile compounds and essential oils have been tested for postharvest disease control in fruit and are promising potential antifungal agents for use as biofungicides in fruit storage disease protection (Sivakumar and Bautista-Banos, 2014). Moreover, since essential oils have low toxicity to mammals, are biodegradable, multifunctional, non-persistent in the environment and are easy and cheap to obtain, the possibility of using them in the protection of stored fruits is considered a very attractive idea in recent years (Pandey et al., 2017).

Different studies have explored the potential of essential oils as antifungal agents (Shehata et al., 2020; Cai et al., 2020). Oregano and thyme essential oils have significant antifungal activity against *B. cinerea* and *P. expansum* infection in stored apples (Rabiei et al., 2011).

Despite the antifungal potential at low concentrations of many volatile compounds tested in vitro, commercial implementation is severely limited due to problems related to decreased *in vivo* efficacy, potential phytotoxicity, and low sensory characterization.

The studies carried out in Romania, regarding the behavior of apples from the varieties Idared, Golden Delicious, Starkimson, claimed that those treated with volatile oils and calcium chloride presented a better resistance to the attack of pathogens and also there was a delay in the appearance storage diseases by 1-2 months (Anghel, 2007).

The studies of Cosoveanu et al. (2013) concluded that essential oils, aqueous and alcoholic plant extracts of different species of *Artemisia* and *Argyranthemum frutescens* show antifungal activity against important pathogens during fruit storage such as *P. expansum, Botrytis cinerea*, *Alternaria alternata*. The results also indicate that the plant extracts used have different antifungal activity depending on the plant species, extract type, concentration and pathogen type.

Also, Groza (2015) studying the effect of some natural products (plant extracts and propolis) on the fungi *Monilia fructigena* and *Penicilium expansum* in different varieties of apples during storage found that propolis tincture had a fungistatic and fungicidal effect on the colonies of *P. expansum*, however, plant extracts had no effect, the fungus developing immediately. However, both the propolis tincture and the extracts had an inhibitory effect on the colony of *Monilia fructigena*.

Effect of beneficial microorganisms on the induction of disease resistance in fruits during storage

Bioagents of the *Trichoderma* genus are extremely versatile in inhibiting post-harvest fruit diseases caused by different phytopathogens *Penicilium expansum* (apples, pears), *Botrytis cinerea* (strawberry, table grape) (Batta et al., 2007; Batta, 2004).

Senthil et al. (2011) support that *Trichoderma harzianum* applied during storage reduces the incidence of blue mould produced by *P.expansum* in grapes and increases the storage time at low temperatures. El-Katatny and Emam (2012) reported that post-harvest treatments with *Trichoderma* suspensions in a concentration of 10^7-10^8 spores/ml increases resistance to *Alternaria alternata*, *Aspergillus* spp. and significantly decrease the incidence of the disease in tomatoes. Other studies have shown that post-harvest treatment with *Trichoderma* in conditioned form leads to a strong reduction in disease intensity caused by blue mold in apples (Quaglia et al., 2011; Batta, 2007). Also, long-lasting protection against infection is obtained and fruit damage is prevented or delayed.

In Romania, there are only a few studies on increasing the systemic resistance of vegetables treated with beneficial microorganisms and highlighting the accumulation of some secondary metabolites as well as the intensification of the activity of antioxidant enzymes. A study on the induction of systemic resistance by *Trichoderma* strains confirmed the intensification of photosynthesis and the increase in the content of assimilatory pigments in tomato plants and thus the reduction of symptoms caused by *Botrytis cinerea* infection (Alexandru et al., 2013).

The exploitation of biopreparations based on microbial biocontrol agents in our country was low due to the low effectiveness compared to chemical fungicides. However, some biopreparations have been realized based on *Trichoderma viride* (Trichosemin 25) used to control gray rot and white rot on sunflower and beans, and based on *Trichoderma viride* (Trichopulvin) used to combat gray rot in grapevine and strawberry (Sesan et al., 1999) which have similar effectiveness to that of chemical products used for the same purpose, presenting the advantage of being classified in the IV toxicity group.

Yeasts have been extensively used to control post-harvest diseases in various fruits, they are safe antagonists, do not produce antibiotics, have no risks and have a low ecological impact, the mechanism of action is based on competition for nutrients and space and by the production of antifungal metabolites (Sharma et al., 2009; Janisiewicz & Korsten, 2002). Induction of resistance in host tissue has been suggested as another mode of action of antagonistic yeasts for inhibition of postharvest diseases in fruit (Sharma et al., 2009; Spadardo et al., 2002).

Numerous yeasts from the genus *Cryptococcus, Pichia, Saccharomyces,* and *Kloeckera* have particularly attracted attention because they can colonize the fruit surface for a long period of time; in dry conditions they produce extracellular polysaccharides and can restrict the areas of colonization and germination of fungal propagules, being very little affected by pesticides. Some studies have reported that different strains of *Candida saltoana*, *Candida oleophila*, *Candida laurentii,* and *Pichia guilliermondii* can induce resistance to numerous postharvest diseases in fruits (Droby et al., 2009).

Wounded (damaged) tissue has been shown to be characterized by a marked presence of reactive oxygen species (ROS) and the ability of antagonistic yeasts to adapt to oxidative stress has been closely related to yeast multiplication in fruit wounds and biocontrol efficacy (Castoria et al., 2003). The increased activity of superoxide dismutase and catalase was closely correlated with the increase in biocontrol efficiency and stress tolerance induced by yeasts, detoxification is dependent on antioxidant enzymes and the increase in their activity is of great importance for the amelioration of oxidative stress.

It is well known that polyphenoloxidase is an important enzyme associated with disease resistance induced by biocontrol yeasts such as *Rhodotorula mucilaginosa* (Liu et al., 2013), *Rhodosporidium paludigenum* (Zhu et al., 2013). The results of Lu et al. (2014) showed that polyphenol oxidase activity in apples treated with *R. paludigenum* combined with 1% chitin was significantly higher than that in apples treated with yeast alone, indicating that the increase in enzyme activity may be part of the mechanism by which resistance to blue mold is induced. Addition of chitin to yeast suspension increases catalase and superoxide dismutase activity and decreases malondialdehyde content. However, most of the antagonistic yeasts when used individually cannot completely control postharvest fruit diseases like synthetic fungicides, thus, many ways have been proposed to improve the effectiveness by combining with other compounds (Zhu et al., 2013). In the same way, it was shown that chitin in a concentration between 0.5-1% can increase the effectiveness of the antagonistic yeasts *Cryptococcus laurentii* (Yu et al., 2008).

Also, some yeasts such as *Rhodotorula glutinis*, *Cryptococcus laurenti, Aureobasidium pullulans* actively contribute to the decrease of patulin accumulation in *Penicilium expansum* infected apples during storage, because they can metabolize this mycotoxin. According to Romanazzi et al. (2013), chitosan can control fungal diseases that damage the quality of fruits during storage. The storage time of papaya fruits was extended by 33 days by using the combined treatment between chitosan and calcium chloride (Romanazzi et al., 2017).

CONCLUSIONS

◘ Post-harvest treatment of fruits with different natural compounds and beneficial microbial agents can maintain fruit quality and induce resistance to different pathogens during storage.

◘ The natural compounds with a role in maintaining post-harvest fruit quality and inducing resistance to diseases frequently used are chitin, chitosan, salicylic acid, essential oils, plant extracts, β-aminobenzoic acid.

◘ The essential oils used to maintain quality and induce post-harvest resistance to fruit are: thyme oil, cinnamon oil, mint oil, clove oil, and oregano oil.

◘ From the beneficial microbial agents most often used in the activation of systemic resistance are various fungal strains: *Trichoderma* spp and different yeasts (*Candida saltoana, Candida oleophila, Candida laurentii, Pichia guilliermondii*).

◘ Plant defense mechanisms can be enhanced by combined application of microbial biocontrol agents and natural compounds versus their individual application. Also, these combined treatments have a superior effect compared to the application of chemical fungicides in diseases control.

◘ Post-harvest treatments represent an eco-friendly, safe, non-toxic alternative for the environment and human health, cheap and easy to apply to fruit during storage.

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SENSORIAL EVALUATION OF ORGANIC STRAWBERRIES AND RASPBERRIES: EFFECTS OF COMPANION PLANTS

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Abstract

This study investigates the effect of companion planting on the sensory quality and consumer acceptance of strawberries (Amandine variety) and raspberries (Kwanza variety). Samples were evaluated based on attributes such as color intensity, aroma, taste, and re-consumption intention. The results showed that control groups of both fruits received higher sensory scores and re-consumption intention, with both strawberries and raspberries intercropped with Borago officinalis L. showing slight changes in taste profiles. Overall, the differences between the control and intercropped samples were minimal, indicating that companion planting had little impact on fruit quality. These findings suggest that while companion planting may not significantly alter sensory qualities, it offers a sustainable agricultural practice that supports biodiversity without compromising consumer preferences.

***Key words**: berry crops; consumer acceptance; intercropping.*

INTRODUCTION

Berry production is influenced by a range of biotic and abiotic stressors, with pests and diseases being primary concerns for growers. Recent trends in agriculture have seen a shift towards more resilient cropping systems that align with consumer demands for high-quality berries, both in taste and nutritional content (Maitra & Gitari, 2020). As global consumer preferences lean towards food safety and natural products (Lelieveld, 2015), and the agricultural sector grapples with the challenges of climate change (Bucur & Dejeu, 2016; Pickering et al., 2014), organic farming practices are becoming increasingly popular. These practices not only help protect the environment but also offer sustainable solutions without compromising crop yields, particularly on soils with high fertility (Rusu et al., 2015). One such method gaining attention is the use of companion plants in berry crops, which can support biodiversity and enhance overall productivity by improving soil health and pest control.

Conservation biological control is a central aspect of this approach, aiming to increase biodiversity while providing sustainable pest management, especially in perennial crops (Landis et al., 2000). For example, in raspberry plantations lasting over 10-15 years, gradually increasing the presence of natural predators can lead to effective long-term pest control. Companion planting offers several benefits for berry crops, such as reducing pest populations, maintaining soil fertility, attracting beneficial insects, and enhancing crop health. Specific companion plants, such as flower strips (e.g., *Borago officinalis* L., *Calendula arvensis* L.), trap plants, and cover crops, each provide unique ecological services that contribute to the overall health of the crop and its surrounding environment.

In the context of strawberry and raspberry cultivation, several technologies have been employed to enhance growth, with varying

degrees of success. Traditional strawberry farming methods, such as wide row spacing, often leave a significant portion of the soil unused (Dane et al., 2016), leading to inefficiencies. With an increased focus on sustainability, intercropping has emerged as a promising solution to better utilize available land (Dane et al., 2016). Similarly, raspberry cultivation has evolved, with high tunnels and protected environments becoming more common for long-cane varieties, although field cultivation remains a challenge for maximizing production (Qiu, 2017; Takeda & Soria, 2011).
Both strawberries and raspberries are highly valued fruits, rich in bioactive compounds and micronutrients that contribute to their unique taste, nutritional value, and organoleptic qualities (Dane et al., 2019; Giampieri et al., 2012). Strawberries, for example, contain a blend of antioxidant and anti-inflammatory compounds beneficial for cardiovascular health (Sparacino et al., 2024), while raspberries are rich in vitamins, phytochemicals, and minerals, making them highly recommended for a healthy diet (Bobinaitė, 2016). Visual quality, including size, color, and freshness, is a key factor in consumer purchasing decisions for both fruits (He et al., 2020). As consumer preferences are heavily influenced by sensory attributes, such as taste and appearance, it is crucial to understand how different cultivation techniques, including companion planting, impact these qualities (Moazzem et al., 2024).
This study aims to assess the effects of various companion plants on the sensory quality and nutritional content of strawberries and raspberries. By evaluating how companion plants influence attributes such as taste, appearance, and consumer acceptance, this research seeks to provide insights into how sustainable practices can enhance berry crop production without compromising fruit quality.

MATERIALS AND METHODS

Samples

Samples of both strawberries (Amandine variety) and raspberries (Kwanza variety) were harvested in September 2022 from the Rodagria Produce Agricultural Cooperative in Ogoru village, Călărași County. All samples were stored under controlled conditions at 1±0.2°C and 90±5% relative humidity for 12 hours prior to being washed and presented to the evaluation panel.
For the strawberry assessment, two sample types were analyzed: a control group and strawberries cultivated with borage as a companion plant (Figure 1). In the raspberry evaluation, the panel assessed four sample types: a control group and raspberries grown with annual, perennial, and borage companion plants (Figure 2).

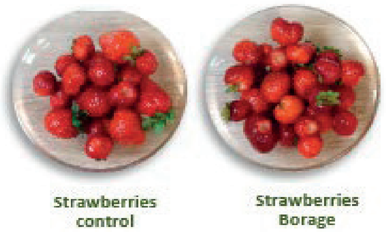

Figure 1. Strawberry samples

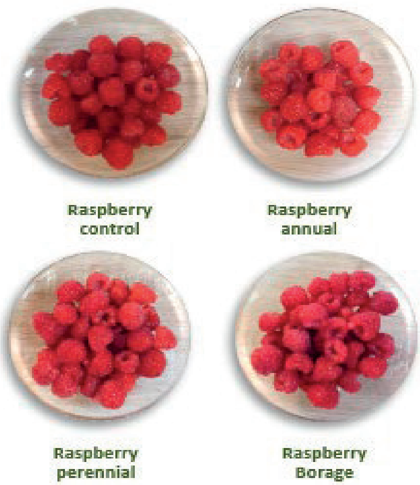

Figure 2. Raspberry samples

Consumer acceptance analysis

Consumer acceptance testing was conducted in the Sensory Analysis Laboratory at the Research Center for Studies of Food Quality and Agricultural Products, part of the University of Agricultural Sciences and Veterinary Medicine of Bucharest (USAMV of Bucharest).
The sensory evaluation of the fruits was conducted to observe the impact of cultivation technology on their sensory characteristics.
The sensory evaluation focused on key properties, including color intensity, aroma,

primary taste components, taste intensity, and aftertaste duration, as well as the percentage of participants indicating an intention for repeat consumption. Assessments were carried out during two separate sessions for strawberries and raspberries, as detailed in Table 1.

Table 1. Sample codification and panel description

Samples	Code	Total number of respondents
Strawberry control	SC	32
Strawberry borage	SB	
Raspberry control	RC	41
Raspberry annual	RA	
Raspberry perennial	RP	
Raspberry borage	RB	

Participants from various age groups were selected to assess the acceptance of fruits cultivated using different agricultural techniques. They evaluated the sensory attributes of the fruits using a 7-point hedonic scale, with ratings ranging from "1 - very unpleasant" to "7 - very pleasant."

The scores for each attribute were calculated as the arithmetic mean ± standard deviation, providing a quantitative measure of sensory acceptance.

RESULTS AND DISCUSSIONS

Sensory evaluations were conducted to assess various attributes of fresh strawberries and raspberries, including color intensity, aroma, primary taste components, taste intensity, after-taste duration, and intention to repurchase (%). The sensory panel for strawberries consisted of 72% female and 28% male participants, while for raspberries, 66% were female and 34% male. As shown in Table 2, the control samples (SC and RC) achieved higher scores in aroma, and taste intensity, particularly in the strawberry control (SC), which scored the highest in aroma (6.13 ± 1.11). In contrast, raspberries intercropped with borage (RB) received higher ratings for aroma (5.32 ± 1.02) than other treatments.

Table 2. Consumer evaluation of key aspects as color, aroma, and taste

Samples analyzed	Color level of the product	Product aroma	Taste intensity level	Post-taste duration level
Strawberries				
SC	5.84±1.28	6.13±1.11	6.00±1.09	5.91±1.01
SB	6.09±0.84	5.16±1.52	5.75±1.52	5.56±1.58
Raspberries				
RC	6.20±0.80	5.28±1.12	5.54±1.27	5.39±1.25
RA	6.37±0.62	5.10±1.16	5.51±1.36	5.24±1.38
RP	6.37±0.65	5.08±1.12	5.61±1.36	5.66±1.26
RB	6.20±1.09	5.32±1.02	5.68±1.02	5.46±1.13

No significant differences were observed between strawberries grown in the control group and those intercropped with borage (*Borago officinalis* L.) (Figure 3). In the control group (SC), the taste profiles were as follows: 6.25% of participants identified a sour taste, 9.37% detected a sweet-tart taste, 37.50% perceived a sweet taste, 3.12% noted a balanced taste, 12.50% found the characteristic strawberry flavor, and 18.75% described it as aromatic. For the strawberries intercropped with borage (SB), the taste profile showed slight alterations: 9.37% of consumers detected a sour taste, 12.50% found a sweet-tart taste, 37.50% a sweet taste, 6.25% described the taste as balanced, 9.37% identified the characteristic strawberry flavor, and 12.50% found it aromatic (Figure 3).

The overall sensory score for strawberries from the control group (SC) was the highest, achieving 23.88 out of 30, indicating the best overall sensory performance. Strawberries intercropped with borage (SB) received a slightly lower score of 22.56 (Figure 5). These results suggest that while borage intercropping did not result in significant changes to the sensory profile of strawberries, it did lead to a slight reduction in overall consumer acceptance.

For raspberries, no significant differences were observed between the control group (RC) and those intercropped with various plants (annual plants, perennial plants, or borage). The primary taste components for raspberries in the control group (RC) were sour (36.58%), sweet-tart (24.39%), and sweet (9.76%). Raspberries intercropped with annual plants (RA), perennial plants (RP), and borage (RB) showed minor variations in taste profiles: 29.51%, 26.83%, and 24.39% of participants, respectively, identified a sour taste. The sweet-tart taste remained prevalent in all variants, though no significant differences were observed in overall flavor descriptors between treatments (Figure 4). Raspberries from the control group (RC) scored 22.40, while those intercropped with annual (RA), perennial (RP), and borage (RB) plants scored 22.22, 22.71, and 22.66, respectively (Figure 5). These results indicate that while intercropping slightly reduced the sensory scores for raspberries, the differences were minor, suggesting limited impact on the sensory quality due to companion planting

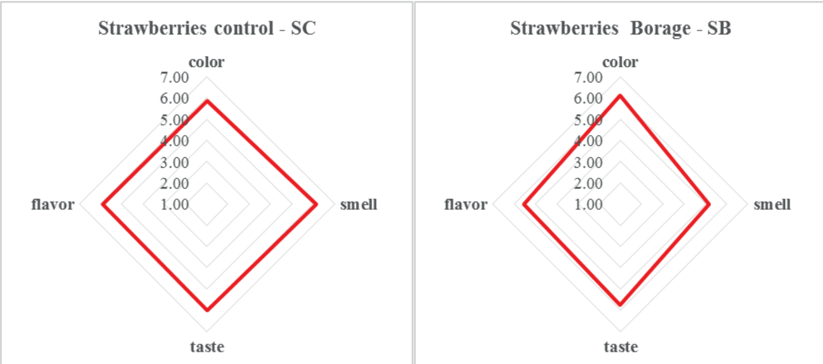

Figure 3. Consumer acceptance evaluation of strawberries using a 7-point hedonic scale

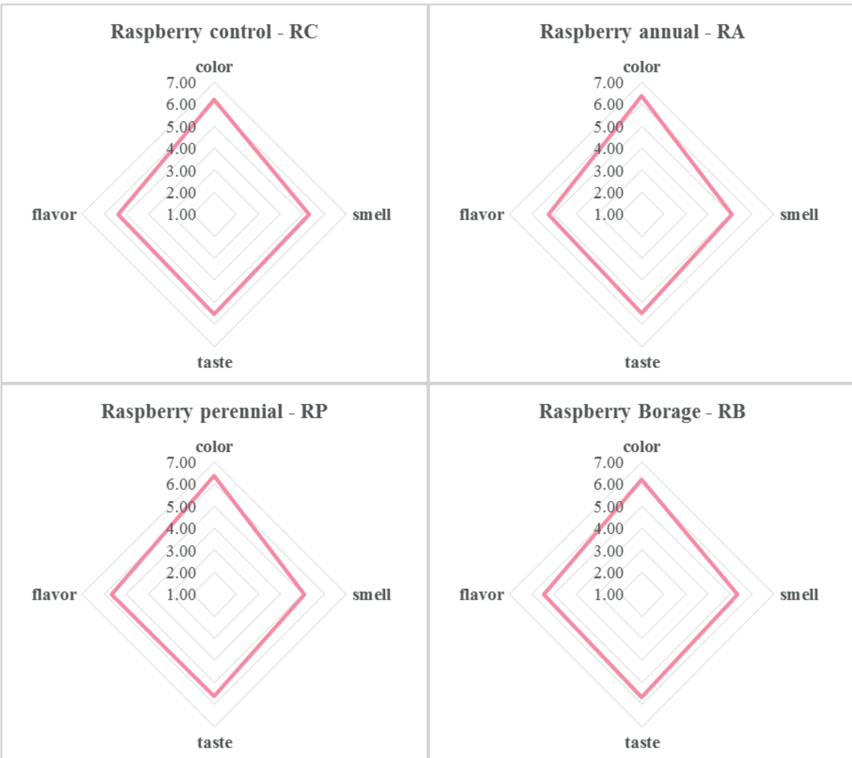

Figure 4. Consumer acceptance evaluation of raspberry using a 7-point hedonic scale

Repurchase intention was evaluated for all treatments. For strawberries, the control group (SC) achieved the highest re-consumption rate, with 93.75% of participants indicating "YES" and only 6.25% selecting "NO." Strawberries intercropped with borage (SB) had a slightly lower re-consumption rate, with 84.38% responding "YES" and 15.63% "NO" (Figure 6). For raspberries, the control group (RC) had a 78.05% "YES" rate, while raspberries intercropped with annual (RA), perennial (RP), and borage (RB) plants showed a reduced re-consumption rate, with 64.29% selecting "YES" for RA and RP, and 78.57% for RB. The reduced repurchase rates for raspberries intercropped with annual or perennial plants suggest that the sensory characteristics of these variants did not meet the expectations of some consumers.

Both strawberries and raspberries demonstrated positive consumer acceptance, with control groups consistently achieving higher scores in sensory evaluations and repurchase intentions. Intercropping with companion plants, such as borage, had minimal impact on sensory attributes. While the use of intercropping aligns with sustainable farming practices, its effect on improving sensory quality was limited. This suggests that environmental benefits associated with intercropping may not necessarily translate into enhanced consumer satisfaction.

In particular, raspberries showed strong performance in visual appeal but had lower scores for sensory attributes like odor and texture. These findings highlight the potential for further improvements through optimized post-harvest handling or additional cultivation techniques. Enhancing these sensory traits could elevate consumer preferences for raspberries, aligning with observations from Ingrassia et al. (2018) and other studies.

This research supports the conclusions of Aoki and Akai (2023) and Sparacino et al. (2024), emphasizing the intricate interplay between cultivation methods, sensory attributes, and consumer behavior. Aoki and Akai (2023) focused on the economic valuation of sustainable farming practices, while Sparacino et al. (2024) identified appearance, health benefits, and sustainability as key consumer drivers. Together, these studies underscore the importance of integrating sensory quality with sustainability practices to enhance consumer acceptance and market success of berry products.

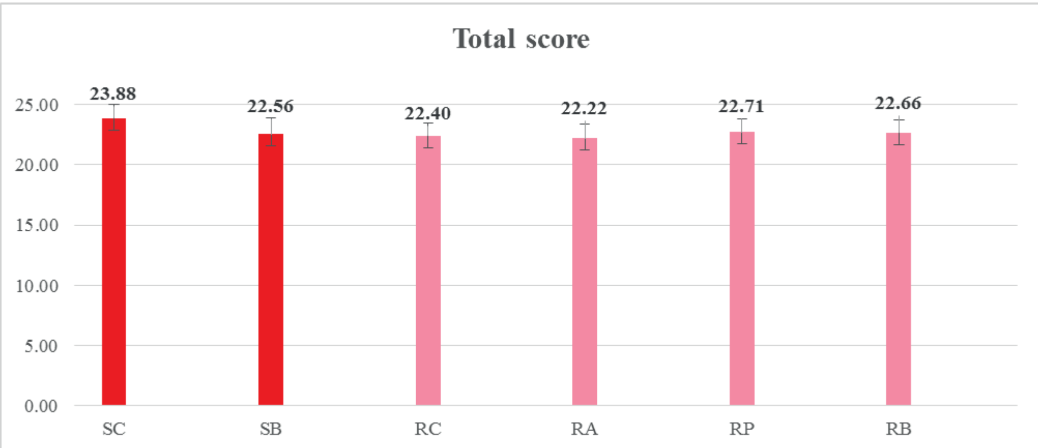

Figure 5. Consumer acceptance evaluation of fresh strawberries and raspberries (total score)

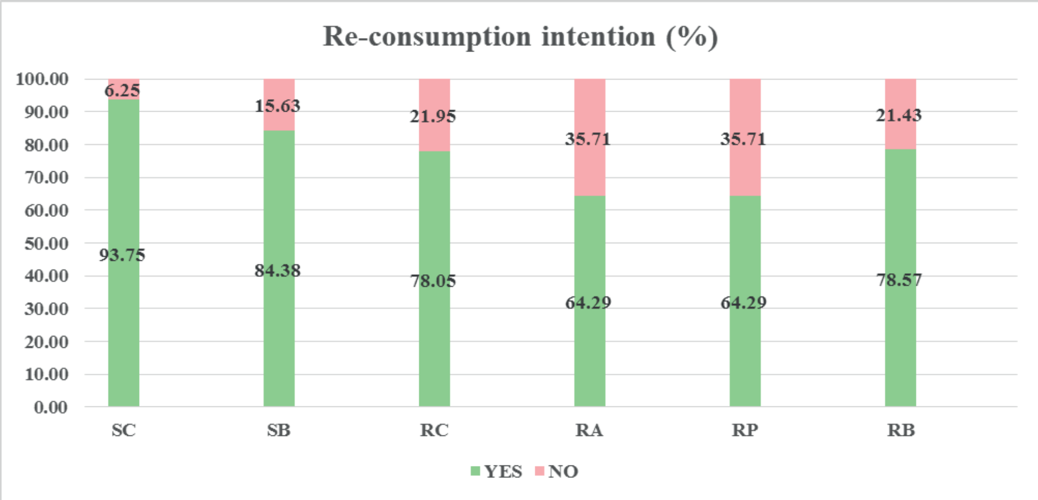

Figure 6. Re-consumption intent of fresh strawberries and raspberries (%)

CONCLUSIONS

The results indicate that control groups of both strawberries and raspberries were preferred by consumers, with higher re-consumption intentions and sensory scores, suggesting a stronger preference for traditionally grown fruits. While the introduction of *Borago officinalis* L. as a companion plant slightly reduced re-consumption intention, the differences in total sensory scores were minimal, indicating that intercropping had little impact on overall fruit quality. However, raspberries intercropped with *Borago officinalis* L. performed better than those with annual or perennial plants. These findings suggest that while companion planting may not drastically affect fruit sensory quality, it can still be a viable sustainable practice in organic agriculture, with potential benefits for biodiversity and environmental sustainability. Future research could explore innovative cultivation methods or advanced post-harvest techniques to further improve sensory attributes, particularly odor and texture, ensuring a balance between environmental sustainability and consumer satisfaction.

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RESEARCH ON THE DEVELOPMENT OF RASPBERRY CULTIVATION IN CONDITIONS OF ORGANIC AGRICULTURE

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Abstract

This paper highlights the evolution of raspberry culture between March and November 2023 on a 1000 m certified organic field in the Bucharest Region. It is based on data obtained during the specified period, monitoring soil parameters at the beginning of the establishment of the crop of two species of raspberries, namely: Opal variety and Delniwa variety. The two varieties were divided into two rows, each occupying half of the total area. The results obtained were evaluated both per variety and per row. There were observed differences between varieties but also between rows in terms of the amount of fruit obtained, in each month of the harvest period. These results support farmers, highlighting varieties suitable for organic crops in the monitored area.

Key words: *raspberries, culture, ecological, development, yield.*

INTRODUCTION

The raspberry (*Rubus idaeus* L.), is a fruit-bearing shrub belonging to the Rosaceae family of the genus Rubus. This family includes fruit trees such as apple, pear, plum, cherry; fruit bushes: raspberry, mulberry but also ornamental shrubs such as rose or other perennial species: Fragaria (strawberry) (Titirică et al., 2023; Veljković et al., 2019; Krauze-Baranowska et al., 2014).

It has been cultivated since ancient times in areas such as Europe, North Asia and North America. In Europe the main raspberry producing countries are Russia, Serbia, Poland, Spain, Ukraine, England (Wróblewska et al., 2020). In Romania most of the raspberry fruits generally come from wild flora, but this is not enough to satisfy the need for domestic consumption and export. This can only be achieved by growing on larger areas of land (Dulf et al., 2012).

According to the FAO in 2022, the main producers of raspberries in the world were Russia, Mexico and Serbia (Figure 1). Romania had an area of 90 hectares cultivated with raspberries and a production of about 170 tons.

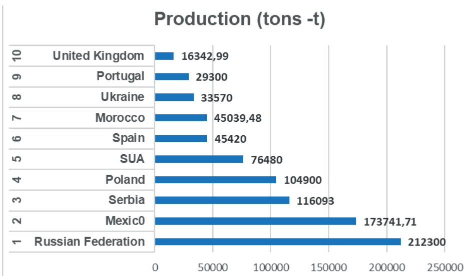

Figure 1. Main producers of raspberries worldwide (https://www.fao.org/faostat/en/#data/QCL/visualize)

In recent years, consumers have started to turn their attention more towards healthy, organic foods that have multiple health benefits. Raspberries can be considered such a food because they are a pleasant-tasting, sweet-sour, aromatic fruit, rich in vitamins, minerals, antioxidants and fibre. It has a low caloric content being an ideal food in diets, whether consumed in its natural state or in various processed products (Bobinaitė et al., 2020;

Jakobek et al., 2007; Koraqi et al., 2019; Ponder et al., 2020; Lopez-Corona et al., 2022). Although organically produced food does not always look pristine or commercial, it costs more, but it is healthier, and this makes consumers increasingly prefer it (Ardelean, 2023).
Productivity and quality of raspberry fruit depends primarily on the variety, but also on various factors such as: applied agricultural techniques, environmental conditions, soil, climate, proper crop management including fertilization (Anttonen & Karjalainen, 2005; Milivojevic et al., 2011; Palonen et al., 2017; Valentinuzzi et al., 2018).
Given the fact that raspberries are a very popular functional food and the increasing need to grow raspberries in Romania to meet the growing needs of consumers, this paper aims to analyse raspberry production for the two varieties studied.

MATERIALS AND METHODS

The raspberry crop was set up on a 1000 m^2 plot of land certified ecologically. within INMA Bucharest.
For the planting of the two varieties of cuttings, the land was first prepared with a scarifier at a depth of 60 cm in order to decompress the deep layers of soil, then shredded and levelled first with a disc harrow and then with a rotary tine harrow.
After soil preparation, 6 soil samples were collected, one from the corners and two from the middle of the field for two depths (0-20 cm and 20-40 cm). Samples were homogenized and analyzed to determine both pH and organic and inorganic soil compounds.
Two raspberry varieties were chosen for this study, namely Opal cuttings and Delniwa cuttings. The planting material was certified (disease free) nursery grown (cuttings, root cuttings) without soil on the roots (bare roots).
The Opal variety (Figure 2 a) was purchased from the Institute for Research and Development in Pomiculture (ICDP) Piteşti-Mărăcineni.
The Delniwa variety (Figure 2 b) was purchased from Nurseries Fundulea and was accompanied by phytosanitary passports and quality documents.
The raspberry cuttings were planted in mid-March 2023. The plot was divided into four rows, the first two rows were planted with Opal and the other two with Delniwa, each row having three 26 m zones delimited by a 4 m crossing zone. The row spacing was 3.3 m and the plant spacing was 0.5 m (Opal) and 0.75 m (Delniwa), according to the staking scheme shown in Figure 3.

Figure 2 a. The Opal variety

Figure 2 b. The Delniwa variety

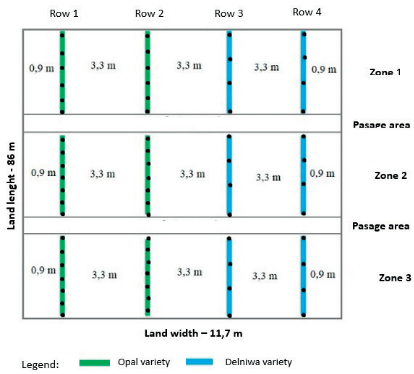

Figure 3. The scheme of picketing the land

Before planting, the planting material was moistened for 2 hours and then introduced into the soil at a depth of 20-25cm using a hand planter (Figure 4), then the hole was covered with loose soil.

Figure 4. Planting raspberry cuttings

Finally, a drip irrigation system was installed and biodegradable mulching film was applied to the cuttings to increase yield and maintain soil moisture (Figure 5).

Figure 5. Laying irrigation system and mulching film

For optimal maintenance of the crop and to ensure the verticality of the raspberry stems in July 2023, a support system (trellising) was installed on the trellises (Figure 6).

Figure 6. Trellis support system

The system is composed of 1.7 m high support poles to which three rows of wires of varying widths are attached, the first wire is 60 cm above the ground and the other two are approximately 30 cm apart (Figure 7).

Figure 7. Placement of the support system on the trellises

The raspberry crop establishment technology is shown schematically in figure 8.

Throughout the vegetation period from planting (mid-March) to dormancy (end of November), the raspberry crop was monitored and maintenance work was carried out. The evolution of the crop in the first year after establishment can be seen in Figure 9.

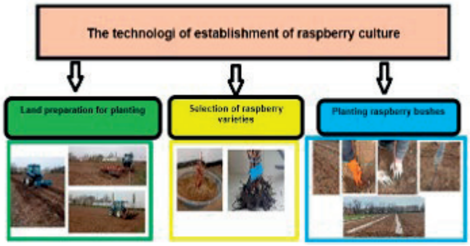

Figure 8. Technology of establishing raspberry culture

Figure 9. The evolution of raspberry culture in the first year after establishment

RESULTS AND DISCUSSIONS

Results from soil samples taken before crop establishment showed that the soil had a pH of 6.54 at 0-20 cm depth, and a pH of 6.63 at 20-40 cm depth. The values of organic and inorganic compounds analysed are highlighted in Table 1.

Table 1. Organic and inorganic compounds analysed

	Organic and inorganic compounds analysed	Content to a depth of 20 cm (mg/kg)	Content to a depth of 20-40 cm (mg/kg)
1	Lead	17.0	17.0
2	Nickel	18.9	18.2
3	Copper	19.6	18.6
4	Cadmium	0.35	0.33
5	Manganese	509.1	493.3
6	Zinc	78.9	79.8
7	Aqueous extract chlorides (1:5)	106.4	88.6
8	Sulphates ($S\text{-}SO_4^{2-}$) aqueous extract (1:5)	96.0	72.0
9	Soluble calcium aqueous extract (1:5)	319.7	269.0
10	Soluble magnesium aqueous extract (1:5)	42.0	31.0
11	Soluble sodium aqueous extract (1:5)	11.0	11.3
12	Exchangeable calcium	5474.0	4310.0
13	Exchangeable magnesium	389.5	400.6
14	Exchangeable sodium	267.9	161.7
15	Exchangeable potassium	335.7	331.6
16	Ammonium ($N\text{-}NH_4^+$) extractable	7.255	5.772
17	Nitrit ($N\text{-}NO_2^-$) extractibil	0.903	0.729
18	Extractable nitrate ($N\text{-}NO_3^-$)	6.497	3.424

Although samples were taken from two different depths, the compound values did not differ significantly.
The obtained values of the analysed compounds are within the parameters for organic crop development.
The first raspberry harvest was on 16 June and the last on 28 November. During this period, depending on the flow of ripe fruit, harvests were made once or twice a week.
The Opal variety started fruiting earlier. The first harvest was directly from the planted cuttings (Figure 10 a) and in September the new shoots started to bud (Figure 10 b). As can be seen the fruits are of medium size but the yield per plant is very high.

Figure 10 a -Harvest from planted cuttings Opal variety

Figure 10 b Harvest from new sprout Opal variety

The Delniwa variety started fruiting later because the planted cuttings did not fruit, but new shoots sprouted from the same root (Figure 11) and fruited in the first year. The fruits of this variety are larger than those of Opal.

Figure 11. Harvest from the Delniwa variety

Fruit yield

As regards raspberry fruit yield in each month of the harvest period, differences were observed between varieties but also between rows (Figure 12).

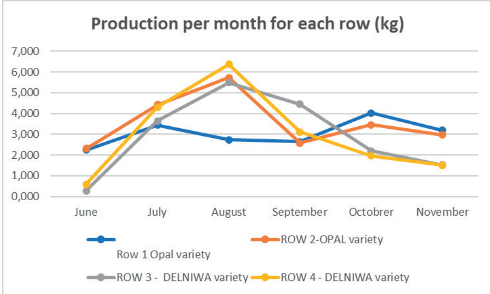

Figure 12. Production per month for each row in kg

At the beginning of the harvest period, the yield was higher in the Opal variety, without too great a difference between rows. In the middle of the harvest period, the yield was higher in Delniwa but also in the second row of Opal, and at the end of the harvest period the yield was again higher in Opal.
Figure 13 shows the total yield per row. The highest yield per row was recorded in Opal with major differences between rows 1 and 2. The Delniwa variety although had a lower yield, the differences between rows were not considerable.

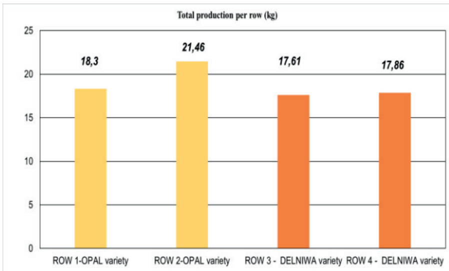

Figure 13. The amount of harvested raspberries in kg

Although the Opal variety recorded higher yields at the beginning and end of the harvest period, it also had the highest yield in row production, in the end the total yield was higher for Delniwa.

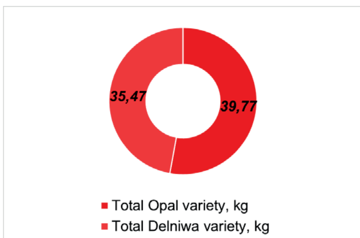

Figure 14. Total production by varieties

CONCLUSIONS

Following the research carried out in the first year since the establishment of the crop we can draw the following conclusions:

The Opal raspberry variety is a variety with bushes with erect stems that fructify in the year of formation. The harvest period is from mid-June to late autumn.

The Delniwa raspberry variety bears fruit later, the first fruits appear on the newly formed shoots. The optimal harvest period is from mid-July to late autumn.

Although there were differences in yield both between varieties and between rows of the same variety, the total yield by variety did not register minor differences (about 4 kg).

Finally, we can say that both varieties are suitable to be grown organically in the monitored area.

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AN ASSESSMENT OF CURRENT STATUS, FUTURE TRENDS AND OPPORTUNITIES FOR IMPROVING EXOTIC AND UNDERUTILIZED POME FRUIT SPECIES PRODUCTION IN ROMANIA

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Abstract

With changing climatic conditions, some of the subtropical and tropical species adapt to the Northern regions finding more favourable conditions for growth and reproduction. In such context, fruit growing in the temperate regions is undergoing an important change in zoning of the species and introducing new ones that can adapt in the new ecological conditions. Species as: goji berry (Lycium chinense Mill.), saskatoon berry (Amelanchier alnifolia (Nutt) Nutt. ex M. Roem), pawpaw (Asimina triloba (L.) Dunal), kiwi (Actinidia deliciosa (A.Chev.) C.F. Liang & A.R.Ferguson), pomegranate (Punica granatum L.), kaki (Diospyros kaki Thunb.), ziziphus (Ziziphus jujuba Mill.), fig (Ficus carica L.) and medlar (Mespilus germanica L.) have a high ecological plasticity and potential to adapt in the new environment.
Also, an opportunity for growing these species into culture is that the Romanian consumers tend to be more curious and willing to try new fresh products so, there are emerging new opportunities for local producers and marketing these pome fruits with high nutritional potential and taste appeal.

Key words: *climate change, zoning, fruit growing, ecological plasticity.*

INTRODUCTION

Climate change significantly contributes to the change of agriculture in our country and thus new species of plants are introduced into culture.

On the other hand, increasing population and consumption in the last century are placing unprecedented demands on agriculture and natural resources (Padulosi S. et al., 2013). In the last decades fruit consumption is becoming more and more varied and this trend leads to the necessity for a more various fruit assortment available on the market (Kariuki L.W., 2013).

Romania is a rich source of genetic variability and diversity of different horticultural crops that refers to plant type, morphological and physiological characteristics and their variations in term of reactions to diseases and pests (resistance, tolerance), adaptability and distribution through the country (Gupta S., 2011).

With an area of 238.397 km^2, Romania develops most diverse conditions for fruit growing in terms of soil types and slopes, altitudes, climatic conditions and insolation.

All these diversity drives to great climatic variations from North to South and from East to West and implicit a great variability among fruit crops. Generally, the Romanian soil is rich in organic matter and has all the necessary characteristics for agriculture.

According to FAO, the total harvested area in Romania, year 2022 is 7457490 ha, from which 142340 ha consist of fruit growing species (2%) (FAOStat, 2024).

Romanian fruit production mainly consists of temperate fruits species as apple, plum, cherries, pear, apricot, peach, berries and dry fruits (walnut, almond, hazelnut, chestnut). A serie of species with high ecological plasticity as ziziphus, medlar, goji, saskaton can also be found in different regions of our country but they are underutilized. These plants are also very important because of their medical value, melliferous potential and are highly decorative.

In the last decades due to the climate change some of the subtropical species find proper condition for cultivation (kiwifruit, persimmon, kaki, pawpaw etc).

These fruits are a valuable source of nutrients as they have important medicinal properties and great potential to contribute to the location specific food chain production. In addition to ensuring the diversification of the food base, different indigenous fruit species produced at

different times of the year, are ensuring the supply of fresh consumption but also raw material for processing throughout the year (Jones M.P. et al., 2009; Thillakawardane T.U., 2009).
The aim of the present study is to analyse the current state, the trends and future opportunities for introducing exotic and underutilized fruit species in Romanian production.

MATERIALS AND METHODS

In order to fulfil the general purpose of the present study we focused on:
- general documentation from specialized literature;
- collecting and centralizing data from the competent authorities;
- data analysis from available data base: FAO (Food and Agriculture Organization) USDA (US Department of Agriculture).

We took into study nine species and they are listed in Table 1.

Table 1. The list of studied species

Popular name	Scientific name	Number of available varieties (Official Catalogue of Cultivated Plants in Romania-2023)
Goji berry	*Lycium chinense* Mill.	6
Saskatoon berry	*Amelanchier alnifolia* (Nutt) Nutt. ex M. Roem	1
Pawpaw	*Asimina triloba* (L.) Dunal	5
Kiwi	*Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R Ferguson	4
	Actinidia arguta (Siebold & Zucc.) Planch.	1
Pomegranate	*Punica granatum* L.	-
Kaki (persimon)	*Diospyros virginiana* L.	5
	Diospyros kaki Thunb.	1
Ziziphus (Chinese date)	*Ziziphus jujuba* Mill.	5
Fig	*Ficus carica* L.	3
Medlar	*Mespilus germanica* L.	-

RESULTS AND DISCUSSIONS

It is known that horticultural activity improves the quality of life for all people of all ages by beautifying neighbourhoods, stimulating social inter-action, producing healthy nutritious food, encouraging self-reliance, conserving resources, and creating opportunities for recreation and education (Abukutsa-Onyango, M.O., 2009) but in the same time these activities are considered economically viable ways of production.
Romania has favourable conditions and old traditions in fruit growing. Even so, there is a continuous need to set up some essential directions for fruit growing (Chitu E. et al., 2023). Among these could be mentioned:
- application of a modern growing technology;
- introduction of new competitive cultivars and planting compact large plots;
- popularization in culture of underutilized fruit growing species, increasing awareness;
- a new zoning in fruit growing species.

There are already a number of varieties (Table 1), listed in the Official Catalogue of Cultivated Plants in Romania, available for culture (www.istis.ro).
Another advantage is the easy production of planting material in nurseries of these species (Pașcu, R. et al., 2018).

Current Status

According to FAOStat, data for 2022, Romania reported 700 ha cultivated with “other berry fruits”, category where part of these species enter.
In southern Romania there are appropriate natural conditions for Chinese date, kiwi, fig and pawpaw cultivation (Chitu et al., 2023). Medlar, goji and saskatoon berry have a larger area of favourability throughout Romania.
There are several places in Romania where the *Chinese date* exists in naturalized populations in Dobrogea area, where it is called Dobrogean date. Trees are extremely drought resistant, and, until now, they seem to have no natural pest enemies and cultivation is not complicated. Advantage of Chinese date culture is that it has no problems with frost, because due to late flowering. Produces small brown olive-like fruits, rich in vitamin C.
In our country it interests as an ornamental species, being planted isolated or in groups, more like an exotic specie.
Medlar is already known to have good adaptation to our temperate continental climate

conditions and also has natural resistance to pest enemies.
Medlar is a traditional medicinal plant from which the fruits, leaves, bark and wood have been used over time. Fruits rich in tannin, proteins, natural organic acids and pectin, promote immunity.
It was used as rootstock for quince. Presently, medlar is used mostly for its ornamental value, healthy aspect of the leaves until late autumn.
Goji berry has become an important fruit crop in the last decade in Europe, due to its sanogenic properties and in this context is becoming more and more popular also in Romania for both producers and consumers.
Saskatoon berries have a high content of antioxidants, flavonoids and vitamin C. It does not require special care, totally resistant to frost, unlike blueberry, it grows well on a much wider range of soils.
Pawpaw is one of the most exotic plants that have been acclimatized in Romania. Is a rustic species that can be successfully cultivated in temperate regions, originating from the eastern part of the United States.
The fruits have a unique taste, a combination of banana, pineapple and mango, but you can also feel flavors of vanilla or chocolate, depending on the variety and degree of ripeness.
Kaki it is grown in many corners of the world, but the largest production of persimmon is in China, where its leaves are also used for tea and anti-aging products.
Fruits contain vitamins, minerals and other beneficial compounds for health, of which we only mention vitamin C, magnesium and zinc (Table 2).
In Romania, persimmons can be cultivated in orchards but also for ornamental purposes, in parks and private gardens, where the temperatures allow it. It is a species sensitive to frost, which is why it is planted in sheltered places in areas of the country where the risk of frost is reduced. Plants are affected by low temperatures below – 18°C but varieties were also created that withstand up to – 32 Kaki ‘Rosseyanka’ (*Diospyros kaki* x *Diospyros virginiana*).
Kiwi it is easy to maintain, it has no diseases and pests and it bears fruit quickly, in one year.
Temperature is a limiting factor for kiwi culture in our country, being a species very sensitive to frost and being planted in areas where the risk of frost is reduced.
Fruits are already appreciated on the market but the possibility of growing them locally is increasing on the recent years.
Fig, being an old Mediterranean species, it requires a mild climate and lots of sun. In Romania is cultivated in south and southwest (Moisescu et al., 2022).
It is a long-lived species, resistant to drought, multiplying without much difficulty, has high economic and ornamental value.
Pomegranate native to Iran, cultivated and naturalized throughout the Mediterranean region, can be cultivated in Romania, especially in areas where the climate is a bit milder in the south and southwest. although sensitive to low temperatures, it blooms between June and August, escapes the danger of late frosts.
The consumption of fresh fruit has many therapeutic indications (Table 2).
it interests as an ornamental species, having a spectacular flowering.
In conclusion, these species resist drought, have no diseases or pests, and the fruits are special both in terms of appearance, taste and phytonutrient components.

Future Trends

As results of the last programs that support fruit growing and ongoing National Strategic Plan (2023-2027) a series of strategies could be highlighted:
- Increasing consumer interest in products obtained in organic farming and through other environmentally friendly agricultural practices. The studied species can be cultivated in small farms, easily assimilated to ecological practices.
- Increasing consumer interest in products obtained in these farms and through other environmentally friendly agricultural practices.
- Development of partnerships for innovation, creation of knowledge bases and new methods in agriculture including new crops and technologies (https://www.afir.info/).

These species have great potential to extend into culture in arid regions because of their resistance, since temperate species face problems of adapting to the new climate changes. Fruit growing is undoubtedly affected by climate change in terms of the decrease in economic yield, in terms of quantity but especially in terms of quality.

Fruits quality is affected mainly by the high temperatures in summer and autumn, cumulated with the effect of lack of precipitation. Symptoms appear more and more often, such as: decalation of fruiting phenophases, rusetting, damage due to sunburn, incomplete staining, cracks on the fruits, accelerated ripening of fruits, unevenness of fruits and their reduced growth (Zlati et al., 2021; Leposavić et al., 2009).

- Implication and implementation in a wide range of research projects involved in surveying, collecting, conserving and studying the genetic diversity of target species to farmers contributing to their maintenance in situ/on-farm, from breeders and experts working to develop new cultivars and technologies to different user groups (Padulosi, 2013).

Table 2. Nutritional facts and health benefits of the species (www.fs.usda.gov/database)

Popular name	Health Benefits	Predominant		Proteins (g/100 g)	Carbo-hidrates	Energy (kcal/ 100 g)
		Vitamins	Minerals			
Goji	Promoting immunity, Improving eyesight, Protecting the liver	A, C	Fe, Ca	4.0	21.6	98
Saskatoon	Antioxidant, Anti-inflammatory	C, A, E, PP, Biotin	Ca, Mg, Fe, Mn, K, Na	1.5	11.4	85
Paw paw	Antioxidant, Anti-inflammatory	A, B, C	Ca, Fe, Mg, Mn, P, K	1.2	18.8	80
Kiwi	Aiding digestion, Improving sleep, Cardiovascular health, Boosting the immune system	C, K, E, B	Cu, K, Mg, Mn, Ca, P	1.2	14.7	61
Pomegranate	Gastrointestinal problems, Supporting cardiovascular health	C, K	K, Mg, Fe, Zn	1.7	18.7	83
Kaki	Lowering blood pressure, Delaying age-related diseases	A, B, C, D, K	Ca, Cu, Fe, Mg, K, Zn	0.6	18.0	71
Ziziphus (Chinese date)	Antioxidant, Immune-boosting, Improve sleep, digestion and brain function	C,	K,	1.0	20.0	79
Fig	Relieving constipation, Managing blood sugar levels	B, K, C	Cu, Mn, K, Mg, Ca, Fe	0.8	19.2	74
Medlar	Forms haemoglobin, Function of muscles, Brain health, Prevent restless leg syndrome, Regulates body temperature	B, A	Ca, Fe	0.5	24.0	88

Opportunities

These species, according USDA Database have important nutritional compounds with significant health benefits due to their high content in antioxidants, carothenoids, pectin etc (Table 2).

The wider implication of this category of phytonutrient-rich fruits consumption could be significant for health promotion, disease prevention and food security at national and international level and (Clevidence B.A., 2010).

New species can be attractive in rural areas from the perspective of the availability and good quality of natural resources (biodiversity, water, soil, landscape, some of these species have melliferous potential.
Could be useful for a future use as rootstocks.
The new direction is towards niche crops, which can be established on small areas, and the incomes obtained from the exploitation of productions are clearly superior.
New species-new plantation-new challenges-new opportunities.

Increasing Awareness
One direction of increasing awareness is by popularizing these species by including them in family organic farms, and also in small private gardens.
Fruit trees have also ornamental value and are well represented by a great diversity of species and varieties while ensuring decor spread throughout the year (Figures 2 and 3).

Figure 2. Including studied species in landscape compositions-proposal 1 (photo: Ina Asiminei)

Figure 3. Including studied species in landscape compositions-proposal 2 (photo: Ina Asiminei)

In addition, activities around the garden (pruning, harvesting) can be a relaxing practice which pleases the viewer on all levels.
Admiring a flowering tree and generally outdoor activities have positive effect on children and in the same time bring them knowledge about nature, raising awareness and connection with nature.

CONCLUSIONS

The material presents valuable growing, yielding and fruit quality characteristics which make them valuable and useful in many aspects.
Based on the analysed data and according to results of this research, the study indicates that the investigated species are valuable sources of desirable genetic characteristics including important morphological and nutritional characteristics of the fruits with significant health benefits.
Taking into consideration our results, we emphasize that the analysed exotic and underutilized pome fruit species are promising for Romanian cultivation. Their cultivation should be extended in integrated and organic farms, and also in small private gardens.
The use of these species for landscape design will bring major benefits to environment in terms of healthy and economic aspects of life.
Even so, there are needed specialized institutions to analyse and monitor climate change long term impacts in this area, especially to track the diversity in terms of loss and movement.

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EFFECT PHYTOTECHNOLOGICAL FACTORS ON TWO RED WINE GRAPE VARIETIES

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Abstract

Among the most extensively grown plants worldwide are grapes. Numerous religions place a high value on grapes and wine. The objective of the experiment was to examine the impact of varying cluster loadings (30% and 50%) on the yield of two distinct grape varieties (‘Zweigelt’ and ‘Fetească neagră’/‘Feketeleányka’), both in terms of quantity and quality. The experiment was carried out Mica village, in Mureș County. From the yield numbers, could be deduct that the control variety yielded the highest. The sugar content was highest and the acid level was lowest at 30% cluster load. The values were pretty close at 50% load. In terms of wine production, the 50% load was the most appropriate to the control. In terms of grape berries number, at ‘Fetească neagră’/‘Feketeleányka’ the highest values were reached at 50 % cluster load and in the case of ‘Zweigelt’ the highest number was recorded at 30% load.

Key words: *cluster load, grape varieties, production.*

INTRODUCTION

Grape is the fruit that is cultivated on a large scale, ranking third in popularity after citrus and banana (Singh et al., 2017). In certain parts of the world with ideal climates, winegrowing and winemaking have a very long history. The wine served as the crown gem of Greek, Roman, and Egyptian cuisine. Because wine was purer than readily available water, it was preferred by nobility for a very long period (Kádár, 1973). For many centuries, wine consumption has been widespread throughout the world (Snopek et al., 2018). Red wine serves as more than just a source of pleasure; it also possesses medicinal properties (Balla et al., 2023). Wine's health advantages were recognised by the ancient Romans, who also popularised it (Lukacs, 2012). It was demonstrated that wine-derived phenolic compounds play a significant role in human health (Antoce & Stockley, 2019). Grosso et al. (2017) research has shown that these compounds have proven effects in reducing the risk of all-cause mortality.

The colour of red wine holds significant importance as it is the primary characteristic that wine consumers notice, and it is closely linked to the wine's overall quality (de Freitas et al., 2017). Viticultural literature defines crop load as the ratio of canopy size to fruit yield, serving as a tool to evaluate source-sink dynamics in relation to vine well-being and sugar content in berries (Reeve et al., 2018). In a study Naor et al. (2002) suggested that the amount of crop load, as rather than the yield, could serve as a more accurate measure of wine quality.

In a previous study is mentioned that the treatment with 8 buds per cane resulted in the highest recorded value for both berry weight and volume, along with the greatest percentage of total soluble solids and T.S.S./Acidity, while also showing the lowest total acidity content (Khamis et al., 2017). Moreover, is also reported that a blend of leaf removal and cluster thinning is increase the quality of grapes and wines (Song et al., 2018). Canopy management encompasses various viticultural techniques, including the implementation of trellis systems, controlling vine vigor, trimming shoots, thinning clusters, and removing leaves, these practices have been extensively employed in grape production worldwide for numerous years (Osrečak et al., 2015; Sivilotti et al., 2016).

In the present paper the aim was to find the greatest cluster load for red wine varieties, to achieve a greater quantity of grapes, moreover a higher quality of wine.

MATERIALS AND METHODS

The experiment was conducted in Mica, Mureș County in a vineyard in 2020. The average temperature was 10°C (Figure 1) and the highest precipitation was in July approximatively ~135 mm and the lowest August when 21 mm (Figure 2).

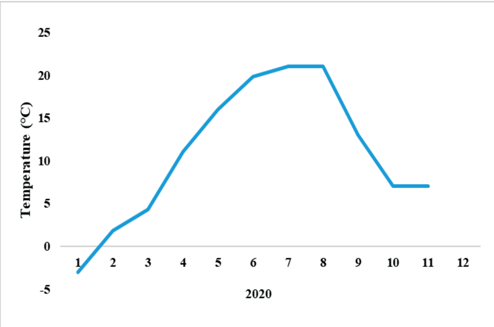

Figure 1. Temperature during the experiment

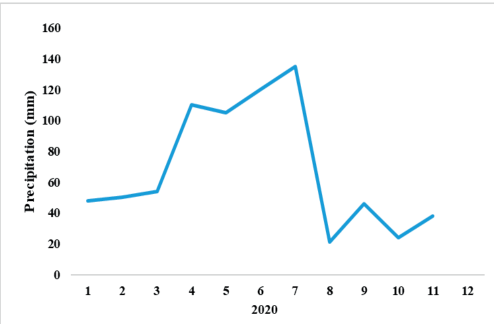

Figure 2. Precipitation during the experiment

The soil type was determined on the experimental site clay-washed, brown forest soil.

US 2) Clay-washed, brown forest soil

Description:

Ap (0-10 cm) clayey – adobe, dark brown, granular structure, frequent root system, coprolitic, loose.

Ao (10-40 cm) clayey – adobe, dark brown, granular structure, frequent root system, coprolitic, loose.

Bt (40-68 cm) clay-washed, yellow-brown, prismatic structure.

Ck (68 cm–) clay-washed, yellow, dense, lime concentration.

US 2) Luvisol

Description:

Ap (0-10 cm) clay-loam texture, dark brown, granular structure, frequent root presence, coprolitic, loose.

Ao (10-40 cm) clay-loam texture, dark brown, granular structure, frequent root presence, coprolitic, loose.

Bt (40-68 cm) leaching clay accumulation (illuviation), yellow-brown, prismatic structure

Ck (68 cm–) leaching clay accumulation (illuviation), yellow, compact, lime concentration.

Table 1. Chemical analysis of US 2) soil

Horizont	Ap	Ao	Bt	Ck
Depth (cm)	0-10	10-40	40-68	68
pH	5.34	5.30	5.90	8.14
Humus	6.50	4.08	1.03	0.59
N %	0,311	0.183	0.076	-
P ppm	7	3	2	-
K ppm	18.5	8.1	10.2	-

Table 2. Physical analysis of US 2) soil
(grain size analysis)

Horizont	Ap	Ao	Bt	Ck
Depth (cm)	0-10	10-40	40-68	68
Coarse sand () 2–0.2 mm	1.9	2.7	0.7	0.2
Fine sand 0.2–0.02 mm	32.7	33.3	18	16
Powder				
0.02–0.002 mm	28	31	29.4	40.1
Clay				

As plant material we have selected ‘Zweigelt’ and ‘Fetească neagră’/‘Feketeleányka’ red wine varieties. In the case of ‘Fetească neagră’/‘Feketeleányka’ the cultivation area is 3 m^2 (2.5 × 1.2 m) and at ‘Zweigelt’ 2.25 m^2 (2.5 × 0.9 m). The ‘Zweigelt’ variety is native to Austria it is a cross-breeding between Blaufränkisch and St. Laurent varieties since 1920. About the ‘Fetească neagră’/‘Feketeleányka’ it is assumed to be native near to the Prut flow.

In the study two types of treatments and one untreated (control) was set up. The treatments were carried out in 4 repetitions on 12 plants per repetition, so the effects of each treatment were examined on a total of 48 plants, per variety. The cluster thinning was made after the flowering stage, when the 50 and 30% cluster loads were set up, moreover in the case of control clusters remained in the same way. At the 50% cluster thinning in every case the shoot first cluster was kept, and the rest of the clusters were removed. In the case of the 30% cluster load, on the grape’s productive bases from the two kept shoots from one of the shoots every cluster was removed, and from the other shoot the second and the third clusters were removed. We have evaluated the grape berry weight (g), cluster weight (g), harvest quantity (t/ha), sugar content (g/L), acid level (g/L), dry matter content (%), and the number of seeds.

The following methods were applied to determine the acidity, sugar, and dry matter content.

Determination of dry matter content

Measurement of volume using a pycnometer for wine and water, along with distillation and subsequent calculations.

Removal of carbon dioxide from the wine through stirring.

Thorough rinsing of the pycnometer with the wine to be analyzed, followed by insertion of the wine and thermometer.

Adjustment of the wine to 20°C.

Filling the pycnometer to the mark, drying it, and weighing it to four decimal places.

Calculation:

Density = (mass of wine - mass of empty pycnometer) / mass of water.

Extract = 1 + wine density - distillate density.

Result interpretation using a reference table.

Calculation of Non-reducing Dry Extract:

Non-reducing dry extract = Total dry extract – residual sugar.

Determination of sugar

Utilization of the Rebelein method

Preparation:

In a flask, combine 10 mL of copper sulphate ($CuSO_4$), 5 mL of Seignette salt, a few grains of pumice stone, and 2 ml of the wine to be analysed.

Boil the solution for 2 minutes and allow it to cool.

Titration:

Add 10 mL of potassium iodide solution, 10 mL of sulfuric acid, and 10 mL of starch to the cooled solution.

Titrate the dark-colored solution with sodium thiosulfate until a yellowish-white color is achieved. Read the sugar content directly in g/L from the biuret. Dilution, if necessary, for sugar content above 28 g/L, dilute the wine with distilled water according to the specified factors.

Determination of total acidity

Employment of the Schliessmann reagent/method

Preparation:

In a flask, prepare 25 ml of the wine to be analysed, ensuring removal of carbon dioxide by heating and subsequent cooling.

Titration:

Titrate with BLAULAUGE 1/3 N solution until a dark green color is reached (indicating neutral pH, confirmed with pH paper).

The amount of sodium hydroxide solution used corresponds to the acidity of the wine.

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

Considering the grape berry weight could be clearly observed that the 30% cluster load is significantly higher compared to the other two (Figure 3). Moreover, no significant differences were observed between the control and the 50% cluster load.

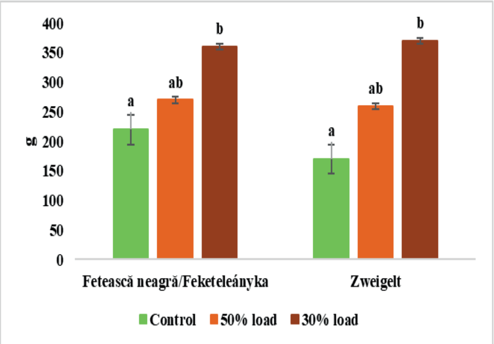

Figure 3. Grape berry weight under the effect of cluster load (50 and 30%). Bars represent the means ± SE (n = 48). Different letters above the bars indicate significant differences between cluster load ($p < 0.05$)

Here again, the greatest cluster weight at both varieties was recorded at the 30% cluster load, which is significantly different compared to the other two treatments (Figure 4). Beside the 50% cluster load also recorded a higher cluster weight when comparing to control. Additionally significant differences were determined also between the 50 and 30% cluster loads.

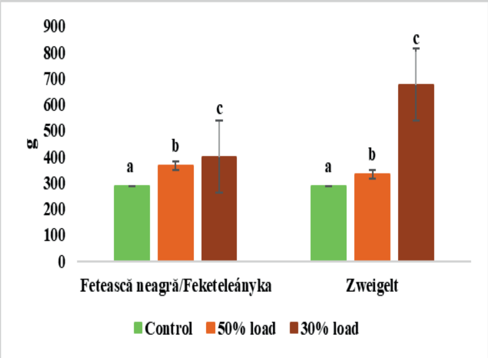

Figure 4. Cluster weight under the effect of cluster load (50 and 30%). Bars represent the means ± SE (n = 48). Different letters above the bars indicate significant differences between cluster load ($p < 0.05$)

As was expected the highest quantity of grapes were determined at the control plants (Figure 5), where significant differences were observed compared to the other two treatments, at both red wine varieties. In the case of the cluster thinning plants no significant differences were recorded.

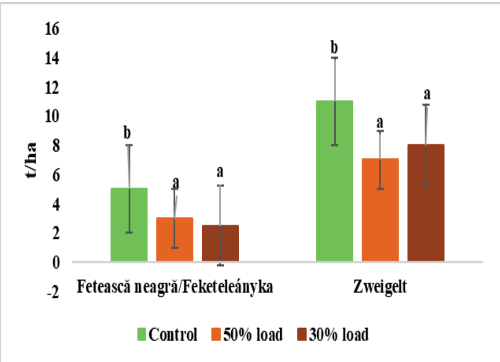

Figure 5. Harvest quantity under the effect of cluster load (50 and 30%). Bars represent the means ± SE (n = 48). Different letters above the bars indicate significant differences between cluster load ($p < 0.05$)

Sugar content did not record significant differences between the cluster thinning methods (Figure 6). Only a small inhibition of sugar content was observed at the 'Zweigelt' variety control treatment, which was not significantly difference compared to 30 and 50% bud load.

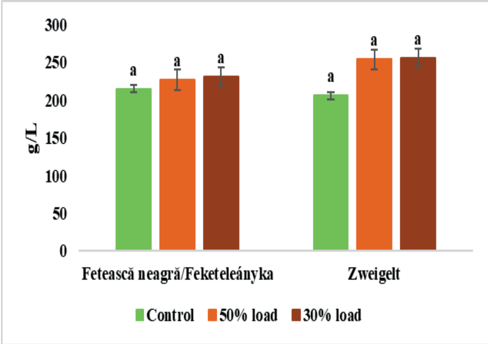

Figure 6. Sugar content under the effect of cluster load (50 and 30%). Bars represent the means ± SE (n = 48). Different letters above the bars indicate significant differences between cluster load ($p < 0.05$)

Once more no significant differences were observed at the acid level (Figure 7). At the 'Feteasca neagră' variety the acid level was approximatively ~ 7 g/L, and in the case of 'Zweigelt' ~ 12 g/L.

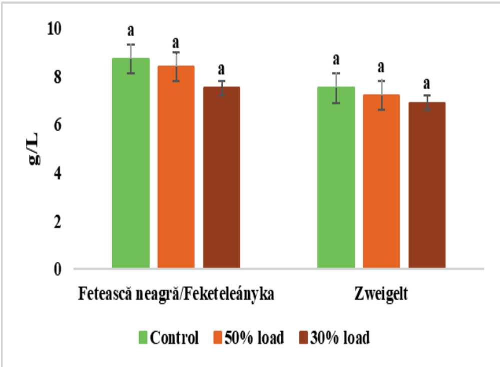

Figure 7. Acid level under the effect of cluster load (50 and 30%). Bars represent the means ± SE (n = 48). Different letters above the bars indicate significant differences between cluster load ($p < 0.05$)

When comparing the dry matter content an inhibition was recorded at the 'Feteasca neagră' variety at control plants compared to the other two treatments (Figure 8). However, in 'Zweigelt' no significant differences were reported, in this case the dry matter was approximately ~ 19%.

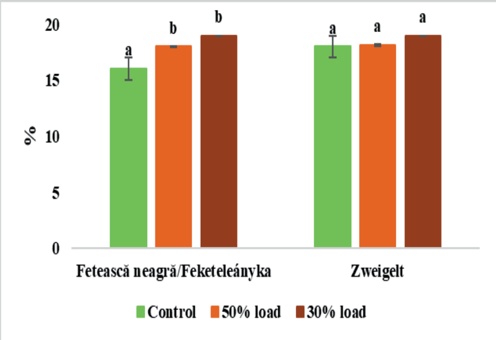

Figure 8. Dry matter content under the effect of cluster load (50 and 30%). Bars represent the means ± SE (n = 48). Different letters above the bars indicate significant differences between cluster load ($p < 0.05$)

At the control and 50% cluster load increase could be observed 3, 4 seed/berry value and decrease are determined at 1 seed/berry (Figure 9). A common value could be observed at 2 seed/berry. The 50 and 30% cluster load an increase is determined at 1 and 2 seed/berry, and decrease at 3, 4 seed/berry.

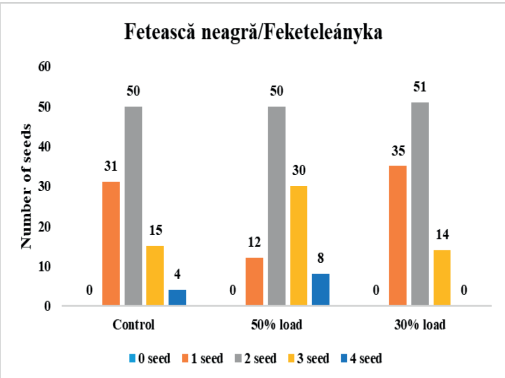

Figure 9. Number of seeds under the effect of cluster load (50 and 30%).

Regarding the 'Zweigelt' variety increase could be determined between control and the two treatments in regard to 3 and 4 seed/berry respectively and decrease at 1 and 2 seed/berry (Figure 10). A similar value could be observed at 2 seed/berry at the 50 and 30% cluster load.

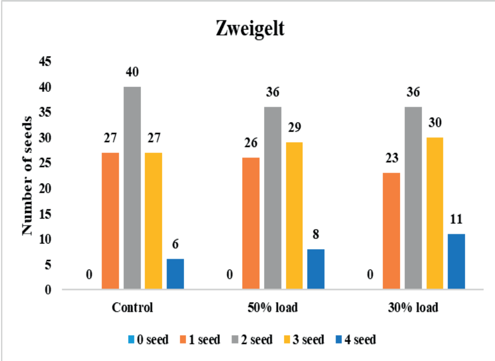

Figure 10. Number of seeds under the effect of cluster load (50 and 30%)

CONCLUSIONS

From the present data could be concluded that in the case of grape berry weight and cluster weight the highest amount was determined at 30% cluster load, moreover the 50% cluster load also achieved a higher quantity compared to control. Regard to the harvest quantity the control treatment reported the highest amount at both red wine varieties. The cluster load did not influence the sugar content and the acid level of the varieties. In the case of number of seed/berries at the 'Zweigelt' the 30% cluster load was the most favoured. However, at 'Feteasca neagră' the 50% cluster load achieved the best results. Concerning the dry matter content the 50 and 30% cluster loads are exceling compared to control. Altogether, at the selected red wine varieties the greatest results were determined at 30% cluster load.

Under our experimental conditions we recommend that cluster thinning could have a positive effect on the grape, however the 30% cluster load achieve a higher achieved greater amount as the other two.

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THE INFLUENCE OF TREATMENTS WITH AMINO-ACIDS AFTER HAIL FALL ON FETEASCĂ NEAGRĂ GRAPE HARVEST

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Abstract

Some fertilizers containing amino acids could help recover grapevine plants after hail stress and on the quality and quantity of harvest. After the hail damage on April 30th, 2019, three treatments with an amino-acids complex were applied to the grapevine canopy - at 20-25 cm, at 35-40 cm shoot length, and berries growth - on Fetească neagră grapevines at Serve Winery, Dealu Mare vineyard. At harvest, the quality and quantity of grape was tested for treated and untreated variant. The total anthocyanins for the treated variant were 211 mg/100 g FW and for the untreated variant - 198 mg/100 g FW. The content of total polyphenols total was 376 g/100 g FW for the treated variant, significantly higher than the untreated variant 320 g/100g FW. The content of flavonoids for the treated variant was of 147 g/100 g FW significantly lower than the untreated variant 130 g/100 g FW. The treatment with amino acids influenced the quantity and the quality of grape harvest affected by the hail, with significant results.

Key words: *Fetească neagră, amino-acids treatments, hail, quality, quantity.*

INTRODUCTION

Climate change poses an additional challenge to wine production at the level world. Historical records reveal significant signals of climate change, including an increase in global average surface temperature of about 0.6°C since 19th century (Trenberth et al., 2007). All these changes are very likely to have an impact significantly on the grapevine (van Leeuwen and Darriet, 2016). Actually, the vine (*Vitis vinifera* L.) is traditionally cultivated in geographical areas where the temperature average growing season is 12-22°C (Droulia and Charalampopoulos, 2021), and climate and climate are among the environmental factors that most influence the yield and quality of wine. Extreme weather events can influence significant yield losses over time how climate change can alter the vine phenological cycle, disease/pest patterns, ripening potential, wine characteristics and yield (Trenberth et al., 2007).

Hail can cause significant damage to the foliage and organs generative. Hail can reduce yield, shoot number and leaf tissue and partially or completely stop plant nutrition. As a result, a hail of over 50% damage suppresses growth and development with up to 20 days, while lighter hail destroys growth processes for about 10 to 14 days. At the same time, secondary buds, dormancy, side shoots from children, begin to develop more intensively. For this reason, apart from the low yield, production small, hail can lead to a poor quality harvest. From this point of view, it is important for the vine to be protected from pathogens and other stress factors after the impact of hail (Baniță et al., 2020).

In terms of agriculture, this implies preserving the environment and public health by cooperating with nature. The "From Farm to Fork" strategy (European Commission, A Farm to Fork Strategy for a Fair, Healthy and Environmentally-Friendly Food System, 2020) is one of the initiatives of the "European Green Deal" and aims to promote a fair, healthy, and environmentally - friendly food system with equitable economic opportunities for all. This approach emphasizes the necessity of a sustainable food production chain by lowering the usage of agrochemical compounds (pesticides and fertilizers) while also guaranteeing the production of wholesome, premium, and reasonably priced food for customers (Artem et al., 2023).

The positive effects of the foliar application of amino acids and bio-stimulants based on amino acids on both the qualitative and quantitative characteristics of different cultivated plants have been reported (Sun et al., 2024).

Foliar application is a widespread practice in the agriculture business despite being highly energy - consuming. Enzymatic hydrolysis of both plant and animal protein hydrolysates produces amino acids that have been employed for foliar usage (Islam et al., 2022).

Abiotic factors are shown to control the synthesis and degradation of primary (sugars, amino acids etc.) and secondary (phenolic compounds) metabolites through the regulation of their biosynthetic pathways at different stages of berry development (Rienth et al., 2021).

The aim of this study was to investigate the influence of Delfan Plus treatment after hail fall on the profile of polyphenolic compounds in Feteasca Neagra grapes. In order to understand how the treatment with amino-acids might regulate the polyphenol metabolism, the appropriate spectrophotometry and high-performance liquid chromatography (HPLC) methods were used to monitor for Fetească neagră grapes after harvest.

MATERIALS AND METHODS

The research was carried out during 2019 on Fetească neagră *Vitis vinifera* L. cultivar - at Serve winery, Ceptura wine growing center, Dealu Mare vineyard. The vines were planted in 2006, with distance of 2.2 m between rows and 0.9 m between vines/row, with a density of 5050 vines/ha. The vines were trained as double Guyot with 24 buds/vine. On April 30th 2019, the hail that felt in Ceptura wine growing center and damaged 90% of shoots. The shoots were 10-15 cm height, uniformly growth and in good phytosanitary shape. In 30 minutes felt an amount of 25 mm/m^2 of rain. On May 2nd, at 2 days after the hail felt, a contact fungicide (Funguran OH, 2 l/ha) was applied. The damaged shoots were cut down to stimulate the growth of dormant, secondary and coronary buds.

Two experimental variants were set on: Control variant (CV) and treated variant (TV) with amino-acids (Delfan Plus) were applied at 20-25 cm shoots length, at 35-40 cm shoots length and at berries growth (treated variant). Also, during vegetative period, nine phytosanitary treatments using contact and systemic pesticides were applied.

At harvest, on September 18, 2019, the grape samples were collected. The analyses consisted on sugar content, total acidity, pH, total polyphenols, anthocyanins and flavonoids.

The quantification of total phenolic concentration in the wine samples was conducted utilizing the Folin-Ciocalteu (FC) method, a widely accepted protocol in analytical chemistry (Girelli et al., 2015). The experimental procedure involved combining 0.1 mL of the wine sample with 5 mL of deionized water, 0.5 mL of Folin-Ciocalteu reagent, and 2 mL of a 20 percent (w/v) Na_2CO_3 solution. Subsequently, the volume was adjusted to 10.00 mL with deionized water. After a specified incubation period of 30 minutes at 40°C, the absorbance of the resulting solution was measured at 760 nm, relative to a blank containing 0.1 mL of water in lieu of the wine sample.

The determination of the total concentration of anthocyanins in wine was facilitated through the distinctive absorption band exhibited by this class of compounds at approximately

520 mn. Given that the color intensity of anthocyanic pigments reaches its zenith at pH 1 and becomes colorless at pH 4.5, a meticulous procedure was employed for sample preparation. Specifically, aliquots of each wine sample were judiciously diluted with a 10 mL solution, composed of 1.49 grams of KCl dissolved in 100 milliliters of deionized water, combined with 67 milliliters of 0.2 molar HCl, and adjusted to pH 1.0. Additionally, a 10 mL aliquot was mixed with a pH 4.5 buffer, consisting of 1.64 grams of sodium acetate dissolved in 100 milliliters of deionized water and adjusted with hydrochloric acid. Spectrophotometric analysis was conducted at 700 mn for degradation product correction and at 520 nanometers (the peak of the visible spectra) for each wine sample, as elucidated by Bora et al. (2018a) and Bora et al. (2018b). It is noteworthy that anthocyanins manifest an absorption band within the 490-550 nanometers range, distinct from the ultraviolet bands characterizing other phenolic compounds. This comprehensive methodology ensures the accurate determination of anthocyanin concentrations in the grape samples.

The quantification of total flavonoids was conducted employing a colorimetric method as described by Kim et al. (2003). The initial step involved the dilution of the extract to a final volume of 5 ml with distilled water. Subsequently, 300 µl of 5% $NaNO_2$ was added to the solution, and the mixture was allowed to stand for 5 minutes. Following this, 300 µl of 10% $AlCl_3$ was introduced, and after an additional 6 minutes, 2 ml of 1N NaOH was incorporated. The resulting solution was thoroughly homogenized, and the absorbance was measured at 510 nm against a blank containing water. This methodological approach, outlined in Kim et al. (2003), ensures the accurate determination of the total flavonoid concentration in the wine samples, providing valuable insights into the compositional aspects of these compounds within the analyzed specimens.

Statistical analysis

The statistical interpretation of the results was performed using the Tukey test, using the SPSS, version 24 (SPPS Inc. Chicago, IL, USA). The statistical processing of the results was primarily made to calculate the following statistical parameters: arithmetic average, standard deviation, average error, using the SPSS version 24 (SPPS Inc. Chicago, IL, USA).

RESULTS AND DISCUSSIONS

Even if the vines were pruned with 24 buds/vine, the hail fall on April 30th, damaged 90% of shoots. In this situation, the number of grapes on vines was low – 3.3 for Control variant and 4.3 for DelfanPlus Treated vines. The chemical analyses of the red grape samples produced in 2019 harvest are present in Table 1. The parameters are reported for the two variants Control and Delfan Plus Treated. The results show that the grape harvest recorded a normal to high sugar content, ranging, in accordance to the experimental variants, from 271 g/l sugar for Delfan Plus Treated Variant to 265 g/l sugar for Control variant, differences being insignificant. The Control variant with reduced number of grapes/vine – 3.3, recorded lower values for the sugar in grapes compared to the Delfan Treated variant, and this could explain the role of this product in increasing the quality of harvest.

Table 1. Grape characteristic - harvest 2019

Variant	No. of grapes/ vine	Sugar content (g/l)	Total acidity (g/l sulphuric acid)	pH
Control Variant	3.3 a	265 a	5.7 b	3.4 a
Treated Variant	4.3 b	271 a	5.9 a	3.5 a

Average values ± standard errors (n=3). The letters in the brackets show the statistical difference among results for $p<0.05$. For the same compound, a common letter for the variants shows no significant difference among them according to Tukey test.

Regarding the total acidity, this parameter was influenced by the treatment, between the two variants being a significant difference, with a 5.9 g/l sulphuric acid for Delfan Plus Treated Variant and a 5.7 g/l sulphuric acid for Control Variant (Table 1).

Table 2. Effect of Delfan Plus Treatment
on the concentration of anthocyanins
in Feteasca neagra grapes at harvest

Anthocyanin types	Delfan PlusTreated variant	Control variant	Sign
Delphinidin-3-O-glucoside	26.83±7.89 B	20.74±5.61 A	***
Cyanidin-3-O-glucoside	13.43±3.32 B	11.45±3.45 A	**
Petunidin-3-O-glucoside	24.62±6.89 B	22.67±5.88 A	**
Peonidin-3-O-glucoside	38.85±6.99 B	36.45±7.76 A	**
Malvidin-3-O-glucoside	95.23±10.03 A	95.31±12.05 A	Ns
Malvidin-3-O-pcumaroilglucoside	12.16±3.57 A	11.67±7.96 A	ns

Average values ± standard errors (n=3). The letters in the brackets show the statistical difference among results for $p<0.05$. For the same compound, a common letter for the variants shows no significant difference among them according to Tukey test.

Anthocyanins are orchestrated within the phenylpropanoid pathway beneath the complex control of all basic chemicals and various administrative qualities at the transcriptional level. Therefore, the most important anthocyanins in red grapes are the 3-O-monoglucosides of cyanidin, peonidin, delphinidin, petunidin, and malvidin (He et al., 2010). Cyanidin in grapes were chemically changed over not as it were to catechins but too to cyanidin-3-glucoside and peonidin-3-glucoside by C3 glucosylation responses catalysed in specific by the action of anthocyanidin/anthocyanin glycosyltransferases. Based on the information gotten after HPLC investigation of anthocyanins, it can be concluded that treatment with Delfan Plus upgraded the quality expression related with anthocyanin biosynthesis, especially within the biosynthesis of cyanidin-3-glucoside. In the case of cyanidin-3-glucoside and peonidin-3-glucoside, an increase of almost 15%, and 7%, respectively. Moreover, in this situation, the existence of directly relationship between the Delfan Plus treatment on the vines and the intensification of anthocyanin biosynthesis in these grapes was confirmed (Table 2).

The HPLC results also show differences between the delphinidin-3-glucoside concentration in the treated grapes and the control grapes were significant. An increase in the concentration level of 23% in the Fetească neagra grapes treated with Delfan Plus was observed, which proves that the application of this products on grapes leads to an enhancement of the enzyme activity of UDP-D-glucose flavonoid 3-O-glycosyltransferase, according to Artem et al. (2023), an increase of about 8% in the petunidin 3-glucoside concentration was observed in the Delfan Plus treated grapes. This could be explained by the statetment that delphinidin 3-O-glucoside underwent two successive methylations, mediated by O-methyltransferases and with S-adenosylmethionine as a methyl donor, at the oxygen in the 30 - and 50 -positions, giving successively petunidin 3-O-glucoside and the malvidin 3-O-glucoside (Artem et al., 2023). For malvidin-3-glucoside, the concentrations were similar in both the control and treated grapes (Table 2). According to He et al., 2012, the most abundant monomeric anthocyanin in red grapes is malvidin-3-O-glucoside. In our study, the major monomeric anthocyanin was malvidin-3-O-glucoside, with values of 95.23 mg/100 g FW (Delfan Plus treated grapes) and 95.31 mg/100 g FW (Control grapes), the differences being insignificant. The anthocyanins, the red pigments of grape, influence the colour shade and intensity through their concentration and their proportion, each anthocyanin having colours which depend on their chemical structure and external factors, such as the pH. Thus, any change in the anthocyanin profile leads to colour change. Some study, showed that anthocyanins like delphinidin, peonidin, petunidin accumulated better in warmer years. Anthocyanins is the class of polyphenols which is the most influenced by the grape yield. In our study, even if the Delfan Plus treated variant had a higher number of grapes – 4.3 grapes/vine, the concentration of total anthocyanins were higher - 221 mg/100 g FW, then Control variant – 3.3 grapes/vine – 198 mg/100 g FW, differences being significant.

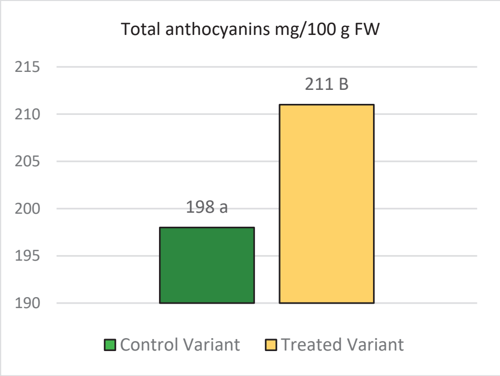

Figure 1. Total anthocyanin content in experimental variants. All the analysis were performed in triplicate. Different letters indicate the existence of significant differences (α≤ 0.05)

The most important flavonoids in wine are anthocyanidins, flavanols (also known as catechins or flavan-3-ols), and flavonols (including quercetin and myricetin). Red wine is a major source of flavonoids, and it is known to reduce cardiovascular events when consumed in moderation (Fernandes et al., 2017) and an appropriate intake of those compounds may be beneficial in preventing, and eventually managing, allergic diseases.

Our results indicate a significant difference of the flavonoids content between the two experimental variants, with values of 130 mg/100 g FW for control grapes and 147 mg/100 g FW for Delfan Plus treated variant (Figure 2). In grape there are two main types of phenolic compounds non-flavonoids, and flavonoids, respectively. The relative amounts and distribution of these compounds depend on many different factors such as grape variety, vineyard location, climate, soil type, agricultural practices, harvest time, production process and wine brewing process (Colleta et al., 2014).

Our results indicate a significant difference of the polyphenols content between the two experimental variants, with values of 230 mg/100 g FW for control grapes and 347 mg/100 g FW for Delfan Plus treated variant (Figure 3), the difference being significant.

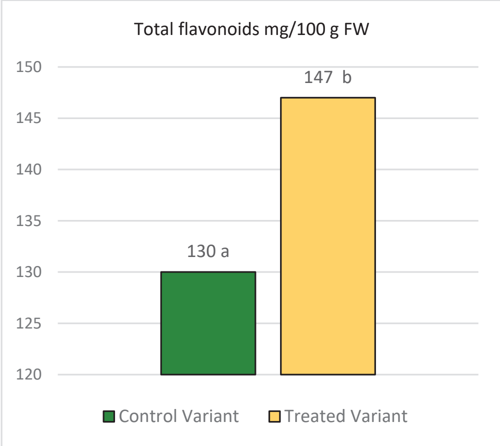

Figure 2. Total flavonoids (mg GAE/L) content in experimental variants. All the analysis were performed in triplicate. Different letters indicate the existence of significant differences (α≤ 0.05)

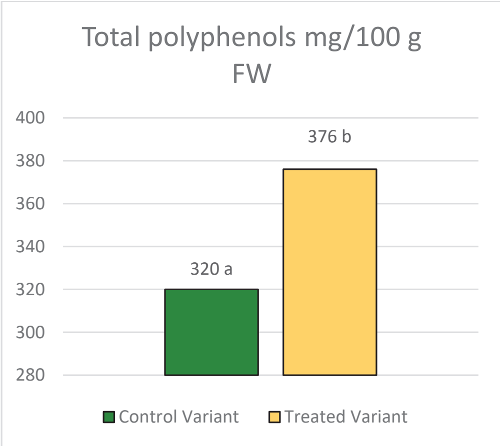

Figure 3. Total polyphenols (mg/100 g FW) content in experimental variants. All the analysis were performed in triplicate. Different letters indicate the existence of significant differences (α≤ 0.05)

CONCLUSIONS

For the first time, at SERVE winery treatments with Delfan Plus was used in treatment grapevines affected by hail. Furthermore, Delfan Plus – a biostimulator containing amino-acids was found to stimulate the grape quality.

It can be concluded that significantly higher concentrations of polyphenols in Fetească neagră red grapes can be achieved by applying Delfan Plus in the preliminary stage of berry development. However, further studies are needed to observe the evolution of the profile of polyphenolic compounds during the applying of biostimulants containing amino-acids to determine the appropriate concentration of chemical compounds required as a quality grape harvest.

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THE BIOLOGY OF THE DEVELOPMENT PROCESS IN SOME GRAPE CULTIVARS FOR RED WINES STUDIED ON THE SANDY SOILS OF SOUTHERN OLTENIA

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Abstract

The research carried out in the period 2020-2022 looked at the biological, productive and quality potential of four grape cultivars for red wines (Băbească neagră, Haiduc, Novac, Arcaș), studied in the ampelographic collection of the Research-Development Station for Plant Culture on Sands Dăbuleni. The obtained results showed that the beginning of bud burst phenophase of these cultivars with grapes for red wines started at temperatures between 11.5-18.2°C, the earliest being the Novac cultivar, which beginning of bud burst between April 16 and May 10. The vegetation period for red wine grape cultivars was between 159-164 days, under the conditions of recording an active heat balance between 3429.4-3491°C. From the point of view of productivity, the Novac cultivar stood out with a grape production of 20479 kg/ha, registering a difference of 6579 kg/ha compared to the control, statistically assured as distinctly significant. The quality recorded at harvest maturity of the grapes revealed values of the content in total sugars between 180 g/l for the Arcaș cultivar and 200 g/l for the Novac cultivar.

Key words: *grapes for wine, grape production, quality.*

INTRODUCTION

The grapevine is a traditional culture for the area of sandy soils in the south of Oltenia, being used since ancient times as a fixing plant for shifting sands. The ecopedological conditions on the unimproved sands were and are quite harsh, being unfavorable for most crops (Baniță, 1983). In this sense, in conditions of low precipitation, with very high temperatures during the summer, low hygroscopicity and the high frequency of days with strong wind exceeding 5-6 m/s, having the effect of increasing the degree of wind deflation and the intensity of evapotranspiration, grapevine was and is one of the species that economically exploit sands and sandy soils (Rățoi et al., 2014). The vine at a certain age, depending on the cultivar, forms its reproductive organs, flowers and fruits. This qualitative jump corresponds to physiological maturity and is the result of a whole series of physiological and biochemical transformations that take place at the level of cells. Starting with the first fruiting and until death, the vine bears fruit every year, being a polycarpic plant, with multiple and repeated fruiting (Popa, 2019). During the vegetation period, the vine goes through several phenophases/vegetation phases, which mark the processes of growth, development and reproduction. In the chronological order of their succession, the vine phenophases are: weeping, beginning of bud burst, beginning of ripening, shoot growth, flowering, berries ripe for harvest, and leaf fall. Weeping, beginning of bud burst, shoot growth and leaf fall are phenophases of vegetative organs, and flowering, berries ripe for harvest and grape ripening are phenophases of fruit organs. The phenophases of the fruit organs take place simultaneously with those of the vegetative organs and are passed by the vine in all types of climate (Irimia, 2012; Manzoor et al., 2023).

Buds exposed to low temperatures (0-10°C), flower faster at 10°C, compared to 20-25°C (Pouget, 1965). According to Carboneanu (1990), the cold requirement for coming out of dormancy is achieved after 7 days of exposure of the buds to positive temperatures lower than 10°C.

The main factor that determines the initiation of the flowering process is temperature. Thus, the initiation of flowering takes place at a temperature of 15-17°C, at 20-25°C the opening of flowers is fast, at 24-27°C the optimal limits are achieved, because at temperatures above 30°C the rate of opening of flowers to decrease (Burzo et al., 2020; Cortázar-Atauri et al., 2017).

Harvesting wine grapes at their optimal level of maturity is the first step in producing high quality wines (Jahnke et al., 2023). Determining the best time to harvest requires both experience and careful assessment of the ripeness of wine grapes. Typical chemical analyzes for determining grape ripeness for wine include monitoring sugar content, titratable acidity and pH (Watson, 2003). Grape ripening is associated with changes in the walls of the pulp cells, due to the action of pectolytic enzymes that hydrolyze the pectins in the cell wall, making them permeable to the changes that occur during winemaking (Amrani and Glories, 1995a; Amrani and Glories, 1995b; Lecas and Brillouet, 1994).

MATERIALS AND METHODS

The study was carried out on four grape cultivars for red wines (Băbească neagră, Haiduc, Novac, Arcaș), from the ampelographic collection of the Research - Development Station for Plant Culture on Sands Dăbuleni. The ampelographic collection was established in 2010. The form of driving the stumps was the classic (low), the planting density was 3787 stumps/ha and the planting distances were 2.2/1.2 m. The experiment was located on a sandy soil with low natural fertility, with an organic carbon content within the limits of 0.24-0.74%, poorly supplied with nitrogen (N_{total}= 0.02-0.03%), well supplied in phosphorus ($P_{extractable}$=74.42-85.56 ppm) and with a low potassium content ($K_{exchangeable}$=36.26-46.93 ppm), according to the supply range established by Davidescu (1981).

The viticultural climate parameters analyzed were: air temperature, thermal balances and precipitation, recorded at the Weather Station of Research - Development Station for Plant Culture on Sands Dăbuleni. The global thermal balance represents the sum of the average daily degrees during the vegetation period; The active thermal balance represents the sum of average daily temperatures higher than 10°C during the vegetation period; The useful thermal balance represents the sum of the differences between the average daily temperature higher than 10°C, and 10, which represents the biological threshold (Georgescu et al., 1986).

Observations and determinations were made regarding the phenological stages: beginning of bud burst, flowering, beginning of ripening, berries ripe for harvest, shoot fertility, grape production and quality. The phenological determinations consisted in the visual observation and noting the initiation of the vegetation phenophases, when they were recorded in at least 30% of the stumps studied within the cultivar.

Production determinations were carried out by weighing the grapes in 4 repetitions, for each cultivar at technological maturity. For quality determinations, samples were taken from the harvested grapes parameters were analyzed in the laboratory: the weight of 100 grapes, by the gravimetric method, the sugar content using the KRUSS digital portable refractometer 0-32% brix and titratable acidity, by the titrimetric method. The obtained results were analyzed statistically using analysis of variance (ANOVA).

RESULTS AND DISCUSSIONS

Climatic data are necessary for the correct zoning of grape cultivars. Each cultivar requires a certain amount of temperatures for its grapes to reach full ripening. The need for thermal resources cultivar considerably from one cultivar to another and is closely related to the length of the vegetation period of the cultivars (Huglin & Schneider, 1998).

In the vineyards of our country, a global thermal balance is recorded between 2700-4000°C, the active one between 2500-3800°C, and the useful one between 1000-1800°C (Georgescu et al., 1986).

From the analysis of the main climatic elements carried out in the period 2020-2022, it is found that the temperature values, expressed through the heat balances, fell within the limited range presented in the specialized literature, so that the global heat balance recorded 3682°C, the active heat balance of recorded 3601°C and the useful heat balance recorded 1888°C (Figure 1).

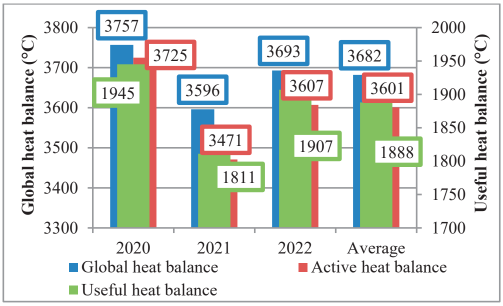

Figure 1. The heat balance recorded in the period 2020-2022

The average temperature during the analyzed period was 12.9°C, higher by 1.42°C compared to the multiannual average from 1956-2022 (11.48°C). The maximum temperature has been increasing since 2020, when 37.3°C were recorded, reaching the maximum of 41.6°C during this period in 2022 (Table 1).

Table 1. The main climatic elements recorded at the weather station* of RDSPCS Dăbuleni during 2020-2022

Climatic elements	**Agricultural year**		
	October 2019-September 2020	**October 2020-September 2021**	**October 2021-September 2022**
Average temperature (°C)	13.6	12.5	12.7
Minimum temperature (°C)	-9.4	-10.6	-10.2
Maximum temperature (°C)	37.3	41.2	41.6
Multiannual mean temperature (1956-2022) °C	11.46	11.46	11.48
Multiannual average precipitation (1956-2022) mm	560.98	562.08	560.06
Annual precipitation (mm)	550.4	547.2	547.2
Precipitation during the vegetation period April-September (mm)	310.4	173.6	300.2
Number of days with rain	102	114	111
Number of days with maximum temperatures > 30°C	79	68	79

*AgroExpert from Adcon Telemetry SRL Romania.

Precipitation during the vegetation period had values between 173.6 mm in 2021 and 310.4 mm in 2020. The average annual precipitation in the period 2020-2022 was lower by 12.66 mm compared to the multiannual average in the period 1956-2022 (560.06 mm), and their correlation with increasing air temperature underlines the increasing drought.

During the analyzed period, beginning of bud burst in grape cultivars for red wines started at temperatures between 11.5-18.2°C. The useful temperature recorded from beginning of bud burst to flowering recorded values between 247.7-276.8°C. To reach maturity, the grapes needed useful temperatures between 734.8-783.6°C, different depending on the cultivar and climatic conditions. The useful thermal balance during the vegetation period recorded values between 1739.7-1765.0°C, and the active thermal balance recorded values between 3429.4-3491.4°C (Table 2).

The specialized literature mentions that during the period of berry growth, the optimum temperature varies between 25 and 26°C (Alleweldt, 1967). Kliewer and Lider (1968), found that temperature of 30°C day and 15°C night reduced berry volume in certain cultivars by 10-20% compared to temperature of 20°C day and 15°C night.

In the 2020-2022 period, the beginning of bud burst phenophase was triggered between April 16 and May 10, the Novac cultivar was the earliest. Flowering took place between May 20 and June 16, approximately 34-36 days after budding (Table 3).

Harvesting of grape cultivars for red wines at the beginning with the Băbeasca neagră cultivar during 13.09-22.10 and Arcaș during 14.09-19.10. The observations made by Constantinescu (1956), on 75 cultivars of vines, proved that the gap between beginning of bud burst of these cultivars varies between 4 and 7 days. However, climatic conditions can cause a delay of 17 to 23 days. The ripening occurs almost suddenly, in about 24 hours, and is marked by changes in the appearance and composition of the berry: the mesocarp loses its firmness, the skin thins, becomes elastic and translucent in white grape cultivars, or reddish in black grape cultivars, and the content of carbohydrates increases rapidly (Irimia, 2012).

In the analyzed period for the red wine grape cultivars, the beginning of destemming lasted

11-12 days, the flowering between 10-13 days, and from the beginning of ripening to the harvest they recorded 66-69 days (Figure 2).

Table 2. Useful heat balance for each phenophase (°C) in the period 2020-2022

Cultivar	Beginning of bud burst	Beginning of bud burst - Flowering	Flowering - Beginning of ripening	Beginning of ripening - Harvest	Useful thermal balance during the growing season	Active thermal balance during the vegetation period
	Limits °C	Σ °t useful	Σ °t useful	Σ °t useful	Σ °t useful	Σ °t active
Băbească neagră	13.9-18.0	272.9	754.8	783.6	1744.2	3445.0
Haiduc	13.9-18.0	276.8	747.3	747.2	1761.1	3472.6
Novac	11.5-14.6	248.5	789.5	734.8	1765.0	3491.4
Arcaș	13.9-18.2	247.7	724.5	767.0	1739.7	3429.4

Table 3. The main phenophases in the period 2020-2022

Cultivar	Beginning of bud burst	Flowering	Berry growth (beginning)	Beginning of ripening	Harvesting grapes
Băbească neagră	18.04-10.05	27.05-12.06	05-14.06	19.07-01.08	13.09-22.10
Haiduc	18.04-07.05	28.05-13.06	06-15.06	23-26.07	19.09-19.10
Novac	16.04-10.05	20.05-10.06	04-13.06	25-27.07	19.09-19.10
Arcaș	18.04-07.05	24.05-10.06	05-13.06	18-26.07	14.09-19.10

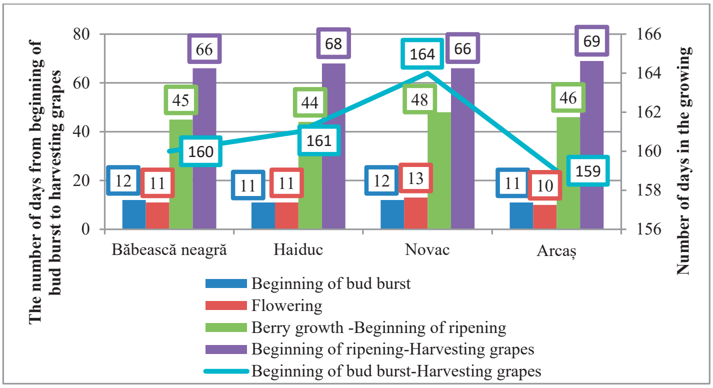

Figure 2. The duration of each phenophase in the period 2020-2022

The vegetation period for red wine grape cultivars was between 159-164 days. Dvornic et al. (1966) analyzing the duration of the flowering period for 5 cultivars of vine cultivated in the southern part of Romania found that this phase lasts 11-12 days and occurs between June 2-12.

The duration of the total growing period of the berries is on average 45 days. Rogiers et al. (2000) studied the process of berry growth in the Shiraz grape cultivar. It was found that the fresh mass of the berries increased for 85 days after flowering, stagnated until the 95th day after flowering, so that later the mass of these decrease.

Fertility and productivity of grapevine cultivars are traits that influence grape production (Table 4).

Table 4. Fertility of shoots in some cultivars of grapevine in the period 2020-2022

Cultivar	Total number of shoots per stump	Number of fertile shoots per stump	The percentage of fertile shoots (%)	Number of inflorescences per stump	Fertility coefficient	
					Relative ≥ 1	Absolute ≥ 1.5
Băbească neagră	27	20	74	23	0.87	1.16
Haiduc	23	19	83	26	1.12	1.62
Novac	28	19	67	27	0.99	1.40
Arcaș	24	20	83	30	1.23	1.50

Fertility is expressed in addition to the percentage of fertile shoots, the proportion of fertile shoots with one or two inflorescences and by the values of the two coefficients of relative and absolute fertility (Belea, 2008). Shoot fertility recorded different values depending on the variety and climatic conditions. The relative and absolute fertility coefficients recorded above-unit values for the Haiduc and Arcaş cultivars.

In the period 2020-2022 for cultivars with grapes for red wines, the average production had values between 13012 kg/ha, for the Arcaş cultivar and 20479 kg/ha, for the Novac cultivar, who recorded made the biggest difference (6579 kg/ha) compared to the Băbeasca neagră cultivar, taken as a control, difference statistically assured as distinctly significant (Table 5).

Table 5. Grape production of some vine cultivars in the period 2020-2022

Cultivar	Average production (kg/ha)	Difference from the witness (kg/ha)	Signification
Băbeascǎ neagrǎ	13900	Mt.	
Haiduc	17662	3762	-
Novac	20479	6579	**
Arcaş	13012	-888	-
DL 5%=3816 DL 1%=5779 DL 0.1%=9283			

The useful heat balance and the active heat balance (°C) influenced grape production, the results obtained being represented by distinctly significant positive correlations (Figure 3 and Figure 4), which underline the increase in grape production with the accumulation in the air of as many thematic resources (r=991**; r=0.998**).

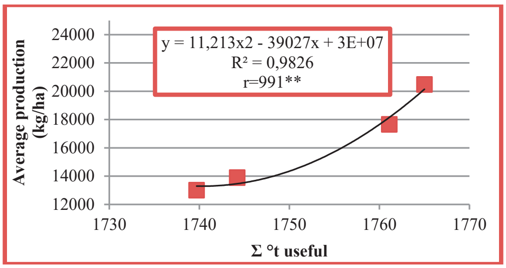

Figure 3. Correlation between average grape production (kg/ha) and useful heat balance °C in some red wine grape cultivars

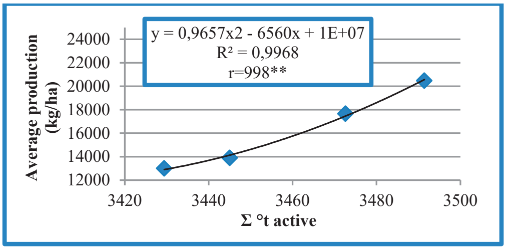

Figure 4. Correlation between average grape production (kg/ha) and active heat balance °C in some red wine grape cultivars

Quality analyzes consisted of determinations of 100 berry weight, total sugar content, and total titratable acidity at harvest. In the period 2020-2022, the weight of 100 berry recorded values between 180 g for the Arcaş cultivar and 263 g for the Novac cultivar, with a difference compared to the witness of 57 g, statistically assured as significant. The sugar content recorded values between 180 g/l for the Arcaş cultivar and 200 g/l for the Novac cultivar. Titratable acidity at harvest expressed in g/l H_2SO_4 recorded values between 3.76 g/l for the Arcaş cultivar and 4.10 g/l for the Novac cultivar (Table 6).

Table 6. Grape quality of some vine varieties in the period 2020-2022

Cultivar	Weight of 100 berries		Total sugar content		Total titratable acidity H_2SO_4	
	(g)	Difference from the witness (g)	(g/l)	Difference from the witness (g/l)	(g/l)	Difference from the witness (g/l)
Băbeascǎ neagrǎ	206	Mt.	197	Mt.	3.98	Mt.
Haiduc	222	16	196	-1	3.94	-0.04
Novac	263	57*	200	3	4.10	0.12
Arcaş	180	-26	180	-16	3.76	-0.21
DL 5%=42			DL 5%=21		DL 5%=1.3	
DL 1%=63			DL 1%=32		DL 1%=2.0	
DL 0.1%=101			DL 0.1%=51		DL 0.1%=3.2	

CONCLUSIONS

The climatic conditions recorded in the area of sandy soils in the south of Oltenia were favorable for the metabolism of red wine grape cultivars, the active heat balance (3429.4-3491.4°C) and the useful heat balance (1739.7-1765°C) falling within the limits presented in the specialized literature.

The obtained results showed that the beginning of bud burst phenophase of these cultivars with grapes for red wines started at temperatures between 11.5-18.2°C, the earliest being the Novac cultivar, which beginning of bud burst registered April 16 and May 10.

The vegetation period for red wine grape cultivars was between 159-164 days, under the conditions of recording an active thermal balance between 3429.4-3491°C.

From the cultivar of vines with grapes for red wines studied on sandy soils, the Novac cultivar stood out with the best production (20479 kg/ha) and quality (200 g/l sugars) characteristics.

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THE EFFECTS OF CLIMATE CHANGE ON VINES IN THE MAIN GROWING COUNTRIES IN EUROPE

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Abstract

Climate change is one of the most urgent problems of contemporary society and has significant consequences for natural ecosystems and economic sectors, including the wine industry. Through this paper, an assessment of the consequences of climate change on vine is proposed, examining how these climate phenomena have influenced the growth cycles, quality and quantity of grape harvest in different European wine-growing regions. Another purpose of this documentation is to analyze available research and data to identify the contributory role of different factors in climate change. It is also intended to identify and analyze the strategies and solutions adopted by winegrowers to face these climate challenges, including the adaptation of grape varieties, the implementation of sustainable agricultural practices and the use of innovative technologies in viticulture. The results of this review underline the need for a proactive approach and international collaboration to manage climate change in the European wine sector.

Key words*: climatic factors, grapes, European region.*

INTRODUCTION

Climate change, also referred to by the acronym CC, is characterized as any change in climatic conditions that is maintained in the long term and is recognized by most researchers as one of the main ecological challenges our society is facing in the 21st century (Pachauri & Reisinger, 2007; Pachauri el al., 2014). A steady rise in temperature, as the main measurable effect of CC, is projected to persist globally, and significant changes are likely to occur in global hydrological and energy cycles (Pachauri el al., 2014; Noguer et al., 2001), resulting in an intensification of radiation as well as the frequency and severity of extreme weather events (Pachauri et al., 2014; Easterling et al., 2000; Bartolini et al., 2008). Europe stands out as a particularly sensitive region to the increase in temperature caused by CC, especially during the warm season, as continued warming is predicted to persist throughout the 21st century on this continent (Giorgi, 2006), where predominant negative impacts are anticipated, including lower harvests, significant variations in agricultural production and a decrease in areas suitable for traditional crops (Olesen & Bindi, 2002). Through this paper, we aim to carry out a comprehensive assessment of the consequences of climate change on the grapevine, the main aspects being: 1) highlighting the significant role of the various factors contributing to climate change in the viticulture industry; 2) assessing how these climatic phenomena have influenced the diversity of growth cycles and shaped the qualitative and quantitative characteristics of the harvest in different European wine-growing regions; 3) identifying and analyzing in detail the strategies and solutions adopted by winegrowers to counteract the negative effects of climate change.

Grapevine (*Vitis vinifera* L.) is one of the most essential crops in Europe, having a significant socio-economic impact. Europe has the largest wine production and the most extensive wine-growing area worldwide, hosting some of the most famous regions and wines. These regions are predominantly located in the Mediterranean area, with a particular concentration in countries with significant wine production, such as Italy, France and Spain (Aurand, 2017). According to the most recent reporting of the

International Organization of Vine and Wine (OIV) (Pachauri & Reisinger, 2007), estimates indicate that the global wine-growing area covers approximately 7.449 million hectares (2018). In the same year, Spain occupied a share of 13% of the world's total wine-growing area, followed by China (12%), France (11%), Italy (9%) and Turkey (6%). These five countries represent approximately half of the entire global vine area. According to the same OIV report (Pachauri & Reisinger, 2007), in contrast to the evolution of the global vine area, world grape production has registered a significant increase in the last two decades, highlighting increases in wine-growing productivity. According to the OIV (Pachauri & Reisinger, 2007), the estimated amount of production reached about 77.8 million tons (103 kg) in 2018, registering a record value, compared to the range of 60-65 million tons at the beginning of the 21st century. The general mode of grape production includes wine grapes (57% of the total), table grapes (36%) and dried grapes (7%). The production of both table grapes and dried grapes is predominant in some countries without a tradition of wine production, such as China (89.7% of total grape production), Turkey (96.8%), India (98.5%), Iran (100%), Uzbekistan (96.3%), Egypt (99.5%) and Brazil (53.5%). In contrast, wine grape production is associated with countries where viticulture is largely dedicated to wine production, such as Italy (86.5%), Spain (96%), France (99.6%), Argentina (93.7%), Australia (90.9%), Germany (99.6%) or Romania (93.1%), listed in descending order in terms of production volume. The four wine producers with the highest production, classified according to the volume obtained, are Italy (54.8 million hectoliters), France (48.6), Spain (44.4) and the USA (23.9), the first three being also the most important exporters globally (Pachauri & Reisinger, 2007). The most significant wine-growing regions worldwide, such as Bordeaux, Burgundy, California, Cape/South Africa, Champagne, La Mancha, La Rioja, Mendoza, Mosel, Porto/Douro, South Australia, Tuscany and others are represented in (Figure 1a). These wine-growing regions are usually located in Recognized Origin areas, while (Figure 1b) highlights the area dedicated to vines in Europe (Kottek et al., 2006; Peel et al., 2007; Fraga et al., 2013).

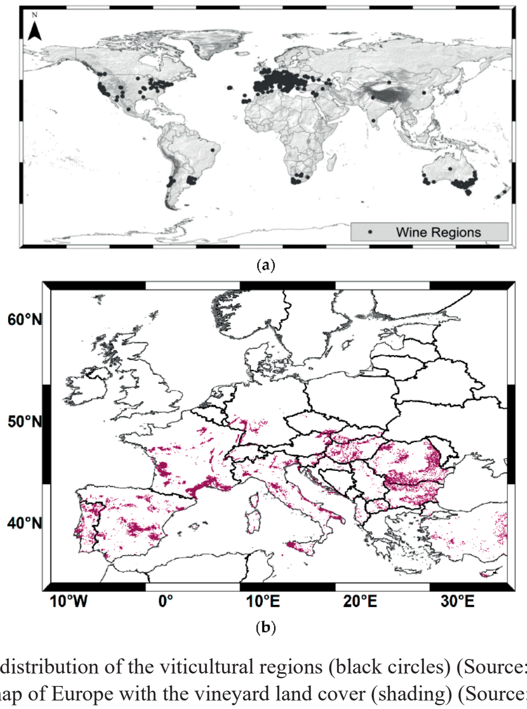

Figure 1. (a) World distribution of the viticultural regions (black circles) (Source: Fraga et al., 2013).
(b) Political map of Europe with the vineyard land cover (shading) (Source: Cover, 2018)

ANALYSIS OF THE ROLE OF CONTRIBUTING FACTORS TO CLIMATE CHANGE IN THE WINE INDUSTRY: AN ASSESSMENT BASED ON RESEARCH AND AVAILABLE DATA

During the 20th century, European regions have experienced significant changes in a varied range of climate factors, showing a large regional variation, according to the (2007) Intergovernmental Panel on Climate Change IPCC report.

Significant changes in temperature were observed during the 20th century, according to research by Santos & Leite (2009). These changes included thermal increases of between 2.3–5.3°C in northern Europe and 2.2-5.1°C in southern Europe, according to the study conducted by Christensen et al. (2007). In fact, changes in the frequency of thermal extremes and precipitation patterns in Europe have been associated with certain atmospheric features, such as the North Atlantic Oscillation (NAO), according to analyzes by Santos & Corte-Real (2006).

By analyzing the results of several simulation models, it is anticipated that the global mean surface temperature will most likely increase by 1°C and 4.5°C, depending on future industrial emissions. The most reliable estimate suggests a warming of between 1.8-2.5°C by the middle of the next century (Carter et al., 1991; Schultz, 2000).

Climate change projections in Europe show a more pronounced warming trend in southern and north-east oriented regions (Christensen & Christensen, 2007; Jacob et al., 2014). Also, significant increases in minimum and maximum temperatures are observed during summer and autumn (Cardell et al., 2019), which coincide with the vine growing season, which takes place between April and October in the northern hemisphere.

The prevalence of continuous temperature increase is a quantifiable factor of climate change, already generating significant changes in global hydrological and energy cycles (Houghton et al., 2001; Pachauri et al., 2014). These transformations, in turn, have amplified the frequency, intensity, and duration of extreme weather events, such as heat waves, droughts or excessive precipitation (Kostopoulou & Jones, 2005; Zeder & Fischer, 2020).

Precipitation and its seasonality also represent an essential atmospheric factor influencing the development of the vine, having a significant control over the soil moisture and the water potential of the vine, especially in non-irrigated wine-growing areas (Huang et al., 2016; Suter et al., 2019). In future perspectives, the current wine-producing regions of southern Europe may experience a reduction in their suitability for viticulture, predominantly due to severe drought (Toth & Vegvari, 2016; Fraga et al., 2018). These regions could face excessively dry conditions for the production of high-quality wines (Kenny & Harrison, 1992), and in some extremely critical situations, may require intensive irrigation (Koundouras et al., 1999; Fraga et al., 2018). Areas such as Andalucía, La Mancha (Spain), Alentejo (Portugal), Sicily, Puglia and Campania (Italy) will likely suffer from severe water shortages. Studies have also indicated that increased summer dryness in southern Europe will lead to a decrease in yield, mainly due to the synergy between warming and drying (Fraga et al., 2016).

Solar radiation also represents an essential element that impacts viticulture. An adequate amount of radiant energy is required, especially during the grape ripening period (Manica, 2006).

Climate change in terms of UV-B radiation has raised concerns in the past, in the context of changes in the protective ozone layer. UV-B radiation influences the composition of grapes, causing changes in secondary metabolites, such as flavonoids, amino acids and carotenoids (Schultz, 2000). Regardless of a possible further increase in UV-B radiation, the combination of high radiation levels and increased temperatures, especially under severe water stress, is often the cause of sunburn damage to both leaves and berries, conditions forecast to become more frequent in Southern Europe (Dinis et al., 2016; Dinis et al., 2018; Santos et al., 2020).

DYNAMICS OF CLIMATIC PHENOMENA AND IMPACT ON GROWTH AND HARVEST CYCLES IN EUROPEAN VITICULTURE REGIONS

Traditionally, vine is grown in geographical regions characterized by an average temperature in the range of 12-22°C during the growing season (Jones, 2010), having an optimal vegetative response to average daily values between 20°C and 35°C (Droulia & Charalampopoulos, 2021). In order to interrupt bud dormancy and initiate the growth/vegetative cycle, winter cooling is required, with a minimum temperature of 10°C (Amerine & Winkler, 1944; Dokoozlian, 1999), essential also for the accumulation of carbohydrate reserves in the perennial structures (roots, trunks and shoots), preparing them for development in the next year (Bates et al., 2002; Field et al., 2009).

The progress in the development of the vine is closely related to the different stages of its vegetative and reproductive cycles. In regions of many traditional wine-growing areas, such as the extratropics, the vegetative cycle of the vine extends over a full year, while its reproductive cycle spans two years. The reproductive cycle exerts control over some significant characteristics, both quantitative and qualitative, such as the number of grape bunches for the following year and the vegetative cycle is composed of two major consistent stages: the dormancy period and the growing season. The phenological evolution of the vine includes multiple stages or phenophases according to (Figure 2). These phases of the vegetative and reproductive cycles of the vine are mainly under the control of atmospheric conditions (Santos et al., 2020).

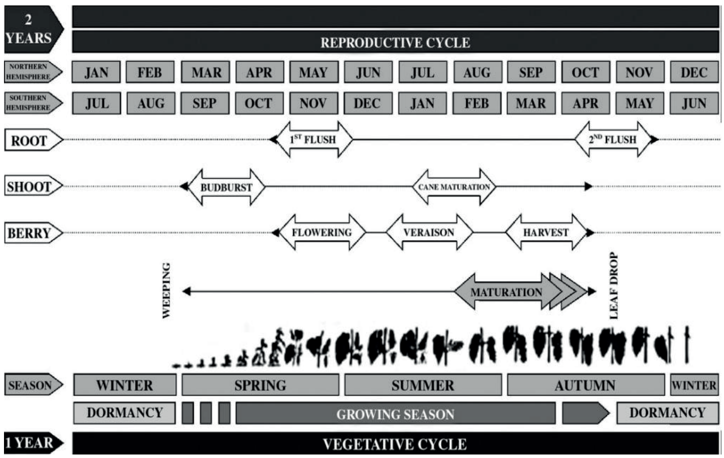

Figure 2. Vegetative and reproductive cycles and vine phenological stages.
Adapted from Eichorn & Lorenz (1977) and Magalhães (2008)

Understanding the influence of high temperatures on the intermediate periods to maturity is essential both for the application of viticultural practices and for the interpretation and identification of trends in the context of climate change. For example, late pruning of vines can be used to delay development and diminish the risk of frost in colder regions (Friend & Trought, 2007). As a general rule, if there is significant damage to the buds in the vine, it is better to keep a larger number of buds at pruning (Sadras & Moran, 2012).

In the time interval between the fall of the leaves and the onset of spring, the vine enters a period of dormancy characterized by the exclusive presence of woody tissue and a reduced physiological activity (Magalhães, 2008). This phase is divided into two sub-periods, controlled by endogenous and exogenous thermal factors, essential for release from the state of dormancy. The first sub-period (endo-dormancy) is initiated by the accumulation of cold (cold units) during autumn and winter, while the second sub-period (eco-dormancy) depends on heat accumulation until budbreak.

Thus, winter cold is a crucial condition for the evolution of vine growth, as low temperatures promote dormancy of buds (Kliewer et al., 1972; Santos et al., 2017), along with other factors such as shortening days and ageing of photosynthetic active parts of the plant. From late winter to early spring, the accumulation of average daily temperatures between 7 and 10°C generally stimulates the end of dormancy and initiation of the vine growth cycle (Amerine & Winkler, 1944).

If the cooling requirement is not adequately fulfilled due to climate change, budding becomes irregular, generating in uneven phenological development in later stages of the season (Tesic et al., 2002; Trejo-Martínez et al., 2009).

There is research suggesting that in the near future, some Mediterranean regions, such as Sardinia and Sicily, could face problems due to excessive temperatures leading to an insufficiency in the accumulation of cooling units and ultimately to the lack of budding. On the other hand, higher temperatures in the latter part of winter influence the eco-dormancy period by reducing the accumulation period of the forcing factor. The cooling requirement in Germany is forecast to be already satisfied at the beginning of winter, but a longer eco-dormancy period is observed until the budding date. In this context, the impact of rising temperatures is more pronounced for European regions characterized by longer dormancy cycles (Leolini et al., 2018).

In the future perspectives, an earlier appearance of budburst and flowering has been forecasted with particular importance in north-eastern Europe. At the same time, the impact of higher temperatures was more pronounced for late compared to very early and early grape varieties in western regions (Leolini et al., 2018).

Phenological diversity in different regions has significant implications in the face of associated extreme events. In Western Europe, where the budburst process occurs earlier, especially in Spain, France and the United Kingdom, there was an estimated increase in the frequency of frost events during budburst (e.g. in Spain: between 9 and 30%, in France: between 3 and 41%, in the United Kingdom: between 3 and 50%). In contrast, Germany and Italy recorded a lower incidence of frost events, ranging between 0% and 16% and between 1% and 11%, respectively (Leolini et al., 2018).

The increased impact of temperatures on budburst is not the only challenge that influences vine phenology. In general, an earlier budburst process leads to an earlier later stage, such as flowering, even if this effect is less pronounced (Fraga et al., 2016).

The initial process of inflorescence differentiation begins near the flowering stage of the previous year (Alleweldt & Ilter, 1969; Morrison, 1991). Warm and sunny periods in this phase favor the development of inflorescence primordia, while cold and cloudy conditions stimulate shoot formation (Buttrose, 2017; Keller 2020). Therefore, the climatic conditions of the previous year have a direct influence on the yield in the following season (Molitor & Keller, 2016). The impact of stressful temperatures during the flowering period is an essential aspect for the final yield (Hale & Buttrose, 1974; Vasconcelos et al., 2009).

Given that the optimal temperature range for the flowering process varies between 20 and 30°C (Kozma et al., 2003; May, 2004), higher or lower temperatures recorded at this stage have a negative impact on flower formation, pollen germination, grape berries formation causing the appearance of several physiological disorders, respectively on the production of grapes (Ewart & Kliewe, 1977).

According to the findings of Ebadi et al. (1995), a 30% reduction in flower size and pollen germination was observed in Chardonnay and Shiraz cultivars under conditions of temperature drops before flowering (Leolini et al., 2018).

Regarding the aspect of quality, high temperatures during the growing season contribute to the accumulation of sugars in the grapes and the breakdown of organic acids, essential factors for the maturity of the grapes. Inadequate temperatures during the growing season can lead to the formation of immature berries, unsuitable for wine production. On the other hand, extremely high temperatures during the ripening period can also affect quality by increasing excessive sugar levels and decreasing acidity, causing low concentrations of anthocyanins and flavonoids (Haselgrove et al., 2000; Downey et al., 2006; Sadras & Moran, 2012), which, in turn, reduce the aromatic characteristics of wines (Jackson & Lombard, 1993; De Orduna, 2010).

Prolonged exposure to extremely high temperatures, such as those above 35-40°C, can have a negative impact on the photosynthetic system of the plant (Berry & Bjorkman, 1980) and can cause damage to the grape skin in the form of burns, thus increasing the incidence of latent fungal infections within the grapes (Steel & Greer, 2006).

Forecasts of future changes in minimum temperatures during the ripening period in the Iberian Peninsula have also been identified, according to studies by Projeção & Portuguesa (2012) and Malheiro et al. (2012), suggesting a possible decrease in wine quality.

According to ProWein's 2019 business report, available at (https://www.prowein.de/) the results of an industry survey of more than 1,700 enterprises indicated that the sensory profiles of wines have changed in recent decades. The conclusion of this study suggests that climate change has the potential to threaten the typicality of wines in traditional wine-producing regions (Molitor & Junk, 2019).

INNOVATIVE STRATEGIES AND SOLUTIONS ADOPTED BY WINEGROWERS TO ADDRESS CLIMATE CHALLENGES IN VITICULTURE

The adaptation measures addressed by winegrowers can be divided into two levels: in the short-term they can be considered as a first protection strategy and should focus on specific threats, especially on changes in crop management practices for example late vine pruning, goblet vine management, planting density, no-till and minimum tillage systems (MIT), use of foliar protection substances against sunburn, use of rootstocks and resistant varieties in drought, irrigation, shade nets, solar screens for leaf protection, while in the long term, a wide range of adaptation measures should be considered for example (creating varieties adapted to future climatic conditions and the migration of vines to higher altitudes).

Winter pruning as a short-term measure, if performed later, the budburst process is slightly delayed by a few days (Friend & Trought, 2007). However, the variations seem to become smaller in the case of later phenological stages. Discrepancies are more notable when pruning is performed when the vine has 2-3 leaves, without however affecting yield or pruning weight in the following season (Moran et al., 2019). Maturity is significantly delayed when the vine is subjected to a second pruning, long after the budburst period (Friend & Trought, 2007; Martínez-Moreno et al., 2019; Petrie et al., 2017). However, this method remains in the experimental stage, and the long-term consequences on vigor need to be studied in depth. Another short-term measure can be the crown management system that proves remarkably resistant to drought and high temperatures, known as the Mediterranean goblet or shrub vine. Through this management mode, it is feasible to grow vines without resorting to irrigation in extremely arid environments, up to only 350 mm of precipitation/year (Deloire, 2012; Santesteban et al., 2017). Certainly, goblet-style vines tend to produce generally lower yields, but enjoy ease of cultivation at low costs per hectare (Roby et al., 2008). In the search for alternative solutions to improve the drought resistance of the vine, the expansion of the space between the rows can be considered. In regions where water deficit is not a major problem, such as Bordeaux, Champagne and Burgundy in France, wide row spacing is a traditional practice. In the context where water becomes a limiting factor, narrowing the space between the rows contributes to a more efficient use of water, as the capture of sunlight provides the necessary energy for the transpiration process (Van Leeuwen et al., 2019).

According to research by Fischer et al. (2007), it is found that the adoption of strategies to reduce the impact, which lead to lower concentrations of greenhouse gases, can lead to a decrease of about 40% in water requirements in agriculture, compared to unfavorable climatic conditions. No-till and minimum tillage practice systems (MIT) are considered the most efficient because the absence of soil surface disturbance promotes carbon retention

and sequestration according to research by (Kroodsma & Field, 2006).
For sunburn, exploring the alternative of using mineral and chemically inert substances to protect leaves against sunburn is considered a significant option (Pelaez et al., 2000; Glenn et al., 2010).
To prevent the effects of drought on vine, an efficient and ecological adaptation consists in the use of drought-resistant rootstocks to maintain yields and prevent quality losses caused by excessive water stress. This choice represents an environmentally friendly strategy and, once implemented, does not generate significant increases in production costs (Van Leeuwen et al., 2019).
Vines varieties show significant variation in drought tolerance (Chaves et al., 2007). This diversity may be associated with how different cultivars regulate their water availability in response to increasing atmospheric demands and fluctuations in soil water content. Another useful indicator for assessing the drought tolerance of cultivars is how they adjust their water use efficiency in the face of drought challenges. Most varieties originating from the Mediterranean basin (such as Grenache, Cinsault, Carignan) are recognized for their resistance to drought, while others, such as Merlot, Tempranillo or Sauvignon Blanc, do not show the same tolerance. There are also reports indicating that certain local varieties from the Mediterranean islands, such as Xinistery from Cyprus, demonstrate particular drought resistance and could be considered for cultivation outside their region of origin. Choosing to plant drought-resistant varieties in arid environments is an effective strategy in adapting to climate change, which is why these varieties deserve more attention (Gowdy et al., 2019).
The reuse of water for irrigation is configured as a viable economic option for agriculture in the Mediterranean region. This practice helps reduce the need to develop new water sources and provides an adaptive solution to climate change. Recycled water, in many arid and semi-arid regions of the Mediterranean, becomes an accessible alternative resource for agricultural, industrial and urban use not for consumption (Lazarova et al., 2001; Angelakis & Gikas, 2014). The potential benefit can be amplified by expanding and optimizing wastewater treatment facilities. For example, in Spain, about 408 hm3/year (13% of the total available water) is reused, of which 79% is dedicated to agricultural irrigation 320 hm3/year (Raso, 2013). Irrigation is proving to be one of the most efficient methods for increasing yield and crop quality in dry regions (Costa et al., 2007; Forbes et al., 2009; Flexas et al., 2009, Romero et al., 2010).
In the context of creating new vine varieties, White et al. (2006) argue that breeding programs should focus on developing varieties resistant to high temperatures. Related to this direction, Duchene et al. (2012) developed a framework for the genetic crossbreeding of new cultivars more efficiently adapted to future climatic conditions, while maintaining some key characteristics of already existing cultivars. In addition, given the significant diversity of existing vine varieties, it is crucial to maintain natural biodiversity to ensure a more effective adaptation to climate change (Tello et al., 2012).
Viticulture is predominantly limited to regions located below 50ºN latitude. In the future, there are prospects that viticulture will expand to areas with latitudes up to 55ºN, opening possibilities for expanding the areas dedicated to the cultivation of vine. However, this expansion may face resistance due to current regulations in Europe and increased interest in other crops such as wheat, barley and maize (Ingram & Porter, 2015). Regarding wine-producing regions in southern Europe, such as Italy, Spain, and Portugal, projections indicate the maintenance of viticulture viability, although its sustainability may be affected (through lower yields) due to accentuated warming and drying conditions (Moriondo et al., 2013, Toth & Vegvari, 2016).

CONCLUSIONS

Climate change has caused a significant change in the phenological stages of the vine during the last decades. If the trend of increasing annual temperatures and global warming persists, according to climate model forecasts, the global wine industry will face a concrete threat in the next period. Future risk assessment aims to develop rational and sustainable strategies for winegrowers, thus contributing to adaptation to new climate conditions (Iglesias et al., 2007).

Climate change leads to higher temperatures, periods of drought and intensification of radiation, especially UV-B radiation. These changes have a significant impact on the cultivation process of vine and wine production, affecting both European countries and viticultural markets globally. However, this review presents various adaptation options, offering growers the opportunity to maintain high-quality wine production with sustainable economic yields in the face of climate change (Van Leeuwen et al., 2017).

Although studies on the possible impact of climate change on viticulture are largely advanced, compared to other agricultural fields, there are still important knowledge gaps (Santos et al., 2020).

Understanding the future evolution by analyzing the present thus becomes a need for important information, but also an urgent procedure, considering that the consequences of climate change presented in this study bring particularly significant social and economic implications for the European wine industry. In this sector, the origin and variety of grapes are essential indicators of the quality and specificity of the products. Information on the already observed impact of climate change can be utilized and integrated into sophisticated and precise climate change analysis tools with the aim of obtaining a deeper understanding and define more precisely the effect of this phenomenon on environmental sustainability in the wine sector in the following decades (Droulia & Charalampopoulos, 2022).

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AMPELOGRAPHIC AND BIOPRODUCTIVE CHARACTERISTICS OF CABERNET SAUVIGNON CLONES IN OLTENIA WINEGROWING REGION, SOUTH-WEST ROMANIA

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Abstract

Polyclonal vineyards are a useful technological alternative in the context of climate changes foreshadowed in the medium and long term, through the premises of ensuring a sustainable viticulture, but also the possibility of obtaining complex wines. In the last decade in Romania, various international clones of wine grape varieties have been introduced in vineyards, whose performance in terms of adaptation or their bioproductive and qualitative potential in Romanian vineyards is little known. In this context, the aim of this study was to evaluate and determine certain phenotypic characteristics, as well as the bioproductive and qualitative performance of five Cabernet Sauvignon clones: two of French origin (15 and 338 ENTAV) and three clones of Italian origin (ISV 105, ISV 117 and R5), in the pedoclimatic conditions of South-West Romania. Good fertility results are shown by clones R5 and 15 ENTAV, with ISV 117 and R5 clones being the most productives. All clones ensure the quality parameters required for Cabernet Sauvignon DOC wines produced in Oltenia winegrowing region.

Key words: *Cabernet Sauvignon, clones, grape quality, phenotypic characteristics.*

INTRODUCTION

Satisfying consumer demands for the most diverse, typical wines with a high degree of naturalness is a permanent concern equally for producers and the scientific environment (Chivu-Draghia & Antoce, 2016; Monteiro et al., 2020; Schäufele & Hamm, 2017).

The evaluation and use of viticultural genetic diversity are intensively explored alternatives worldwide in the context of the challenges generated by climate change (Bigard et al., 2018; Carvalho et al., 2020; Romero et al., 2023; Tortosa et al., 2020) and meeting the diversified demands of consumers of grapes and wine (Töpfer & Trapp, 2022).

Recent studies that targeted the wine-growing areas in the S-W Romania region, indicated that the changes and trends detected (1961-2021 period) for a series of climatic indices can generate pressure regarding the obtaining of quality wines from certain varieties, against the background of the increased temperature and reduced amounts of precipitation during the growing season (Bucur & Babeș, 2016; Irimia et al., 2018; Vlăduț et al., 2023). Polyclonal grapevine plantations are a useful technological alternative in the context of climate changes foreshadowed in the medium and long term, through the premises of ensuring a sustainable viticulture ((Marković et al., 2017), but also the possibility of obtaining some complex wines (Cichi et al., 2022).

Obtaining quality wines, competitive on the domestic and foreign markets, is an important goal for Romanian wine producers (Mircea, 2020; Muntean et al., 2018).The strategies in the Romanian vine and wine sector during the last two decades has generated important qualitative leaps regarding the areas cultivated with *V. vinifera* L. varieties for wine (Cichi et al., 2021; Mălăescu et al., 2022). Of the approx. 95 thousand ha cultivated with *V. vinifera* L. varieties for wine in Romania, 61% represent the varieties for white wines and 29% the varieties for red wines. The top three varieties for red and rosé wines as vineyard area in Romania are represented by: 'Merlot'

(11.74%), 'Cabernet Sauvignon' (4.76%) and 'Fetească neagră' (3.36%).

Improving the vine-growing techniques and the qualitative grape composition of the 'Cabernet Sauvignon' cultivar for obtained of high quality wines is an important concern (Băducă Câmpeanu et al., 2020; Drenjančević et al., 2017; Nistor et al., 2022; Wang et al., 2019).

In the last decade in Romania, various international clones of wine grape varieties have been introduced in vineyards, whose performance in terms of adaptation or their bioproductive and qualitative potential in Romanian vineyards is little known. In this context, the aim of this study was to evaluate and determine certain phenotypic characterristics, as well as the bioproductive and qualitative performance of five 'Cabernet Sauvignon' clones cultivated in South-West Romania.

MATERIALS AND METHODS

Location and climatic characteristics. The study was conducted for two consecutive years (2022-2023) in wine grape vineyard, located in Didactic Research Station of the University of Craiova (Dealurile Craiovei vineyard, Hills of Muntenia and Oltenia winegrowing region), in the south-west part of Romania. The studied vineyard area is located between the parallels of 44°29' north latitude and 23°87' east longitude (190 m elevation). The main climatic characteristics during the study period are shown in Table 1. The climatic data were obtained from the following source Klein Tank et al, 2002 (data available at http://www.ecad.eu for the Craiova meteorological station-44°13' latitude and 23°52' longitude, 192 m altitude, Dolj County).

Plant material. Five 'Cabernet Sauvignon'clones: two of French origin (clones 15 and 338) and three clones of Italian origin (ISV 105, ISV 117 and R5) grafted on SO4 rootstock were used. The study was conducted on 6-7-year-old vines. The vines were cultivated under the same growing conditions, on reddish-brown soil, with 2.2 x 1.2 m spaces, semi-tall shape of the stem (with a trunk of 0.8 m), Cordon spur pruned, 30 buds/vine, without irrigation. The viticultural management (fertilizer application, pest, diseases and weed control, etc.) was applied for all clones in the same way. The randomized experimental design was used. Ten vines per clones were selected for the study, in three replications.

Table 1. Main climatic indexes of the experimental site

Climatic Index	2022 Year	Class	2023 Year	Class
SAT (Sum of average daily temperature > 10°C, April 1st to September 30th)	3633	Normal for region	3585	Normal for region
Winkler Index (April 1st to October 31th)	2032	Temperate	2000	Temperate
Huglin`s heliothermal index (IH)	2583	IH5-Warm	2484	IH5-Warm
Annual Rainfall (mm)	473.5	Normal for region	575.2	Normal for region
Rainfall in the growing season (mm, April 1st to September 30th)	365.4	Normal for region	318.2	Normal for region
De Martonne Aridity Index (I_{DM}, year)	20.16	Semi-arid	24.52	Moderately-arid
De Martonne Aridity Index growing season (I_{DM}, April 1st to September 30th)	15.55	Semi-arid	10.69	Semi-arid
Nights cold Index CI (°C)	12.21	Cool nights	15.11	Temperate nights

Agrobiological, quantitative and qualitative characteristics. The phenotypic traits were defined and recorded in accordance with OIV standardized descriptors and methods (OIV, 2009) and the standard protocol for phenotyping established by Rustioni et al. (2014). Percentage of fertile shoots, absolute and relative fertility index (Afi and Rfi) were established and calculated according to Cichi et al., 2022.

Sampling measurement and analyses. Five bunches for each clone, 10 berries from the middle part of bunches, in 3 replicates, were used for measurements and analyses of bunch and berry traits, at full maturity/ harvest. For each clone studied, sugar content (°Brix values) was measured using Kruss Optronic Hand Refractometer Hrot 32. Total acidity of must (g/L H_2SO_4) was determined by the titration method, NaOH 0,1N until pH 7.0. Sugar content and total acidity of must measurements were done in three replicates. ***Statistical analysis***. Each variable was examined by analysis of variance (One-way ANOVA). The morphometric, biochemical and productive characteristics are presented as means and standard deviation of each variable. All variables that were significant in the F test were analysed by HSD Tukey`s test to means separation and to establish if there were significant differences among the clones.

RESULTS AND DISCUSSIONS

Climatic resources, along with other terroir factors, have a primary role in the growth and development of grapevine varieties (Gutiérrez-Gamboa et al., 2021), influencing their fruit set and biochemical compounds metabolism in grapes, with implications in the production and quality of grapes, the raw material for wine-making, and implicitly in the quality of wines. In terms of climatic resources, the studied years were favorable for the vine growing but, there is observe a semi-arid aspect during the 2022 year and growing seasons (2022 and 2023), based on De Martonne Aridity Index (Table 1).

Agrobiological characteristics. In what concerns the vegetative growth, among the French clones, the clone 338 ENTAV stands out with the highest number of shoots/vines, the differences being significant compared to clone 15 ENTAV (p<0.01); between the Italian clones there are no significant differences (Table 2). The R5 clone stands out for its good fertility, the differences were significant compared to the 338 ENTAV clone both in terms of the percentage of fertile shoots and the relative fertility index (number of grapes in relation to the total number of shoots/vine, R.f.i.). However, the French clone 15 ENTAV stands out by a higher number of grapes on fertile shoots (A.f.i.).

Table 2. The agrobiological characteristics (2022-2023)

'Cabernet Sauvignon'clones	Total number of shoots/vine	CV %	Percentage of fertile shoots (%)	CV %	R.f.i.	CV %	A.f.i.	CV %
15 ENTAV	$25.89{\pm}4.48^{c}$	17.30	$68.92{\pm}8.57^{ab}$	12.43	$0.84{\pm}0.14^{ab}$	16.66	$1.21{\pm}0.06^{a}$	4.96
338 ENTAV	$40.27{\pm}1.92^{a}$	4.77	$58.05{\pm}6.93^{b}$	11.94	$0.61{\pm}0.11^{b}$	18.03	$1.10{\pm}0.04^{b}$	3.63
ISV 105	$35.39{\pm}5.17^{ab}$	14.61	$67.77{\pm}5.62^{ab}$	8.29	$0.77{\pm}0.06^{ab}$	7.79	$1.14{\pm}0.02^{ab}$	1.75
ISV 117	$34.35{\pm}2.19^{abc}$	6.38	$68.56{\pm}9.08^{ab}$	13.24	$0.79{\pm}0.10^{ab}$	12.65	$1.16{\pm}0.05^{ab}$	4.31
R5	$29.44{\pm}2.37^{bc}$	8.05	$80.31{\pm}4.13^{a}$	5.14	$0.93{\pm}0.03^{a}$	3.22	$1.18{\pm}0.03^{ab}$	2.54

Note: Means± sample std. dev.; Means separation by HSD Tukey`s test at p≤ 0.05; CV%- Coefficient of variation; Means with the same superscript are not statistically significant. In the column: the small letters indicate the significance of the differences between the clones for the same variable and vintage; capital letters represent the significance of the differences between the vintages for the same clone.

Morphometric traits. The highest average length of the bunch was recorded in clone 15 ENTAV (162.28 mm) in 2023, the differences being significant compared to clones 338 ENTAV and clone R5. In 2022, all clones had clusters shorter in length compared to 2023, statistically significant differences between the two years being noted only in clones 15 ENTAV and ISV 117 (Table 3).

In terms of bunch width, no statistically significant differences were recorded between the five clones in any year. With the exception of clones ISV 105 and R 5, all clones have clusters uniform in length and width. In 2022, clone ISV 117 recorded the highest weight of a bunch, the differences being significant compared to clone 15 ENTAV, which recorded the lowest weight (106.82 g). In 2023, no statistically significant differences were recorded between clones regarding the clusters weight. Significantly positive differences in cluster weight were recorded in 2023 compared to 2022 only for clone 338 ENTAV (Table 3). The clones 338 ENTAV and ISV 117 also stand out in terms of clusters weight homogeneity (CV% < 10%).

Clusters and berries size is influenced by the genetic (Tello et al., 2015) and the metabolic particularities of grapevine cultivars (Pisciotta et al., 2018), but also can be influenced by others factors, such as thermal resources, water status and cultural practices (Costea et al., 2015; Holt et al., 2008; Stroe et al., 2020). Clone 15 ENTAV had the smallest berry length, however the differences are statistically significant only compared to clone R5 in year 2022 (Table 4). There were no significant differences between the two years for berry length in either clone. It can also be noted in all clones that both the length and the width of the berry are homogeneous in both years studied (CV% < 10%).

Table 3. Morphometric characteristics of bunch at full maturity

‘Cabernet Sauvignon’clones	Vintage	Bunch length (mm)	CV %	Bunch Width (mm)	CV %	Bunch Weight (g)	CV %
15 ENTAV	2022	112.10 ± 11.78^{aB}	10.5	69.83 ± 10.83^{aA}	15.50	106.82 ± 13.04^{bA}	12.20
	2023	162.28 ± 15.05^{aA}	9.27	81.20 ± 9.52^{aA}	11.72	118.09 ± 26.31^{aA}	22.27
338 ENTAV	2022	107.33 ± 9.33^{aA}	8.69	77.33 ± 10.44^{aA}	13.50	117.10 ± 5.37^{abB}	4.58
	2023	127.00 ± 19.87^{bA}	9.27	73.40 ± 9.42^{aA}	12.83	139.94 ± 6.15^{aA}	4.39
ISV 105	2022	115.00 ± 23.84^{aA}	20.73	84.17 ± 16.54^{aA}	19.65	121.43 ± 13.10^{abA}	10.79
	2023	139.20 ± 26.22^{abA}	18.83	78.20 ± 5.54^{aA}	7.08	124.34 ± 18.97^{aA}	15.26
ISV 117	2022	115.83 ± 9.47^{aB}	8.17	76.00 ± 10.13^{aA}	13.33	142.53 ± 5.25^{aA}	3.68
	2023	134.00 ± 4.16^{abA}	3.10	80.80 ± 8.52^{aA}	10.54	134.58 ± 9.76^{aA}	7.25
R5	2022	108.17 ± 16.25^{aA}	15.02	86.00 ± 19.27^{aA}	22.40	134.03 ± 14.19^{abA}	10.59
	2023	123.20 ± 21.80^{bA}	17.69	83.60 ± 9.96^{aA}	11.91	148.18 ± 14.96^{aA}	10.10

Note: Means± sample std. dev.; Means separation by HSD Tukey`s test at p≤ 0.05; CV%- Coefficient of variation; Means with the same superscript are not statistically significant. In the column: the small letters indicate the significance of the differences between the clones for the same variable and vintage; capital letters represent the significance of the differences between the vintages for the same clone.

The berry width ranged from 10.47 mm (15 ENTAV, 2023 vintage) to 11.77 mm (338 ENTAV, 2023 vintage).

The berry length/width ratio represents a useful indicator in appreciating the shape of the berry. It shows values between 0.99 (338 ENTAV) and 1.05 (clones 15 ENTAV and ISV 105). Significant differences between clones, regarding the berry length/width ratio, were recorded in 2023 alone (Table 4), respectively clone 338 ENTAV compared to ISV 105 ($p < 0.05$) and ISV 117 ($p < 0.05$). Based on the length/width berry ratio it can be appreciated, however, that all clones have spherical berries in shape.

The berry weight was between 0.90 g (clone 15 ENTAV, 2023 vintage) and 1.19 g (R5 clone, 2022 vintage). Significantly negative differences regarding the berry weight were highlighted in clone 15 ENTAV in vintage 2023 (compared to the other clones, $p < 0.01$). Except for clone ISV 105, there were no significant differences in average berry weight between the two study years (Table 4).

Gil et al. (2015) reported that berry size influences the color of the Cabernet Sauvignon wines obtained from grapes from the same vineyard. Specifically, the smaller the berry size, the more intense the colour and the higher the concentration of anthocyanins and proanthocyanidins. Selecting grapes by size could be an interesting tool for the wine industry, especially to improve wine colour (Gil et al., 2015).

The number of seeds per berry was between 1.57 in clone 338 ENTAV (2023 vintage) and 2.43 in clone R5 (2023 vintage). Between the two study years no significant differences were noted for any of the clones regarding the number of seeds in the berry (Table 4). The highest number of seeds in the berry was recorded in clones R5 and ISV 117, the differences being significant compared to clone ISV 105 in 2022 vintage and to clone 338 ENTAV in 2023. If in clones 15 ENTAV, 338 ENTAV and ISV 105 the frequency of berries with 1-2 seeds is over 80%, in clones ISV 117 and R5 over 90% of berries had 2-3 seeds (Figure 1).

Seed number can influence berry size and calcium, potassium and magnesium contents, with effects on mineral must and wine composition (Boselli et al., 1995).

ISV clones 105 and R5 had the longest seeds, the differences being significant compared to ENTAV clones 15 and 338.

Clones 15 ENTAV and R5 have the lowest 100-seed weight, but relative to berry weight, seed weight expressed as a percentage is significantly higher in clones ISV 105, R5 and ISV 117 (Table 5).

Quantitative and qualitative characteristics. Of the two years, the year 2023 stands out for the highest productions (kg/vine) in all clones, the differences being significant compared to the year 2022.

Table 4. Morphometric characteristics of berries at full maturity

'Cabernet Sauvignon' clones	Vintage	Berry length (mm)	CV %	Berry Width (mm)	CV %	Lenght/ Widht berry	CV %	Number of seeds/berry	CV %	Berry Weight (g)	CV %
15 ENTAV	2022	10.87 ± 0.63^{bA}	5.80	10.82 ± 0.22^{aA}	2.03	1.05 ± 0.02^{aB}	1.90	1.88 ± 0.19^{abA}	10.11	0.99 ± 0.06^{aA}	6.06
	2023	10.77 ± 0.53^{aA}	4.92	10.47 ± 0.45^{bcA}	4.30	1.01 ± 0.02^{abA}	1.98	1.97 ± 0.25^{abA}	12.69	0.90 ± 0.20^{bA}	22.22
338 ENTAV	2022	11.25 ± 0.46^{abA}	4.09	11.12 ± 0.62^{aA}	5.58	1.00 ± 0.06^{aA}	6.00	1.85 ± 0.14^{abA}	7.56	1.16 ± 0.15^{aA}	12.93
	2023	11.87 ± 0.35^{aA}	2.95	11.77 ± 0.32^{aA}	2.72	0.99 ± 0.02^{bA}	2.02	1.57 ± 0.15^{bA}	9.55	1.06 ± 0.21^{aA}	19.81
ISV 105	2022	11.32 ± 0.40^{abA}	3.53	10.54 ± 0.08^{aB}	0.76	1.05 ± 0.01^{aA}	0.95	1.77 ± 0.15^{bA}	8.47	0.96 ± 0.02^{aB}	2.08
	2023	11.66 ± 0.57^{aA}	4.89	11.29 ± 0.29^{aA}	2.57	1.04 ± 0.02^{aA}	1.92	2.07 ± 0.32^{abA}	15.46	1.09 ± 0.18^{aA}	16.51
ISV 117	2022	11.60 ± 0.13^{abA}	1.12	11.19 ± 0.11^{aA}	0.98	1.03 ± 0.01^{aA}	0.97	2.30 ± 0.26^{aA}	11.30	1.12 ± 0.12^{aA}	10.71
	2023	11.08 ± 0.51^{aA}	4.60	10.94 ± 0.27^{bA}	2.47	1.03 ± 0.03^{aA}	2.91	2.33 ± 0.29^{aA}	12.44	1.14 ± 0.16^{aA}	14.03
R5	2022	11.75 ± 0.54^{aA}	4.60	11.13 ± 0.34^{aA}	3.05	1.03 ± 0.03^{aA}	2.91	2.30 ± 0.20^{aA}	8.69	1.19 ± 0.09^{aA}	7.56
	2023	11.62 ± 0.32^{aA}	2.75	11.75 ± 0.49^{aA}	4.17	1.01 ± 0.01^{abB}	0.99	2.43 ± 0.32^{aA}	13.17	1.12 ± 0.23^{aA}	20.54

Note: Means ± sample std. dev.; Means separation by HSD Tukey`s test at $p \leq 0.05$; CV % - Coefficient of variation; Means with the same superscript are not statistically significant. In the column: the small letters indicate the significance of the differences between the clones for the same variable and vintage; capital letters represent the significance of the differences between the vintages for the same clone.

Figure 1. Frequency of the number of seeds per berry in ‘Cabernet Sauvignon’ clones

Table 5. Morphometric characteristics of seeds at harvest (2023 vintage)

‘Cabernet Sauvignon’ clones	Seed Length (mm)	CV %	100 seeds Weight (g)	CV %	% Seeds in berry	CV %
15 ENTAV	5.68 ± 0.05^{b}	0.88	2.38 ± 0.22^{c}	9.24	4.78 ± 0.54^{ab}	11.30
338 ENTAV	5.67 ± 0.01^{bc}	0.18	2.42 ± 0.26^{b}	10.74	3.50 ± 0.49^{b}	14.00
ISV 105	5.97 ± 0.08^{a}	1.34	2.68 ± 0.22^{a}	8.21	5.52 ± 1.09^{a}	19.74
ISV 117	5.84 ± 0.03^{ab}	0.51	2.62 ± 0.21^{a}	8.02	5.28 ± 0.44^{a}	8.33
R5	5.93 ± 0.11^{a}	1.85	2.33 ± 0.22^{c}	9.44	5.34 ± 0.59^{a}	11.05

Note: Means± sample std. dev.; Means separation by HSD Tukey`s test at p≤ 0.05; CV% - Coefficient of variation;
In the column: means with the same superscript are not statistically significant.

The highest production (4.26 kg/vine) was recorded in the R5 clone. The lower productions in 2022 can also be explained by the effect of the prolonged drought in the growing season 2021 and late harvest (end of October 2021).

In 2022, among the Italian clones, the clone R5 stands out with the highest sugar accumulation potential (24.3°Brix), while the ISV 117 clone had the lowest sugar content among all clones studied (21.5°Brix). Under the conditions of the 2023 vintage, the Italian clone ISV 105 stood out with the highest accumulation potential in sugars (25.8°Brix), the differences being significant compared to the other clones. Significant differences between clones in 2023 are also noted in relation to the content of total acidity in the must, the French clone 15 ENTAV had the highest content in total acidity (5.94 g/L H_2SO_4), the differences being significant compared to the Italian clones ISV 105 and ISV 117 (Table 6).

The climatic conditions specific to the ripening period of the two viticultural years significantly influenced the content of sugars in must only in clones 15 ENTAV and ISV 105 (Table 6).

Table 6. Main quantitative and qualitative characteristics of Cabernet Sauvignon clones at harvest

'Cabernet Sauvignon' clones	Vintage	Yield (kg/vine)	CV %	Sugar content (° Brix)	CV %	Potential alcohol (% vol.)	CV %	Total acidity of must (g/L H_2SO_4)	CV %
15 ENTAV	2022	1.89 ± 0.27^{abB}	14.28	23.73 ± 0.80^{abA}	3.37	14.04 ± 0.28^{abA}	1.99	5.57 ± 0.26^{aA}	4.67
	2023	3.18 ± 0.25^{aA}	7.86	21.63 ± 0.59^{bB}	2.73	12.77 ± 0.40^{bB}	3.13	5.94 ± 0.11^{aA}	1.85
338 ENTAV	2022	1.57 ± 0.19^{bB}	12.10	23.50 ± 0.80^{abA}	3.40	13.87 ± 0.46^{abA}	3.32	5.42 ± 0.28^{aA}	5.17
	2023	4.07 ± 0.52^{aA}	12.77	23.27 ± 0.50^{bA}	2.15	13.77 ± 0.38^{abA}	2.76	5.38 ± 0.35^{abA}	6.5
ISV 105	2022	1.75 ± 0.09^{abB}	5.14	24.09 ± 0.28^{aB}	1.16	14.26 ± 0.26^{aB}	1.82	5.21 ± 0.25^{aA}	4.80
	2023	3.52 ± 0.62^{aA}	17.61	25.80 ± 0.69^{aA}	2.67	15.33 ± 0.46^{aA}	3.00	5.07 ± 0.18^{bA}	3.55
ISV 117	2022	2.44 ± 0.32^{aB}	13.11	21.50 ± 0.31^{cA}	1.44	12.53 ± 0.45^{cA}	3.59	4.68 ± 0.44^{aA}	9.4
	2023	3.51 ± 0.14^{aA}	3.98	21.87 ± 0.76^{bA}	3.47	13.37 ± 1.03^{bA}	7.70	4.37 ± 0.31^{cA}	7.09
R5	2022	2.05 ± 0.18^{abB}	8.78	24.30 ± 0.23^{aA}	0.95	14.24 ± 0.24^{aA}	1.68	4.81 ± 0.52^{aA}	10.81
	2023	4.26 ± 0.59^{aA}	13.85	23.40 ± 1.22^{bA}	5.21	13.87 ± 0.75^{abA}	5.41	5.48 ± 0.07^{abA}	12.77

Note: Means± sample std. dev.; Means separation by HSD Tukey`s test at $p \leq 0.05$; CV% - Coefficient of variation; Means with the same superscript are not statistically significant. In the column: the small letters indicate the significance of the differences between the clones for the same variable and vintage; capital letters represent the significance of the differences between the vintages for the same clone.

Although there are differences from one year to another in terms of the must acidity content of the five clones, they are not statistically significant.

CONCLUSIONS

Results showed that the clone had a significant influence on most of the analyzed ampelographic traits. The year significantly influenced bunch weight (in 338 ENTAV clone), bunch length (in clones 15 ENTAV and ISV 117), berry weight and width (in ISV 105 clone), yield (in all clones), sugar content and potential alcohol (in clones 15 ENTAV and ISV 105).

Good fertility results are shown by clones R5 and 15 ENTAV, with ISV 117 and R5 clones being the most productive.

Considering the compositional atributes of the grapes for each clone, it emerged that all clones are suitable for obtaining DOC/PDO top quality red and rose wines. Although the data presented by us are in the context of a young vineyard, the clones are of interest from the perspective of their productive and qualitative potential and must be further explored from the perspective of establishing an optimal viticultural technique for a quantity-quality balance, of their potential phenolic in climatic contexts specific and their ability to produce diverse wines types.

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ASSESSMENT OF SOME HYBRID ELITES OF TABLE GRAPES OBTAINED AT INCDBH STEFANESTI-ARGES WITHIN THE ROMANIAN BREEDING PROGRAM

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Abstract

This paper presents two new genotypes obtained at INCDBH Ştefăneşti-Argeş through controlled hybridization of Muscat de Pölöskei x Victoria (coded RVC 24) and Coarnă neagră x Victoria (VMD 24) varieties. Both the hybrid elites studied and the control Victoria were characterized and evaluated according to standardized international methodology. Phenotypic, agro-biological, and technological determinations were carried out throughout the years 2020-2023, following the 'OIV Descriptor list for grapevine varieties and Vitis species'. The RVC 24 genotype stood out for its superior agro-biological and technological potential compared to the control: high fertility at 67%, absolute and relative fertility coefficients (1.2 and 0.76, respectively), average grape weight of 476.24 g, absolute and relative productivity indices: 555 and 364 respectively, sugar content of 157 g/l. The VMD 24 genotype was notable for its high to very high fertility at 78%, average grape weight of 443.30 g, absolute and relative productivity indices: of 474 and 373 respectively, and sugar content of 155 g/l. The results obtained were correlated with the climatic data recorded during the study period, which showed significant fluctuations from one year to another.

***Key words**: genotypes, genetic resources, vine, ampelographic descriptors.*

INTRODUCTION

The objectives of the national program for improving the assortment of table grape varieties have been aimed at creating new genotypes with superior agro-biological and technological potential: different ripening epochs to ensure consumption in fresh condition over a longer period of time, stenospermocarpy and parthenocarpy, increased ecological plasticity, high adaptability to different biotope conditions, resistance to major diseases, pests, or critical climatic conditions.

It is appreciated that the success of any breeding program is largely determined by the genetic variability sources available to the breeder (Savatti et al., 2004).

Understanding the ontogenetic and phylogenetic aspects of the material used, as well as a profound theoretical basis, represents the basic premises for breeders to efficiently carry out practical work aimed at obtaining new competitive varieties.

The criteria for choosing suitable parents are, consequently, diverse, considering phenotypic, biochemical, physiological, ecological, or genetic aspects. Without diminishing the importance of other aspects, genetic factors are fundamental in assessing the value of initial breeding material as accurately as possible.

The more abundant and diversified the germplasm sources are, the greater the possibility that, following thorough studies, the forms containing the most valuable genes can be retained, whose accumulation is targeted in the new organisms created (Leonte C., 2011).

In obtaining valuable hybrid descendants, from which elite plants have been selected, an important role has been attributed to the choice of parents. In plant breeding, it is a requirement for the initial material to exhibit a sufficiently large variability to identify and multiply those genotypes with new characteristics or traits, which truly allow the creation of new varieties. Variability is defined by the total differences between the characters and traits of individuals within a specific population. Characters encompass all morphological, physiological, and biochemical characteristics and can be grouped according to the parameters used for their expression into qualitative and quantitative traits (Denisa Mihaela Coman, 2010).

MATERIALS AND METHODS

The initial material used for the hybridization work consisted of the following grape varieties: *Muscat de Pölöskei* (R 10), *Victoria*, and *Coarnă neagră*, all part of the Grape Germplasm Collection at the National Research and Development Institute for Biotechnologies in Horticulture Ştefăneşti-Argeş.
The intraspecific hybridizations carried out at the National Research and Development Institute for Biotechnologies in Horticulture Ştefăneşti-Argeş, within the *Vitis vinifera* species, aimed at transmitting and fixing in offspring intermediate characters between parents (quantity - quality), the type of blended inheritance (grape color), and the sex conversion with a pronounced heterozygous characteristic. The following scheme was applied: **E1 x E2 → F1 (E1 E2) - selection of hybrids in F1.**
Using *Muscat de Pölöskei* (R 10) x *Victoria* as the parental forms, the aim was to obtain genotypes with large, crunchy berries and increased tolerance to biotic and abiotic factors. Muscat de Pölöskei is a variety recognized as belonging to the group of resistant varieties. Its main qualities include highly fertile pollen, a favorable ratio between sugar content and acidity, pleasant grape flavor, crunchy berries, and suitability for winter storage. It is resistant to powdery mildew and gray mold, moderately resistant to downy mildew. The Victoria variety was chosen as the parent in this combination due to its outstanding qualitative characterristics: large grapes, large berries, pleasing commercial appearance, and medium ripening time. The hybrid *RVC 24* was obtained as a result of these hybrid combinations (Figure 1). Using the black *Coarnă neagră* variety as the maternal donor, it was aimed at its very good resistance to drought and frost (-20... -22°C), to gray mold of grapes, grape moths, as well as good tolerance to downy mildew, powdery mildew, and phylloxera. As a result of hybridizing the *Coarnă neagră* x *Victoria* varieties, the hybrid *VMD 24* was obtained (Figure 2). According to the 'OIV Descriptor list for grapevine varieties and *Vitis* species', phenotypic, agro-biological, and technological determinations were carried out throughout the years 2020-2023. ***The main phenological descriptors used in*** describing the genus *Vitis* are budburst, flowering, veraison, and full grape ripening. ***The main agro-biological descriptors*** evaluated were: shoot growth vigor, percentage of fertile shoots, fertility coefficients: absolute and relative, and productivity index: absolute and relative. ***The main technological descriptors*** are: cluster weight, berry weight, weight of 100 berries, grape production per hectare, yield, must sugar content and acidity.
The results obtained from the analyses and determinations were correlated with the climatological data recorded by the institute's own iMetos 3.3 station, within the institute - the Fitotron Complex.

Figure 1 - RVC 24 hybrid elite

Figure 2 - VMD 24 hybrid elite

RESULTS AND DISCUSSIONS

Under the climatic aspect, the Vine growing Center Ştefăneşti-Argeş belongs to the moderately warm-semi-humid Zone II. Within this zone, the territory occupies the IV th subzone and the class of weakly excess hydroclimatic balance. The pedoclimatic characteristics of the Vine growing Center Ştefăneşti are specific to soils with umbrisol, with loamy-sandy and loamy-clayey textures, without gravel. In the climatic conditions of this area, the average annual temperature ranges between 9.8°C and 10.1°C. The average temperature per season is 22.2°C in summer, 11.4°C in autumn, 9.5°C in spring, and -0.9°C in winter. The absolute maximum temperature is 39.2°C in July, and the absolute minimum is -27.6°C in January. The duration of the interval with temperatures above 10°C is 186-192 days, and the sum of active daily average temperatures is 3164°C in the western zone and 3364°C in the eastern zone. The average sunshine duration sums up to approximately 2177 hours/year. The length of the vegetation period is approximately 183 days. Analyzing the length of the vegetation period over a long period, years (1960-1990), we observe a very large variation, ranging from 162 days to 215 days in 1989. Regarding the water regime, the area is characterized by average annual precipitation between 450-700 mm. As can be seen from Table 1, in 2020, high temperature values were recorded compared to 2021, 2022, and the multi-year average, both in terms of the overall thermal balance, active and useful, as well as the average minimum temperature with a value of 7.7. The vegetation period lasted 193 days compared to 2021 (167 days) and 2022 (186 days) (Table 1).

Table 1. Synthesis of the main climatic elements in the Vine growing Ştefăneşti Center

Climatic elements analyzed	Multi-year average**	2020	2021	2022
Global thermal balance, ∑t°g, °C*	**3285.6**	3516.6	3347.6	3495.1
Active thermal balance, ∑t°a, °C*	**3198.3**	3775.2	3225.4	3393.7
Useful thermal balance, ∑t°u, °C*	**1454.0**	1754.1	1633.4	1690.3
Average monthly temperature, °C	**10.6**	14.6	13.3	13.9
Average temperature in vegetation period, °C	**17.6**	19.5	18.6	19.4
Minimum temperature, °C	**6.2**	7.7	0.3	7.1
Maximum temperature, °C	**16.3**	23.7	30.2	22.1
∑ annual rainfall (mm)	**689.6**	415.2	544.4	559.9
∑ rainfall in active period (mm)	**416.9**	122.4	356.8	508.7
Active period (no. days)	-	193	167	186
Hydrothermic coefficient, CH	**3.0**	3.2	5.1	3.3
Real heliothermic index, IHr	**2.32**	2.51	2.40	2.66
Viticultural bioclimatic index, Ibcv	**8.0**	8.6	7.7	8.2
Oenoclimatic aptitude index, IAOe	**4655.2**	4780.1	4573.2	4736.1

As observed in Table 2, the number of days with temperatures exceeding 32°C is significantly increasing from year to year, particularly during the summer months. Much higher temperatures than the normal and multi-year average are also recorded in the months of September-October, characterized by a dry climate. Additionally, the hydrological regime recorded during the winter is deficient, with precipitation in recent years occurring in the form of rain and sleet but in much lower quantities than the multi-year averages.

Table 2. Frequency occurrence of abiotic stress factors in the period 2020-2022

Stress factor	2020			2021			2022		
	Month	**No. days**	**F %**	**Month**	**No. days**	**F %**	**Month**	**No. days**	**F %**
Winter frost average min. T < -15°C	-	-	-	-	-	-	-	-	-
Spring frost average min. T< -2°C	-	-	-	III	5	16	III	4	13
	-	-	-	IV	3	10	-	-	-
Drought Abs. max. T .> 30°C	V	7	23	-	-	-	V	8	26
	VI	18	60	VI	10	33	VI	23	77
	VII	27	87	VII	31	100	VII	18	58
	VIII	29	94	VIII	27	87	VIII	18	58

***RVC 24* Hybrid Elite**

Morphological characterization

The **rosette** is hairy and scaly, with a greenish-white color (Figures 3, 4). **The tip of the shoot** is completely open, without anthocyanin pigmentation, and the density of fine hairs is moderate (Figure 5). **The flower** is a normal hermaphrodite, type 5, with fully developed stamens and pistils (Figure 6). **Young leaves** are green on the upper side, and the density of long hairs on the lower side, between veins, is moderate (Figure 7). The shoot is smooth, and green with red streaks on the sunny side. The nodes are long, bifurcated, yellowish-green in color, and their distribution is discontinuous. **The mature leaf** is large, wedge-shaped, and pentagonal. **The teeth** are long with straight edges. The color of the blade is dark green, moderately embossed, with a straight profile in cross-section. The upper lateral sinuses are open, with highly overlapping lobes, U-shaped, and the lower sinuses are deep. The petiolar sinus is open in a U shape (Figure 8). **The bunches** are large, averaging 192 mm in length, with variations ranging between 179 and 201 mm. In terms of compactness, it is lax or very lax (degree of expression 1-3), with very visible pedicels. **The shape** of the grape is cylindrical-conical, uniaxial, with an average of 3-4 wings (degree of expression 3). **The grape** has an average weight of 434.49 g. **The peduncle** is long, averaging 86 mm.

The berry is large, averaging 22 mm, with variations ranging between 21 and 24 mm. The weight of a berry is 4.8-5.4 g. The shape is elongated elliptical (degree of expression 4), with a greenish-yellow color, and the skin is lightly pruinose. The pulp does not exhibit anthocyanin coloration, being very firm. The berries are difficult to separate from the pedicel. On average, they contain 3 - 4 seeds. **The weight of the grape**: 457.80-483.70 g, 100 berries: 487-546 g, must concentration in sugars: 155-160 g/l, acidity 3.4-3.9 g/l H_2SO_4 (Figure 9).

Figure 3 - Bud BBCH 00

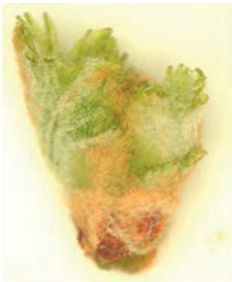
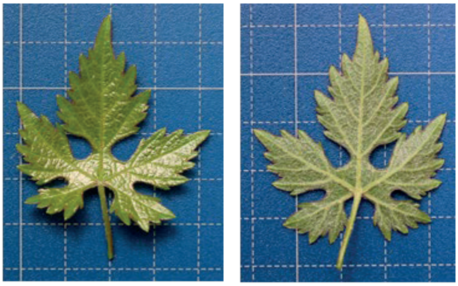


Figure 4 - Rosette

Figure 5 - Young shoot

Figure 6 – Inflorescence



a. b.

Figure 7 - Young leaf (a. upper side; b. lower side)

Figure 8 - Mature leaf

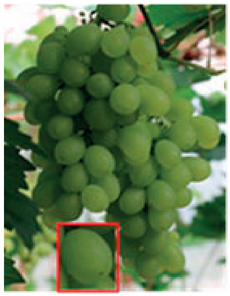


Figure 9 - Bunch

***VMD 24* Hybrid elite**

Morphological characterization

It buds through a green **rosette** with very sparse hairs (Figures 10, 11). **The tip of the shoot** is semi-open, with very sparse long hairs (Figure 12). **The shoot** is green with red stripes, with meristems covered with sparse fine hairs. **The inflorescence** is cylindro-conical, medium-sized (Figure 13). The **young leaves** are green, glabrous on both the upper and lower surfaces, without anthocyanin pigmentation (Figure 14).

The mature leaf is medium-sized, wedge-truncated, pentalobed, green, and glabrous. **The petiolar sinus** is open in the shape of a U or lyre, and the **upper lateral sinuses** are slightly overlapping with the base in a V shape. **The teeth** are short and have one concave and one convex edge. **The leaf blade** has medium undulation, with generalized waviness, and the cross-section profile is revolute.

The petiole is shorter than the length of the main vein (figure 15).

The grapes are large, with an average length of 202 mm, ranging between 193 and 212 mm. In terms of compactness, they are loose to very loose (expression grade 3-5), with very visible pedicels. **The shape** of the bunch is cylindrical-conical, uniaxial, with an average of 3-4 wings (expression grade 3). **The bunch** has an average weight of 443.30 g. **The peduncle** is long, with an average length of 99 mm (Figure 16).

The berry is medium to large-sized, with an average diameter of 21 mm, ranging between 18 and 23 mm, unevenly. The weight of a berry is 4.2-4.5 g. **The weight of the bunch** ranges from 411.10 to 487.22 g, 100 berries weigh between 405.30 and 427.81 g, the must concentration in sugars ranges from 153 to 157 g/l, acidity is between 3.4 and 3.9 g/l H_2SO_4.

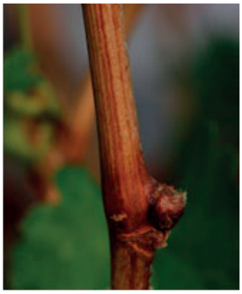


Figure 10 - Bud BBCH 00


Figure 11 - Rosette

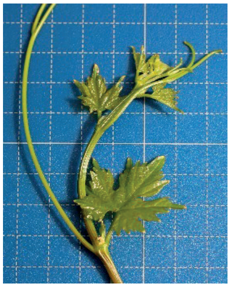


Figure 12 - Young shoot


Figure 13 - Inflorescence

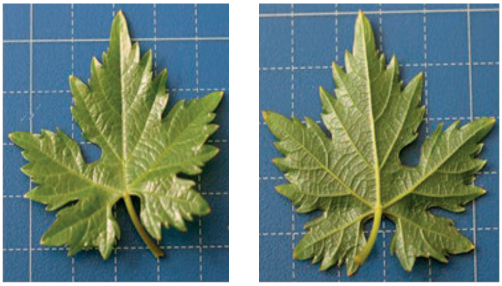

a. b.
Figure 14 - Young leaf (a. upper side; b. lower side)


Figure 15 - Mature leaf


Figure 16 - Bunch

As indicated in Table 3, in the year 2020, due to the high temperatures in the months of February, March, and April, the four studied phenological stages occurred earlier compared to the year 2022, with significant deviations from the year 2022. In the year 2021, both bud burst, veraison, and grape ripening were much delayed compared to the year 2020, with a significant deviation of 46 days (veraison) and 26 days (full ripening).

Evaluation of agro-biological descriptors during the period 2020-2022

Based on the obtained results, it was observed that both the fertility of the studied genotype, which recorded an average value of 67% (medium to high), and the values of AFA and RFA (Table 4) were above unity with an average of 1.2 and 0.76, respectively compared to the control variety which recorded lower values (Figure 17).

Table 3. Monitorization of the phenological spectrum of the RVC 24 hybrid elite during 2020-2022

	Year	Budburst OIV 301		Full bloom OIV 302		Veraison OIV 303		Full maturity 304	
		Date	UTB (ºC)	Date	UTB (ºC)	Date	UTB (ºC)	Date	UTB (ºC)
***RVC 24* hybrid elite**	2020	08 - 11.04	78.0	09 - 13.06	396,7	17 - 20 .07	431.4	01 - 05.09	220.3
	2021	26 - 29.04	25.0	10 - 14.06	303.1	31.08 - 03.09	429.6	05 - 08.10	212.2
	2022	15 - 18.04	61.2	15 - 18.06	374.6	21 - 25.07	401.8	09 - 12.09	191.8
***Victoria* control**		19 - 21.04	61.2	13 - 18.06	374.6	27 - 31.08	401.8	14 - 18.08	191.8

Table 4. Evaluation of the fertility characteristics of the RVC 24 hybrid elite in the period 2020-2022

***RVC 24* hybrid elite**	**Year**	**Total no. of shoots**	**No fertile shoots**	**Coefficient of fertile shoots (LF) %**	**No. inflorescence s**	**Fertility coefficients**	
						AFC	**RFC**
	2020	24	13	54	16	1.2	0.66
	2021	28	20	71	23	1.2	0.82
	2022	27	20	74	22	1.1	0.81
Average		**26**	**18**	**67**	**20**	**1.2**	**0.76**
***Victoria* control**		**23**	**14**	**60**	**16**	**1.1**	**0.69**

Table 5. Evaluation of the productivity characteristics of the studied hybrid elite

***RVC 24* hybrid elite**	**Year**	**Fertility coefficients**		**Average weight of bunch at full maturity (g)**	**Productivity index**	
		AFC	**RFC**		**API**	**RPI**
	2020	1.2	0.66	457.80	549	302
	2021	1.2	0.82	483.70	580	396
	2021	1.1	0.81	487.22	536	394
Average		**1.2**	**0.76**	**476.24**	**555**	**364**
***Victoria* control**		**1.1**	**0.69**	**420.33**	**462**	**290**

The results included in Table 5 reveal the high to very high productivity potential of the *RVC 24* hybrid, with absolute productivity index values ranging between 536 and 580, and 302-394 for the relative productivity index.

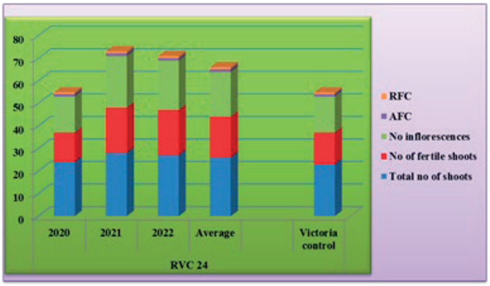


Figure 17 - Fertility descriptors of the new genotype and the control variety

The values recorded by the control variety are lower compared to those obtained by the studied genotype (Figure 17).

In the eco-pedoclimatic conditions of the years 2020-2022, it was observed that at the time of measurements (during flowering), the vegetative growth based on the number of buds sprouted showed higher values in 2020, with an average value of 21.8 cm compared to 2022 when the average value of shoot growth was 14.5 cm. According to descriptor OIV 351, the average value was 17.9 cm, receiving a score of 7, indicating high vigor.

The *Victoria* variety also exhibited high growth vigor with an average of 15.5 cm, also scored 7 (Table 6). The vigor of the varieties is expressed by the amount of wood removed during pruning and the length of the vegetative growth per vine (OIV 351).

In the climatic conditions of the years 2020-2022, the assessment of the biological potential of the analyzed genotype as well as the control variety was carried out according to the OIV Descriptor List for Grapes, 2009 edition. Regarding the studied period, the winters were mild with minimum temperatures above normal averages. As a result, the buds were not affected, and their viability was 100%, representing a very high resistance level for both the hybrid and the control variety (Table 7).

Relative to the climatic conditions of the years 2020-2022, a period characterized by dry summers, the hybrid elite *RVC 24* exhibited high and very high tolerance (rating 7 and 9, respectively). The control variety also showed a high level of tolerance. Even though the precipitation during the growing season was deficient and unevenly distributed, the studied hybrid did not exhibit specific symptoms of water stress (Table 8).

Table 6. Biometric determinations of the studied genotype (length of shoot growth on the trunk)

	Year	No shoot/trunk	Length of shoot (cm)			Vigor of shoot growth (OIV 351)
			maximum	**minimum**	**average**	
***RVC 24* hybrid elite**	2020	24	29.5	14.2	21.8	9
	2021	28	22.8	11.9	17.4	7
	2022	27	17.6	11.5	14.5	7
Average		**26**	**23.3**	**12.6**	**17.9**	**7**
***Victoria* control**		**21**	**22.7**	**12.8**	**15.5**	**7**

Table 7. Evaluation of the biological potential of the hybrid elite regarding frost

	Year	Viable buds %	Degree of resistance
***RVC 24* hybrid elite**	2020	100	9
	2021	100	9
	2022	100	9
Average		**100**	**9**
***Victoria* control**		**100**	**9**

Table 8. Evaluation of the biological potential of the hybrid elite regarding drought tolerance (OIV 403)

	Year	Codex OIV 403	Expression level
***RVC 24* hybrid elite**	**2020**	7	High
	2021	9	Very high
	2022	9	Very high
Average		**7-9**	**High - Very high**
***Victoria* control**		**7**	**High**

Evaluation of technological descriptors during the period 2020-2022

The values of the analyses and determinations are presented in the table below (Table 9). As can be seen, the new genotype stood out compared to the control variety through the descriptors of the average weight of a bunch, with values that varied between 457.80 and 487.22 g and the weight of 100 grains where *RVC 24* recorded values between 513.12 and 546.01 g. Figure 18 reveals the fact that in terms of the sugar content of the must, the hybrid was superior to the control with an average value of 157 g/l.

Regarding yield evaluation, according to the data in Table 10, the *RVC 24* genotype recorded higher values compared to the control for grape quantity per vine expressed in kg/trunk as well as for grape yield expressed in t/ha. The average yield was 5.3 kg/trunk and 23 t/ha, respectively. Marketable yield ranged between 82 and 87%.

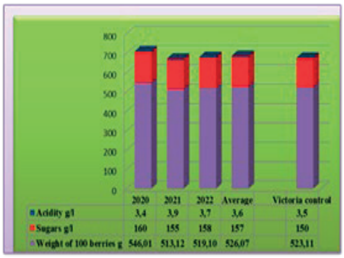


Figure 18 - Quality descriptors of *RVC 24* compared to the Victoria control

Table 9. Evaluation of the quality of the harvest for the period 2020-2022

	Year	Average weight of the bunch (g)	Weight of 100 berries (g)	Sugars g/l	Acidity g/l	*Ripening index
***RVC 24* hybrid elite**	2020	457.80	546.01	160	3.4	4
	2021	483.70	513.12	155	3.9	3
	2022	487.22	519.10	158	3.7	4
Average		**476.24**	**526.07**	**157**	**3.6**	**4**
***Victoria* control**		**420.33**	**523.11**	**150**	**3.5**	**4**

*De Cillis ripening index. Its value ranges from 1 to 1.5 at veraison and from 3 to 6 at full grape ripening.

Table 10. Evaluation of the harvest during 2020-2022

RVC 24 hybrid elite	Year	Harvest		Productivity index		
		Kg/trunk	t/ha	API	RPI	Yield Goods %
	2020	5,5	24	549	302	87
	2021	5,0	22	580	396	82
	2022	5,1	23	536	394	86
Average		**5,3**	**23**	**555**	**364**	**85**
***Victoria* control**		**4,6**	**21**	**462**	**290**	**87**

VMD 24 Hybride elite

Evaluation of elite phenological descriptors during the period 2020-2022

According to the data from Table 11, the year 2020 recorded values above normal averages in the months of February, March, and April. The budburst phase occurred earlier compared to the years 2021 and 2022, with a deviation of 20 days. Also, regarding flowering, it started 15 days earlier compared to the other years. The ripening of the grapes was much delayed in 2021 and 2022 compared to the year 2020, with a significant deviation of 27 days.

Evaluation of agrobiological descriptors during the period 2020-2022

Following the obtained results, it was found that both the fertility of the studied *VMD 24 hybrid*, which recorded an average value of 78% (high), and the CFR value (with an average of 0.84), were above standard compared to the control variety, which recorded lower values (Table 12 and Figure 19).

The results included in the table below reveal the high productive potential of the *VMD 24* hybrid, with absolute productivity index values ranging between 411 and 536, and relative productivity index values between 362 and 395 (Figure 20). The values recorded for the control variety are lower compared to those obtained by the studied genotype (Table 13).

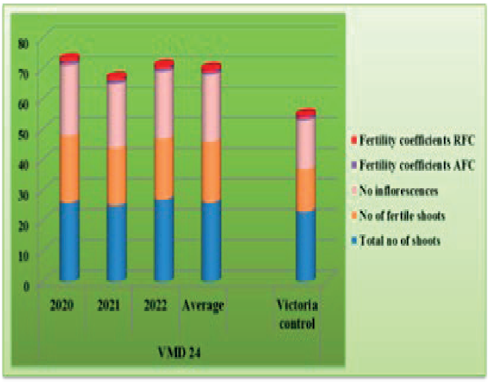


Figure 19 - Fertility coefficients of *VMD 24* compared to the control variety

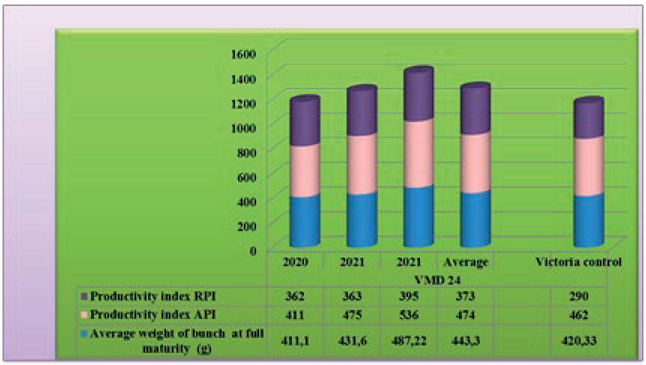


Figure 20 - Productivity descriptors of the new genotype and the control variety

Table 11. Monitorization of the phenological spectrum of the *VMD 24* hybrid elite during 2020-2022

VMD 24 hybrid elite	Year	Budburst		Flowering		Veraison		Full maturity	
		Date	UTB (°C)	Date	UTB (°C)	Date	UTB (°C)	Date	UTB (°C)
	2020	03 - 06.04	78.0	30.05 - 03.06	396.7	21 - 26.07	431.4	28.08 - 05.09	220.3
	2021	20 – 24.04	25.0	11 - 14.06	303.1	23 - 26.07	429.6	23 - 26.09	212.2
	2022	23 - 26. 04	61.2	14 - 17.06	374.6	26 - 29.07	401.8	22 - 27.09	191.8
Victoria control									

Table 12. Evaluation of the fertility characteristics of the *VMD 24* hybrid elite in the period 2020-2022

VMD 24 hybrid elite	Year	Total no of shoots	No. fertile shoots	Coefficient of fertile shoots (LF) %	No. inflorescence	Fertility coefficients	
						AFC	RFC
	2020	26	22	84	23	1.0	0.88
	2021	25	19	76	21	1.1	0.84
	2022	27	20	74	22	1.1	0.81
Average		**26**	**20**	**78**	**22**	**1.1**	**0.84**
Victoria control		**23**	**14**	**60**	**16**	**1.1**	**0.69**

Table 13. Evaluation of the productivity characteristics of the studied hybrid elite

VMD 24 hybrid Elite	Year	Fertility coefficients		Average weight of bunch at full maturity (g)	Productivity index	
		AFC	RFC		API	RPI
	2020	1.0	0.88	411.10	411	362
	2021	1.1	0.84	431.60	475	363
	2022	1.1	0.81	487.22	536	395
Average		**1.1**	**0.84**	**443.30**	**474**	**373**
***Victoria* control**		**1.1**	**0.69**	**420.33**	**462**	**290**

In the eco-pedoclimatic conditions of the years 2020-2022, it was observed that at the time of measurements (during flowering), the vegetative growth was higher in 2020 with an average value of 23.0 cm compared to the year 2022 when the average value of shoot growth was 14.1 cm. According to descriptor OIV 351, the average value was 17.7 cm, receiving a score of 7, which represents high vigor. The Victoria variety also exhibited high growth vigor with an average of 15.5 cm, receiving a score of 7 (Table 14). In the climatic conditions of the years 2020-2022, the assessment of the biological potential of the analysed hybrid elite was conducted according to the Descriptor List for Grapes by OIV, 2009 edition.

Regarding the studied period, winters were mild, with the minimum temperatures above normal averages. As a result, the buds were not affected, and their viability was 100%, representing a very high level of resistance both in the studied genotype and in the control variety (Table 15). In the vegetation period, the studied hybrid elite showed fluctuating drought tolerance, exhibiting specific manifestations of water stress (Table 16) during the grapevine growth period and berry dropping during the veraison - maturation period. The negative effects related to soil moisture are caused by both water deficit and excess moisture.

Table 14. Biometric determinations of the studied genotype (length of shoot growth on the trunk)

VMD 24 hybrid elite	Year	No shoot/trunk	Length of shoot (cm)			Vigor of shoot growth (OIV 351)
			maximum	minimum	average	
	2020	26	32.0	14.1	23.0	9
	2021	25	21.9	11.5	16.0	7
	2022	27	17.1	11.1	14.1	7
Average		**26**	**23.6**	**12.2**	**17.7**	**7**
***Victoria* control**		**21**	**22.7**	**12.8**	**15.5**	**7**

Table 15. Evaluation of the biological potential of the hybrid elite regarding frost

	Year	Viable buds %	Degree of resistance
***VMD 24* hybrid elite**	2020	100	9
	2021	100	9
	2022	100	9
Average		**100**	**9**
***Victoria* control**		**100**	**9**

Table 16. Evaluation of the biological potential of the hybrid elite regarding drought tolerance (OIV 403)

	Year	Codex OIV 403	Expression level
***VMD 24* hybrid Elite**	2020	5	Medium
	2021	5	Medium
	2022	5	Medium
Average		**5**	**Medium**
***Victoria* control**		**7**	**High**

*According to the OIV descriptor list for grape varieties and *Vitis* species, 2nd edition, 2009.

Evaluation of technological descriptors during the period 2020-2022

The results obtained from the analyses and determinations reveal that the genotype *VMD 24* recorded higher values compared to the control variety *Victoria* regarding the technological descriptors: average weight of the bunch, sugars g/l, acidity g/l but not for the weight of 100 berries g (Table 17 and Figure 21).

Regarding the production evaluation, the *VMD 24* genotype recorded a slightly higher value than the control for the average grape yield, 4.6 and respectively 4.7 kg/trunk.

As concerns the harvest per hectare as well as the marketable yield, expressed in percentage, both the new genotype and the control presented similar values, respectively 21 t/ha and 87% (Table 18).

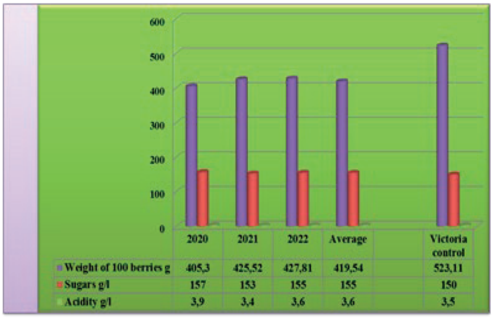


Figure 21 - Quality descriptors of *VMD 24* compared to the Victoria control

Table 17. Evaluation of the harvest during 2020-2022

	Year	Average weight of the bunch g	Weight of 100 berries g	Sugars g/l	Acidity g/l	*Ripening index
***RVC 24* hybrid elite**	2020	457.80	546.01	160	3.4	4
	2021	483.70	513.12	155	3.9	3
	2022	487.22	519.10	158	3.7	4
Average		**476.24**	**526.07**	**157**	**3.6**	**4**
***Victoria* control**		**420.33**	**523.11**	**150**	**3.5**	**4**

*De Cillis ripening index. Its value ranges from 1 to 1.5 at veraison and from 3 to 6 at full grape ripening.

Table 18. Evaluation of the harvest during 2020-2022

	Year	Harvest		Productivity index		
		Kg/trunk	t/ha	API	RPI	Yield Goods %
***VMD 24* hybrid elite**	2020	4.8	22	411	362	88
	2021	4.5	20	475	363	85
	2022	4.7	21	536	395	87
Average		**4.7**	**21**	**474**	**373**	**87**
***Victoria* control**		**4.6**	**21**	**462**	**290**	**87**

CONCLUSIONS

The objective pursued through the respective hybridizations was to obtain new genotypes for table grapes with resilience capacity, remarkable agro-biological and technological characterristics (clusters with large berries and desirable appearance, resistance to transportation, resistance to skin cracking, different ripening periods, high tolerance to major fungal diseases, extreme climatic conditions).

The new genotypes obtained at INCDBH Ştefăneşti - Argeş are highlighted by relevant biological and economic characteristics and traits.

Selection criteria for *RVC 24* genotype were: the weight of the bunch: 457.80-483.70 g; the berry is large, averaging 22 mm, with variations ranging between 21 and 24 mm the weight of a berry is 4.8-5.4 g, the pulp is very firm; 100 berries: 487-546 g, must concentration in sugars: 155-160 g/l, acidity 3.4-3.9 g/l H_2SO_4 and the harvest is high between 5.0-5.5 kg/trunk; the harvest per hectare is very high between 22-24 t.

The elite stood out for the size of the cluster and its berries, which gives it a particularly pleasant commercial appearance, and high tolerance to stress factors (frost and drought), high powdery mildew and downy mildew.

Selection criteria for *VMD 24* genotype were: large bunches, with an average length of 202 mm, ranging between 193 and 212 mm. It is loose to very loose with very visible pedicels. The bunch has an average weight of 443.30 g; the must concentration in sugars is high with values between 153-157 g/l; the color of skin berry is rose - red, slightly pruinose, and very attractive; the harvest is 4.5-4.8 kg/trunk; the harvest per hectare is between 20 and 22 t.

The elite stood out for the size of the cluster, as well as their color, which gives it a particularly pleasant commercial appearance, earliness, and good resistance to stress factors (frost and drought).

ACKNOWLEDGEMENTS

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ASSESSMENT AND PREDICTION OF GRAPE AND WINE TRACEABILITY: A CASE STUDY OF FETEASCĂ NEAGRĂ AND PINOT NOIR CULTIVATED IN VARIOUS WINE-GROWING REGIONS

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Abstract

The study was conducted between 2022 and 2023 with the aim of tracing specific elements of chemical composition from grapes to wine, focusing particularly on the 'Fetească Neagră' and 'Pinot Noir' varieties. These grape varieties were cultivated in five distinct wine-growing regions across Romania. A significant part of the grape's substances is proportionally transferred into the wine, blending with other beneficial compounds resulting from the conversion of grape must into wine. The correlation between the chemical composition of wine and its derivation from the chemical composition of the grape is a critical factor in forecasting wine quality. The chemical composition of the grapes was assessed at full maturity, and included the following elements: water, proteins, total lipids, ash, dietary fibers, and sugars. The corresponding components were subsequently traced within the wine. The examination took place subsequent to the stabilization of the wines, carried out in anticipation of bottling and consumption. The determination of both grape and wine samples' chemical composition adhered to specific standards validated by the Association of Analytical Chemists.

Key words: *chemical composition, grapes, quality, traceability, wine.*

INTRODUCTION

Grapes are widely considered as important and valuable fruits and foodstuffs for human consumption mainly due to their complex chemical composition (Kim et al., 2020). The compounds found in grapes have various roles in human body, including providing vitamins, energy, minerals and overall invigoration (Aubert and Chalot, 2018). Moreover, certain compounds from grape berries are believed to have therapeutic properties, contributing to their importance in traditional medicine practices (Urbi et al., 2014). A considerable rate of compounds found in grapes is stored in both grape must and wine (Nemzer et al., 2021). During grape must fermentation, these compounds are supplemented by additional substances further enhancing the nutritional and health benefits of wine; many researchers consider wine to be the healthiest natural alcoholic beverage for human consumption (Wimalasiri et al., 2022). Understanding the relationship between the chemical composition of wine and the precursor grape is critical for forecasting wine quality (Ferrero-del-Tesso et al., 2020). Winemakers can acquire significant insights into the potential qualities and traits of a wine by analysing its chemical elements. This knowledge enables the development of high-quality wines that fulfil consumer tastes and standards (Simhizu et al., 2020). The wine industry has recently evolved into a highly efficient and competitive field, with competitors required to offer wines of great quality at economically viable prices in order to gain and sustain a market presence (Masset et al., 2023). To achieve this, viticultural practices must yield grapes with specifically chemical compositions that are matched to the demands of winemaking methodologies and facility, infrastructure and, most significantly, the desired wine type and

market niche (Rainer, 2021). Furthermore, viticulture has significant challenges due to climatic changes, particularly the ongoing increase of temperature. This phenomenon has resulted in an excessive accumulation of sugars in grape must, resulting in the production of highly alcoholic wines, which is significant concern for winemakers (Dobrei et al., 2023). 'Feteasca Neagra' is not just a grape variety, but also the most well-known Romanian wine in both domestic and international markets, setting the standard for Romanian viticulture and vinification processes (Dobrei et al., 2018). Benefiting from climatic fluctuations over the last 10-15 years, it has experienced a rapid expansion, currently spreading across almost all of Romania's wine-growing regions. Conversely, 'Pinot Noir' is a renowned and highly esteemed grape variety used in winemaking, particularly associated with Burgundy, France, but also grown in various wine regions worldwide, including the United States, New Zealand, and Australia It is considered one of the oldest grape varieties, with a long history dating back to ancient times (Nistor et al., 2019).
The main aim of this research was to investigate the distinct elements of chemical composition from grapes to wine, specifically emphasizing the 'Fetească Neagră' and 'Pinot Noir' varieties. By delving into the chemical makeup of grapes and its implications for wine quality, this study also aims to offer valuable insights for tackling challenges and leveraging opportunities within the Romanian wine industry.

MATERIALS AND METHODS

The research was carried out in the 2022-2023 growing seasons with the objective of tracing certain elements of chemical composition from grapes to wine, focusing on the 'Fetească Neagră' and 'Pinot Noir' varieties. These varieties were cultivated in five distinct wine-growing regions, encompassing some of Romania's most prominent wine-producing areas: Iasi, Recas, Dealu Mare, Sarica Niculitel and Blaj (Figure 1). The selection of the two grape varieties was motivated by distinct factors. 'Fetească Neagră', renowned as Romania's most esteemed grape variety among winegrowers and vintners, was chosen for its perceived value within the local viticultural community. In contrast, 'Pinot Noir' was selected as a comparative reference due to its global prevalence and prominence in the wine industry.

Figure 1. Viticultural areas for grapes and wine origin

The chemical composition analysis of the grapes was conducted at full ripeness, encompassing parameters such as water content, proteins, total lipids, ash, dietary fibers and sugars, along with macro and microelements including phosphorus, potassium, copper, iron and sodium. Subsequently, the same constituents were assessed in the resulting wines derived from these grape varieties, with analyses performed following wine stabilization in preparation for bottling and consumption.
The determination of chemical compositions in both grape and wine samples adhered to standardized protocols validated by the Association of Analytical Chemists (AOAC). Specifically, the AOAC 925.09 method was employed for water content, AOAC 985.29 for dietary fiber, AOAC 920.39C Gravimetry (ether extraction) for feeding fibers, AOAC 945.38F for total sugars, and a portable ZEISS refractometer for refractometric sugar analysis. Furthermore, phosphorus, potassium, copper, iron and sodium levels were assessed using the AOAC 994.02 standard, ISO 12193, or AOCS Ca 18c-91 Atomic Absorption methodologies. All results were reported per 100 grams of sample.
Statistical analysis of data was done by using XLSTAT by Addinsoft (2018) Statistical and Data Analysis Solution Version 2018.7.5., which facilitate Pearson correlations calculation which is essential for assessing the relationships between variables in the dataset. Additionally,

was computed the principal component analysis (PCA) diagram by transforming high-dimensional data into a lower-dimensional space.

RESULTS AND DISCUSSIONS

Irrespective of the prevailing climatic conditions and the inherent characteristics of the cultivation regions, the chemical composition of both grape varieties exhibited relatively narrow variations. This suggests the stability inherent to these varieties concerning their chemical makeup across different cultivation environments. Specifically, the content of dietary and food fibers (Figure 2) showed minimal divergence, both between the two grape varieties and across the five cultivation regions, falling within the ranges commonly reported in specialized literature. This consistent profile observed both temporally and spatially, indicates the stability and adaptability of these grape varieties regarding fiber content. This adaptability positions them favorably for inclusion in varietal assortments in vineyards, particularly in anticipation of changing climatic conditions.

In contrast, the sugar content measured at grape harvest, while exhibiting relatively similar values, displayed less consistency across cultivation regions (Figure 3). Naturally, the regions renowned for their tradition and aptitude in cultivating grape varieties for red wines (such as Dealu Mare, Recaș and Sarica Niculitel) recorded the highest sugar concentrations. Additionally, noteworthy is the attainment of sufficiently high sugar concentrations in the grapes from Iași and Blaj, areas previously regarded primarily for white wine production, lacking grape varieties suited for red wine production in their assortments. This suggests a notable shift in the viticultural landscape, with previously white wine-focused regions now demonstrating potential for high-quality red wine production.

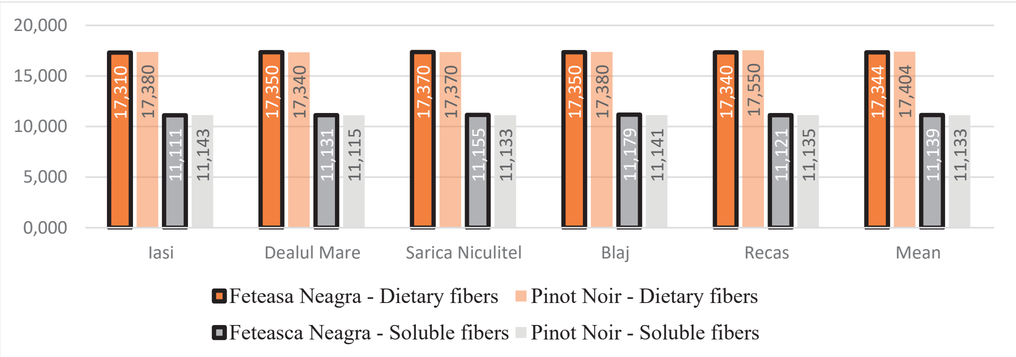


Figure 2. Berry grapes composition in dietary and soluble fibers (g) (mean for 2022-2023)

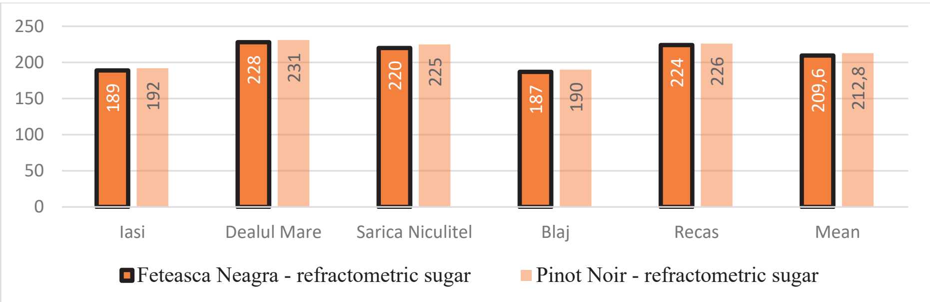


Figure 3. Total soluble solids (g/l) in grape must (mean 2022-2023)

Climatic disturbances, particularly the recent rise in temperatures, have shifted the dynamics of sugar accumulation in grapes (Suter et al., 2021). Continuation of this climatic trend poses the threat of excessive sugar accumulation, resulting in wines with elevated alcohol content

(Leolini et al., 2019). Presently, across the majority of wine-growing regions in Romania and other countries with abundant solar radiation (such as Italy, France, Spain and Portugal), there is a noticeable uptrend in the alcohol content of wines (Vaquero et al., 2022). Rarely do wines exhibit alcohol levels below 12-13%. In light of these concerns, the two grape varieties under consideration emerge as promising options due to their early maturation stage, allowing for the attainment of technological grape maturity. Across all five regions, grapes reached technological maturity as early as the second decade of August. This timing minimizes the accumulation of excessive heat units and sunlight exposure, thereby limiting sugar accumulation to manageable levels. Specifically, sugar concentrations ranged from a maximum of 224-228 g/l for the 'Fetească Neagră' variety and 226-231 g/l for the 'Pinot Noir' variety. These moderate accumulations afford winemakers the opportunity to craft balanced wines during the winemaking process, ensuring that the final products do not exhibit excessive alcohol levels.

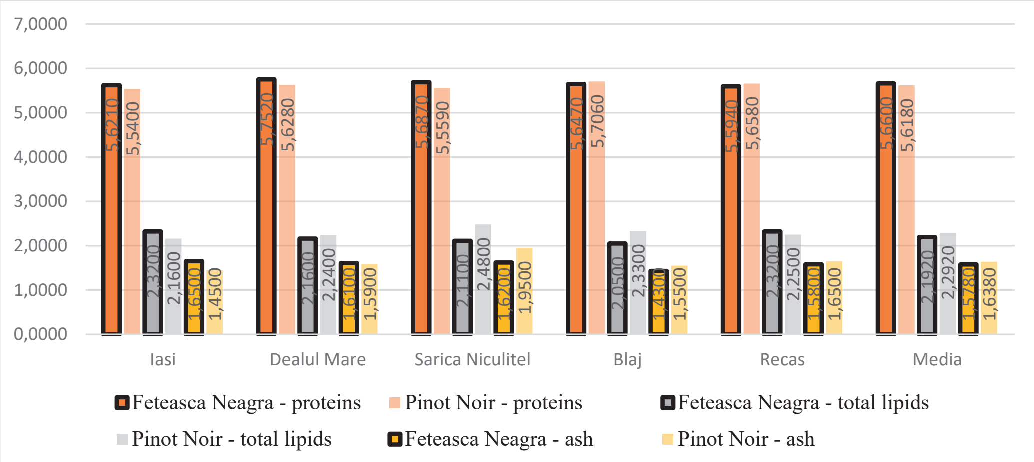


Figure 4. Berry grape composition in proteins, total lipids and ash (g) (mean 2022-2023)

Proteins, total lipids and ash constitute crucial components of grape composition, exerting significant influence on the sensory and biochemical characteristics of wines (Ovcharova et al., 2016). In this regard, both the 'Fetească Neagră' and 'Pinot Noir' varieties exhibited stability across all five regions, with recorded values (Figure 4) closely aligning with the ranges documented in specialized literature (Frîncu et al., 1019; Senila et al., 2020). Similarly, the levels of proteins, total lipids and ash in the resultant wines displayed consistent balance and proximity across all regions. However, it is noteworthy that the values observed in wine samples were naturally slightly lower compared to those in grapes, as wine is recognized for its relatively low protein and lipid content.

Qualitative compounds from grape berries encompass a wide range of chemical constituents that significantly influence the sensory characteristics of wines (Sam et al., 2021). The correlation among these qualitative compounds is complex and multifaceted. Understanding the interplay among these qualitative compounds is essential for winemakers to produce wines of consistent quality and style. By analyzing and managing these correlations, winemakers can optimize grape growing practices, fermentation techniques and aging processes to achieve desired sensory outcomes in the final wine product. The correlations between the variables that characterize 'Fetească Neagră' cultivated in several vineyards from Romania are presented in Table 1.

Table 1. Pearson's correlation between qualitative variables of 'Fetească Neagră' variety for 2022-2023 growing seasons

Variables	DF Fn	SF Fn	TSS Fn	RDS Fn	W Fn	P Fn	TL Fn	A Fn	P Fn	K Fn	Cu Fn	Fe Fn	Na Fn
DF Fn	**1**	0.677	0.418	0.508	**0.850**	0.511	-0.745	-0.272	-0.069	-0.289	0.359	0.434	0.778
SF Fn	-	**1**	**0.815**	-0.259	0.287	0.214	**-0.931**	-0.794	-0.322	-0.289	**0.828**	0.538	0.712
TSS Fn	-	-	**1**	-0.233	0.255	0.127	-0.722	**-0.958**	-0.025	-0.562	**0.991**	0.327	**0.824**
RDS Fn	-	-	-	**1**	**0.840**	0.431	0.099	0.423	0.400	-0.214	-0.339	-0.100	0.342
W Fn	-	-	-	-	**1**	0.426	-0.360	-0.079	0.349	-0.318	0.145	0.285	0.749
P Fn	-	-	-	-	-	**1**	-0.551	0.143	-0.583	-0.765	0.141	-0.537	0.419
TL Fn	-	-	-	-	-	-	**1**	0.611	0.532	0.512	-0.747	-0.249	-0.726
A Fn	-	-	-	-	-	-	-	**1**	-0.049	0.324	**-0.957**	-0.481	-0.671
P Fn	-	-	-	-	-	-	-	-	**1**	0.304	-0.141	0.401	0.117
K Fn	-	-	-	-	-	-	-	-	-	**1**	-0.564	0.520	-0.640
Cu Fn	-	-	-	-	-	-	-	-	-	-	**1**	0.275	0.759
Fe Fn	-	-	-	-	-	-	-	-	-	-	-	**1**	0.301
Na Fn	-	-	-	-	-	-	-	-	-	-	-	-	**1**

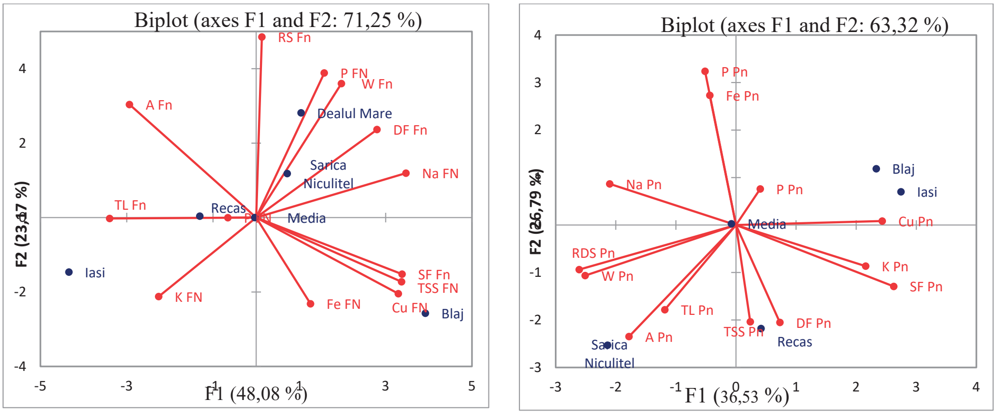
Fn – Feteasca Neagra; DF - Dietary fiber (g); SA - Soluble fiber (g); TSS – Total soluble solids (g); RDS - Refractometric Dry Substance (g/l); W - Water (g); P - Protein (g); TL - Total lipids (g); A - Ash (g); P - Phosphorus (mg); K - Potassium (mg); Cu - Copper (mg); Fe - Iron (mg); Na - Sodium (mg).

The correlation coefficients indicate the strength and direction of the relationships between the variables. For instance, variables such as dietary fibers (DF) and water (W) show a strong positive correlation (0.85), while total lipids (TL) and soluble fibers (SF) exhibit a strong negative correlation (-0.931). Positive correlations indicate that as one variable increases, the other variable tends to increase as well, whereas negative correlations suggest that as one variable increases, the other variable tends to decrease. It's essential to note that correlation does not imply causation. While the analysis identifies associations between variables, it doesn't establish a cause-and-effect relationship. The statistical analysis provides insights into the relationships between different variables related to the 'Fetească Neagră' variety, which can inform further research and decision-making in viticulture. The correlation matrix (Table 2) provides valuable insights into the relationships between different variables associated with 'Pinot Noir' grapes.

Table 2. Pearson's correlation between qualitative variables of 'Pinot Noir' variety for 2022-2023 growing seasons

Variables	DF Pn	SF Pn	TSS Pn	RDS Pn	W Pn	P Pn	TL Pn	A Pn	P Pn	K Pn	Cu Pn	Fe Pn	Na Pn
DF Pn	**1**	0.274	0.532	0.209	0.200	0.303	-0.172	0.013	-0.589	0.251	0.360	-0.503	-0.332
SF Pn	-	**1**	0.226	-0.778	-0.710	-0.058	-0.031	-0.202	-0.530	0.801	0.667	-0.421	-0.758
TSS Pn	-	-	**1**	0.234	-0.039	-0.633	-0.252	0.116	-0.365	-0.288	0.489	**-0.931**	0.277
RDS Pn	-	-	-	**1**	**0.927**	-0.071	0.275	0.595	-0.068	-0.682	-0.623	-0.133	0.626
W Pn	-	-	-	-	**1**	0.200	0.515	0.703	-0.208	-0.411	-0.798	0.050	0.352
P Pn	-	-	-	-	-	**1**	0.010	-0.220	-0.008	0.483	-0.176	0.637	-0.563
TL Pn	-	-	-	-	-	-	**1**	**0.897**	-0.582	0.234	-0.744	-0.039	-0.199
C Pn	-	-	-	-	-	-	-	**1**	-0.612	-0.094	-0.712	-0.327	0.113
P Pn	-	-	-	-	-	-	-	-	**1**	-0.535	0.016	0.630	0.526
K Pn	-	-	-	-	-	-	-	-	-	**1**	0.266	0.046	**-0.987**
Cu Pn	-	-	-	-	-	-	-	-	-	-	**1**	-0.399	-0.260
Fe Pn	-	-	-	-	-	-	-	-	-	-	-	**1**	-0.068
Na Pn	-	-	-	-	-	-	-	-	-	-	-	-	**1**

High water content is positively correlated with reducing sugars (0.927), indicating ripe grapes with higher sugar levels, similar with the results found by Geng et al. (2022). Relatively high and negative correlation (-0.778), suggesting that grapes with higher sugar content may have lower fiber content. Total soluble solids show a strong negative correlation with iron (-0.931), implying that grapes with higher sugar content may have lower iron levels. Copper shows mixed correlations with different variables, indicating its complex relationship with grape composition. These insights can inform decisions related to irrigation, fertilization and harvest timing to achieve desired grape characteristics and wine quality.



a b

Figure 5. PCA diagram for 'Fetească Neagră' (a) and 'Pinot Noir' (b) varieties from several vineyards from Romania Fn – 'Fetească Neagră'; Pn – 'Pinot Noir'; DF - Dietary fiber (g); SA - Soluble fiber (g); TSS – Total soluble solids (g); RDS - Refractometric Dry Substance (g/l); W - Water (g); P - Protein (g); TL - Total lipids (g); A - Ash (g); P - Phosphorus (mg); K - Potassium (mg); Cu - Copper (mg); Fe - Iron (mg); Na - Sodium (mg)

The PCA diagram (Figure 5a) reveals distinct clusters of data points representing 'Fetească Neagră' grapes from different vineyards. Component 1, which accounts for 48.08% of the variance, appears to primarily differentiate the vineyards based on variables related to refractometric sugar, water content, dietary fiber, sodium and phosphorus. Component 2, contributing 23.17% of the variance, further separates the vineyards based on variables such as iron, soluble fiber, TSS and copper content. Notably, grapes from Dealu Mare vineyards exhibit the highest levels of refractometric sugar, water, dietary fiber, sodium and phosphorus, while those from Blaj vineyards display higher concentrations of iron, soluble fiber, TSS and copper. In contrast, grapes from Iasi vineyards demonstrate lower values across several variables, including refractometric sugar, water, dietary fiber, sodium, phosphorus, potassium and total lipids. Analysis of the PCA highlights significant variability in grape composition among different vineyards, reflecting variations in environmental conditions, soil characteristics and cultivation practices. Grapes from Dealu Mare vineyards exhibit characteristics associated with higher sugar content and nutrient levels, due to favorable climatic conditions and soil composition in the region. Conversely, grapes from Iasi vineyards show lower values across multiple variables, suggesting potential differences in agricultural practices or environmental factors that impacted grape quality and composition. The distinct clusters observed in the PCA diagram underscore the importance of considering geographical and environmental factors in grape cultivation and their influence on grape composition and quality. The PCA diagram for 'Pinot Noir' grapes (Figure 5b) shows distinct patterns among various vineyards, with Principal Component 1 representing 36.53% and Component 2 representing 26.79% of the variance. Grapes from the vineyards of Iasi and Blaj display higher levels of phosphorus and copper, suggesting potential differences in soil composition or fertilizer application practices in these regions. However, they exhibit lower levels of RDS, water, ash and total lipids, which may impact flavor profile and overall grape quality. In contrast, grapes from the Sarica Niculitel vineyard contain higher levels of ash and total lipids, indicating potential differences in soil fertility and vineyard management practices. Grapes from the Recas vineyards, while rich in sugar and dietary fiber, display deficiencies in essential nutrients such as phosphorus, iron and sodium, highlighting potential challenges in soil fertility or nutrient management strategies in this region.

The correlations between various chemical elements in grape berries (denoted with "bFn") and their corresponding elements in wine (denoted with "wFn") for the 'Fetească Neagră' variety are shown in table 3.

Table 3. Pearson's correlation between grapes must and wine qualitative variables of 'Fetească Neagră' variety for 2022-2023 growing seasons

Variables	P bFn	K bFn	Cu bFn	Fe bFn	Na bFn	P wFn	K wFn	Cu wFn	Fe wFn	Na wFn	TanFn	ProtFn	LipFn	AshFn
P bFn	1	0.304	-0.141	0.401	0.117	**0.757**	-0.141	0.035	-0.613	0.223	-0.085	-0.541	0.657	-0.031
K bFn	-	1	-0.564	0.520	-0.640	-0.209	0.666	**-0.787**	0.287	-0.529	0.126	**-0.921**	0.544	-0.199
Cu bFn	-	-	1	0.275	**0.759**	0.524	-0.567	**0.742**	-0.099	0.541	0.558	**0.737**	-0.566	-0.217
Fe bFn	-	-	-	1	0.301	0.450	-0.171	0.092	0.306	0.329	0.277	-0.344	-0.070	0.035
Na bFn	-	-	-	-	1	0.662	**-0.961**	**0.977**	-0.065	**0.955**	-0.077	0.649	-0.629	0.407
P wFn	-	-	-	-	-	1	-0.587	0.598	-0.609	0.627	0.177	0.085	0.144	-0.062
K wFn	-	-	-	-	-	-	1	-0.962	0.062	**-0.985**	0.342	-0.599	0.600	-0.607
Cu wFn	-	-	-	-	-	-	-	1	-0.147	**0.920**	-0.125	**0.758**	-0.636	0.400
Fe wFn	-	-	-	-	-	-	-	-	1	0.000	-0.159	-0.011	-0.603	0.418
Na wFn	-	-	-	-	-	-	-	-	-	1	-0.332	0.481	-0.568	0.626
TanFn	-	-	-	-	-	-	-	-	-	-	1	0.105	0.095	**-0.886**
ProtFn	-	-	-	-	-	-	-	-	-	-	-	1	-0.757	0.100
LipFn	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.468
AshFn	-	-	-	-	-	-	-	-	-	-	-	-	-	1

A significant positive correlation of 0.757 was found between phosphorus content in grape berries (P bFn) and wine (P wFn), indicating a potential relationship between nutrient levels in the grapes and the surrounding environment. Such a correlation indicates that the availability of phosphorus in the soil or vineyard environment may play a crucial role in determining the phosphorus content of grape berries. Grapes grown in environments with higher phosphorus levels may exhibit increased phosphorus content, which is then reflected in the phosphorus levels of the wine produced from those grapes (Piccin et al., 2017).

Conversely, a negative correlation of -0.141 was observed between potassium content in grape berries (K bFn) and wine (K wFn), indicating a weak negative relationship between the two. High potassium levels in the soil may decrease potassium absorption by grapevines, resulting in reduced potassium content in both grape berries and the resulting wine (Hu et al., 2023). Environmental conditions, such as temperature, rainfall patterns, and sunlight exposure, also play a crucial role in potassium uptake and accumulation in grapevines. Extreme weather events or drought conditions may hinder potassium absorption, resulting in fluctuations in potassium levels in grapes and wine (Villette et al., 2020). Additionally, fermentation methods, yeast selection, and clarification techniques impact the extraction, retention, or removal of potassium during winemaking processes (Payan et al., 2023). A weak positive correlation of 0.035 was noted between copper content in grape berries (Cu bFn) and wine (Cu wFn), suggesting a consistent copper presence across different parts of the grape. The vineyard management practices and agricultural inputs, such as copper-based fungicides or fertilizers, can impact copper levels in grape berries. Regular application of copper-based products to control fungal diseases like mildew or botrytis may lead to the accumulation of copper in grape tissues, subsequently influencing its presence in the wine. Factors such as maceration, pressing and fermentation methods may influence the release, extraction, or retention of copper during winemaking, thereby influencing its concentration in the wine (Donici et al., 2019). A moderate negative correlation of -0.613 was found between iron content in grape berries (Fe bFn) and wine (Fe wFn), indicating a moderate negative relationship between the iron levels in the two. Soil characteristics, such as pH levels and soil composition, can affect the availability of iron to the grapevine roots. Additionally, microbial activity in the soil can influence the solubility and availability of iron, thereby affecting its uptake by grapevines (Zebek et al, 2021). During winemaking, processes such as pressing, fermentation and clarification can impact the transfer of iron from grape berries to wine. A moderate positive correlation of 0.223 was also observed between sodium content in grape berries (Na bFn) and wine (Na wFn), suggesting a moderate positive association between sodium levels in grape berries and wine. Various correlations ranging from -0.886 to 0.758 were noted for tannins, proteins, lipids, and ash; tannin content is positively correlated with iron in wine, indicating a potential influence on the sensory characteristics of the wine; proteins exhibit strong negative correlations with potassium in berries and wine, indicating a potential antagonistic relationship affecting protein metabolism in the grapes. Lipids in berries show positive correlations with lipids in wine and negative correlations with other minerals, suggesting a complex relationship influenced by multiple factors (variety, growing conditions, climate, soil, viticultural practices, winemaking techniques, etc). All findings provide insights into the associations between the chemical compositions of grape berries and wine, indicating potential impacts on wine quality.

The strong negative correlation between potassium levels in berries and wine suggests that higher potassium content in berries leads to lower levels in wine, which could impact the taste and quality of the wine. Moderate positive correlations for phosphorus, sodium and iron between berries and wine suggest that these elements are consistently transferred from berries to wine during winemaking processes. The weak correlations for tannins and lipids indicate that there may be factors other than berry composition influencing their presence in wine

Table 4. Pearson's correlation between grapes must and wine qualitative variables of 'Pinot Noir' variety for 2022-2023 growing seasons

Variables	P bPn	K bPn	Cu bPn	Fe bPn	Na bPn	P wPn	K wPn	Cu wPn	Fe wPn	Na wPn	TanPn	ProtPn	LipPn	AshPn
P bPn	1	-0.535	0.016	0.630	0.526	-0.080	-0.563	0.264	0.174	0.508	-0.267	0.609	0.273	-0.783
K bPn	-	1	0.266	0.046	-0.987	-0.747	0.384	-0.782	-0.088	-0.116	0.891	0.080	-0.210	0.024
Cu bPn	-	-	1	-0.399	-0.260	-0.331	0.270	0.207	-0.631	0.154	0.012	-0.423	-0.473	-0.500
Fe bPn	-	-	-	1	-0.068	-0.423	-0.445	-0.537	0.629	0.342	0.457	0.990	0.603	-0.543
Na bPn	-	-	-	-	1	0.681	-0.276	0.793	0.107	0.000	-0.909	-0.086	0.109	-0.006
P wPn	-	-	-	-	-	1	-0.284	0.646	-0.072	0.041	-0.744	-0.475	0.245	0.480
K wPn	-	-	-	-	-	-	1	-0.188	0.145	-0.893	0.138	-0.425	-0.373	0.279
Cu wPn	-	-	-	-	-	-	-	1	-0.502	0.115	-0.960	-0.537	-0.366	0.053
Fe wPn	-	-	-	-	-	-	-	-	1	-0.395	0.254	0.596	0.703	-0.014
Na wPn	-	-	-	-	-	-	-	-	-	1	0.040	0.311	0.217	-0.502
TanPn	-	-	-	-	-	-	-	-	-	-	1	0.464	0.205	-0.153
ProtPn	-	-	-	-	-	-	-	-	-	-	-	1	0.502	-0.501
LipPn	-	-	-	-	-	-	-	-	-	-	-	-	1	-0.175
AshPn	-	-	-	-	-	-	-	-	-	-	-	-	-	1

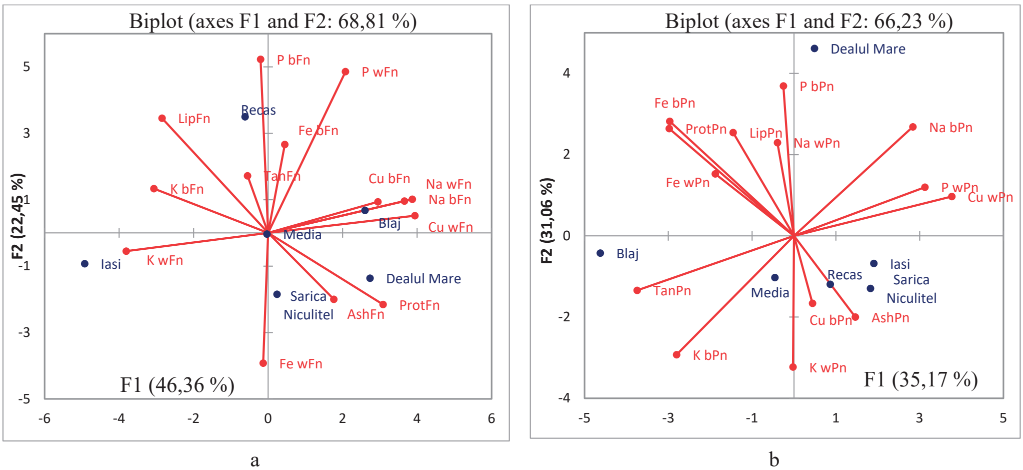


Figure 6. PCA diagram for 'Fetească Neagră (a) and 'Pinot Noir' (b) varieties from several vineyards from Romania Fn – 'Fetească Neagră'; Pn – 'Pinot Noir'; A - Ash (g); P - Phosphorus (mg); K - Potassium (mg); Cu - Copper (mg); Fe - Iron (mg); Na - Sodium (mg); Prot – Proteins; Lip – Lipids, Tan- Tannins

The PCA diagram (Figure 6a) indicates that the first principal component (F1) contributes 46.36% to the overall variability, while the second principal component (F2) contributes

22.45%. These components collectively explain a substantial portion of the variability observed in the dataset. For the 'Fetească Neagră' variety, notable patterns emerge in the accumulation of certain compounds across different vineyards. In the Blaj vineyard, there is a pronounced accumulation of copper and sodium, both in the grape berries and the resulting wine. This suggests that environmental or soil conditions in the Blaj vineyard may favor the uptake and retention of these elements by grapevines, leading to higher concentrations in both grapes and wine. Conversely, the Recas vineyard exhibits greater accumulations of phosphorus in grape berries and wine, along with a lesser extent of lipids. This contrasts with the patterns observed in the Blaj vineyard, indicating distinct soil compositions or management practices that influence nutrient uptake by grapevines. Additionally, the protein and ash content in the wine show higher accumulations in the Sarica Niculitel and Dealu Mare vineyards. This suggests that factors specific to these vineyards, such as soil composition, microclimate, or viticultural practices, may contribute to the enrichment of proteins and ash in the resulting wine. Interestingly, across all vineyards, the accumulation of iron in the wine is observed to be minimal. This consistent pattern suggests that iron levels in the soil or grapevines may be inherently lower or that winemaking processes may result in the removal or reduction of iron compounds in the final wine product. In the PCA graph, it is observed that the first principal component (F1) accounts for 35.17% of the variability, while the second principal component (F2) contributes 31.06%. Analysis of the 'Pinot Noir' variety reveals distinct patterns in nutrient accumulation across various vineyards. In the vineyard located at the Dealu Mare, notably high concentrations of phosphorus are recorded in grape berries, alongside elevated levels of sodium in both the berries and the resulting wine. Additionally, copper and phosphorus concentrations in the wine from this vineyard are observed to be notably high. These findings suggest that environmental factors or soil characteristics in the Dealu Mare vineyard may contribute to the enrichment of these elements in grape berries and wine. In the vineyards of Recas, Iasi, and Sarica Niculitel, the wine exhibits large accumulations of ash, copper and potassium in grape berries. This indicates that these vineyards may possess soil compositions or viticultural practices that favor the uptake and retention of these elements by grapevines, subsequently influencing their concentrations in the resulting wine. In the Blaj vineyard, the concentration of tannins in the wine and potassium in the berries is notably low. This suggests that environmental or management factors specific to the Blaj vineyard may result in lower levels of these compounds in grape berries and wine.

CONCLUSIONS

Both studied varieties exhibited grapes with highly comparable chemical compositions regardless of the vineyard of origin, indicating that the varieties are stable and adaptable to pedoclimatic conditions in all cultivated areas. Both the 'Fetească Neagră' and 'Pinot Noir' varieties reach maturation much earlier than other varieties from vineyards and accumulate amounts of sugars that allow them to produce balanced wines without the risk of accumulating excessive amounts of sugars, which would result in wines that are overly alcoholic. The chemical composition of the wines produced from the two types was also balanced and uniform, regardless of the vineyard of origin. The analysis of correlations among different components in grape must and wine for both varieties reveals valuable insights for vineyard management and winemaking practices. The distinct patterns observed in the PCA diagrams emphasize the importance of considering geographical and environmental factors in grape cultivation and their impact on grape composition and quality. Further research could explore the specific factors driving these differences and their implications for viticulture practices and grape quality optimization in each region. Additionally, targeted interventions such as soil amendments or nutrient management strategies may be warranted to address nutrient deficiencies and optimize grape quality in specific vineyards.

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THE IMPACT OF COPPER APPLICATION TIMING AND THE FREQUENCY OF TREATMENTS ON PHYSIOLOGICAL, BIOCHEMICAL, AND PRODUCTIVE PARAMETERS IN A VINEYARD

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Abstract

The research carried out between 2021 and 2023 was primarily focused on managing the timing and frequency of copper application - an essential treatment in the control of diseases and pests. The grape varieties (Victoria, Muscat de Hamburg, Merlot, Cabernet Sauvignon, Fetească neagră) involved in the research were for table grapes and wine, with peculiar tolerance to frost, diseases, and pests. Experimental plots were organized based on several copper treatments correlated with disease and pest management. Several grape yield parameters, as well as berry composition and canes (berry sugar and vitamin content; cane carbohydrate content), were tracked for each of the six experimental plots within each grape variety. Simultaneously, an examination of how the vines survived during the winter as regards of wood maturity and bud viability was carried out, taking into account the major influence of copper on these parameters. The results showed that the grape varieties behaved differently from year to year, depending on the individual qualities of each variety.

Key words*: viticulture, copper, cane maturation, grapes, quality.*

INTRODUCTION

Grapevine is one of the most vulnerable cultivated plants to disease and pest attacks (Hnatiuc et al., 2023). According to the majority of winegrowers, the most widespread and dangerous disease is downy mildew, which is caused by the fungus *Plasmopara viticola* (Peng et al., 2024). Copper-based treatments were previously the most widely utilised and effective products for downy mildew control (Dobrei et al., 2020). Copper was initially used as a fungicide in agriculture in the 17th century to treat wheat seed against downy mildew before sowing (Beckerman et al., 2023). Copper was applied for downy mildew control in vine treatments in 1882, in France, by Millardet, who applied copper as a fungicide in the Bordeaux mixture (Morton & Staub, 2008). Much later, in 1956, the first copper-based insecticide for grapevine mildew control was approved (Rajwade et al., 2024). Since then, the number of copper-based treatments approved for downy mildew control on vines has expanded substantially, making it nearly impossible to develop an efficient strategy to control diseases and pests on vines that should not be found in copper-based products (Puelles et al., 2024). Copper serves various roles in vine metabolism beyond its conventional use as a pesticide (Moine et al., 2023; Betancur-Agudelo et al., 2023). It contributes to essential processes such as protein and lignin synthesis, chlorophyll production, respiration, absorption, and winter resistance (Oyebamiji et al., 2024). As the concept of organic viticulture is more spread and vine-cultivated areas expand under organic systems, the significance of copper has increased (Jez et al., 2023; Vršič et al., 2023). Copper-based products are among the few chemical treatments approved for use in organic viticulture, underscoring their importance in sustainable grape cultivation practices (Döring et al., 2015). The proper application of copper in viticulture represents a critical and extensively investigated aspect in recent years (Volkov et al., 2023). Research findings have highlighted its multifaceted role beyond solely downy mildew control (Moine et al., 2023; Maddalena et al., 2023). Copper exhibits beneficial effects and unintended yet effective side effects, including mitigating powdery mildew incidence by impacting cleistotheca and mycelium, decreasing grey rot (*Botrytis cinerea*) susceptibility through thickening the skin of the berries, postponing leaf fall to promote shoot tissue maturation, and enhancing winter resilience (Widmer & Norgrove, 2023; Bleyer et al., 2023). Optimal utilization of copper in viticulture necessitates an integrated approach, involving the rotation of different copper formulations or its alternation with other products. This strategy is crucial to prevent the development of resistance in target pathogens (Trentin et al., 2023). Moreover, the timing of copper application holds significant importance and must be carefully synchronized with climate variability, grape variety, growing stage, and the purpose of application (Moine et al., 2023).

The objective of the paper was to investigate the management of timing and frequency of copper application in grapevine, particularly focusing on its influence on grape yield parameters, vine survival during winter, disease and pest control.

MATERIALS AND METHODS

The study was conducted in the Recaș vineyard area over the growing seasons of 2021 to 2023, with the main objective of improving copper treatment timing, amount, and frequency. Experimental plots were established to investigate various copper application protocols, which included different numbers of treatments and scheduling strategies, as part of overall disease and pest management strategies (V_1 - one treatment with copper (0.76 kg/ha Cu,) applied after veraison (Control); V_2 - one treatment with copper (0.76 kg/ha Cu) applied before flowering; V_3 - one treatment with copper (0.76 kg/ha Cu) applied at berry leg; V_4 - two treatments with copper (0.76 kg/ha Cu) applied at berry leg + (0.76 kg/ha Cu) applied after veraison; V_5 - two treatments with copper (0.76 kg/ha Cu) applied before flowering + (0.76 kg/ha Cu) applied after veraison; V_6 - three treatments with copper: (0.76 kg/ha Cu) applied before flowering + (0.76 kg/ha Cu) applied at berry leg + (0.76 kg/ha Cu) applied after veraison.

Cuproxat Flowable product was used (190 g/litre Cu derived from tribasic copper sulphate) in the copper treatments. The varieties chosen for research (for wine and fresh consumption), have variable resilience to frost, diseases and pests.

Observations were made and determined on varieties and experimental plots regarding: the percentage of matured annual wood, the fertile buds percentage, the carbohydrates composition of annual canes (at the onset of dormancy and at the end of physiological dormancy), the grape production, the sugar concentration in grape juice, the amount of assimilated sugar per hectare and the berry composition in vitamins. The latter was assessed in accordance with specific standards endorsed by the Association of Analytical Chemists (AOAC) and subsequently expressed per 100 grams of sample. The statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software for Windows version V22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated for all variables. Normality of data distribution was assessed using the Shapiro-Wilk test. For inferential statistics, a two-tailed t-test was performed to compare the means of two independent groups, with significance set at $p < 0.05$. The t-value was calculated for the difference between the means. The p-value was calculated for the probability of obtaining the observed difference between groups. A p-value less than 0.05 was considered statistically significant. The statistical analysis was conducted using XLSTAT 2018 software (Addinsoft, Paris, France). Principal Component Analysis (PCA) was performed to analyze the qualitative characteristics of the data.

RESULTS AND DISCUSSIONS

The majority of viticultural practices involve 1-2 copper treatments as part of disease and pest management strategies, which are often applied after veraison (Jez et al., 2023). During the three-year study period, climate conditions varied significantly throughout the growing seasons, ranging from very favourable in 2021 to moderately favourable in 2023 (Table 1). Therefore, the average results attained during these years can be considered consistent and reliable.

Table 1. Influence of treatments on one year old wood maturation in table grape varieties
(Mean 2021-2023 growing seasons)

Experimental plot	Cu (kg/ha)	No of days till the first hoar-frost			Percentage of one year old wood maturated (%)	Difference to the control (%)
		Treat. 1	Treat. 2	Treat. 3		
Victoria						
V_1(Control)	0.76	97	-	-	69	-
V_2	0.76	156	-	-	74	5^{NS}
V_3	0.76	121	-	-	76	7*
V_4	1.52	121	97	-	83	14^{**}
V_5	1.52	156	97		85	16^{**}
V_6	2.28	156	121	97	88	19^{**}
Muscat Hamburg						
V_1(Control)	0.76	69	-	-	60	-
V_2	0.76	156	-	-	69	9^{*}
V_3	0.76	94	-	-	71	11^{*}
V_4	1.52	94	69	-	76	16^{**}
V_5	1.52	156	69		78	18^{**}
V_6	2.28	156	94	69	82	22^{***}

The percentage of annual matured wood serves as a significant indicator profoundly impacting vine winter resilience and bud viability (Dobrei et al., 2021). Despite minimum temperatures across all three years remaining above the frost resistance threshold for vines, the percentages of one year old matured wood, particularly among table grape varieties, ranged between 60 and 85%. Bondok et al. (2012) found in the Roumi Ahmar table grape, that old wood have a significant role in grape yield increasing, bunch weight and berry quality. In both table grape varieties, the ranking of experimental plots was near similar, differing primarily in the percentage of matured one-year-old wood. The control plots recorded less matured wood, while plots receiving higher copper doses and extended copper application durations showcased elevated percentages of ripened wood. These differences compared to the control were statistically significant.

Table 2. Influence of treatments on one year old wood maturation in wine grape varieties
(Mean 2021-2023 growing seasons)

Experimental plot	Cu (kg/ha)	No of days till the first hoar-frost			Percentage of one year old wood maturated (%)	Difference to the control (%)
		Treat. 1	Treat. 2	Treat. 3		
Fetească neagră						
V_1 (MT)	0.76	68	-	-	73	-
V_2	0.76	156	-	-	75	2^{NS}
V_3	0.76	95	-	-	79	6^{NS}
V_4	1.52	95	68	-	84	11^{*}
V_5	1.52	156	68		85	12^{*}
V_6	2.28	156	95	68	91	18^{**}
Merlot						
V_1 (MT)	0.76	64	-	-	74	
V_2	0.76	156	-	-	77	3^{NS}
V_3	0.76	84	-	-	80	6^{NS}
V_4	1.52	84	64	-	85	11^{*}
V_5	1.52	156	64		86	12^{*}
V_6	2.28	156	84	64	90	16^{**}
Cabernet Sauvignon						
V_1 (MT)	0.76	61	-	-	83	-
V_2	0.76	156	-	-	83	-
V_3	0.76	79	-	-	84	1^{NS}
V_4	1.52	79	61	-	90	7^{*}
V_5	1.52	156	61		91	8^{*}
V_6	2.28	156	79	61	96	13^{*}

In Muscat Hamburg variety, renowned for its frost sensitivity and wood maturation challenges, the beneficial influence of copper surplus compared to the control was notably evident.

Despite the inherent good properties of wine grape varieties concerning the maturation of one-year-old wood and winter resilience, certain experimental plots exhibited lower percentages of wood maturation (Table 2). Nonetheless, across all plots, the wood demonstrated satisfactory maturation and remained undamaged by winter's low temperatures. Comparatively, the differences observed between experimental plots for wine grape varieties were less significant than those observed in varieties for table grapes. In plots with higher doses of copper treatments application, had increased percentages of matured wood. The timing of the copper application was also important: applications made too early, prior to flowering, or too late, at the onset of veraison, demonstrated lesser efficacy than applications made during the berry leg stage. In all three varieties, the most efficient treatment as indicated by wood maturation was in the V_6 plot, which received the highest copper dosage, applied in all three critical stages: before flowering, during berry leg development and at the onset of veraison. Among the wine grape varieties, the smallest differences between treatments were recorded in the Cabernet Sauvignon variety, known for its favourable properties regarding wood maturation and winter cold resistance. After two copper treatments application in Riesling variety (during and after flowering) for *Plasmopara viticola* control, Meissner et al. (2019) discovered significantly higher copper content in both leaves and vines wood, which may inhibit or even delay the maturation of cane wood.

The carbohydrate content of the canes during dormancy and at the winter dormancy was an indicator directly influenced by both the doses and timing of copper application. It should be noted that the varieties considered being less resistant to frost recorded lower carbohydrate concentrations in the canes compared to the varieties for wine, which are typically more resilient to frost. In all varieties, the influence of the treatment from the experimental plot on the carbohydrate content of the canes was similar, with all experimental plots recording values superior to the control. This indicator is significantly influenced by both the quantity of copper applied and the timing of application.

The highest carbohydrate content in the canes was observed in plot V_6, where the highest copper dose was applied in three rounds. The percentage of fertile buds was also obviously influenced by copper, with all varieties following a similar pattern of experimental plot scaling. Although the recorded minimum temperatures during the three winter seasons did not drop below the resistance limit of the varieties as outlined in specialized literature and practiced in viticulture significant percentages of buds affected by frost were noted in certain experimental plots (Nistor et al., 2023) (Table 3).

Table 3. Treatments influence on grape production and fertile buds percentage in table grape varieties (Mean 2021-2023 growing seasons)

Experimental plot	**Grape production (kg/ha)**	**Sugar (g/l)**	**Sugar (kg/ha)**	**Canes carbohydrates g %**		**Fertile buds percentage**	**Difference to Control (%)**
				15.XI	**1.III**		
Victoria							
V_1 (Control)	16220	150	1703.1	10.6	8.1	61	-
V_2	15750	147	1620.6	10.7	8.2	69	8^{*}
V_3	16953	157	1863.1	10.9	8.5	71	10^{*}
V_4	18230	161	2054.5	12.8	10.6	76	15^{**}
V_5	17570	159	2066.8	12.9	10.8	79	18^{**}
V_6	18810	163	2146.2	13.4	11.4	83	22^{***}
Muscat Hamburg							
V_1 (Control)	12530	165	1447	11.9	8.0	52	-
V_2	11254	160	1260	12.4	8.3	62	10^{*}
V_3	13457	171	1611	12.9	8.4	65	13^{*}
V_4	14838	174	1807	14.8	9.9	71	19^{**}
V_5	14325	172	1724	15.1	10.2	75	23^{***}
V_6	15740	180	1983	16.2	11.1	80	28^{***}

Table 4. Treatments influence on grape production and fertile buds percentage in wine grape varieties (Mean 2021-2023 growing seasons)

Experimental plot	Grape production (kg/ha)	Sugar (g/l)	Sugar (kg/ha)	Canes carbohydrates g %		Fertile buds percentage	Difference to Control (%)
				15.XI	1.III		
Fetească neagră							
V_1 (Control)	9875	194	1341	13.6	11.2	74	-
V_2	9611	189	1271	13.7	11.6	75	1^{NS}
V_3	10276	198	1424	14.0	12.1	79	5^{NS}
V_4	10916	207	1581	15.7	12.5	83	9^{*}
V_5	10518	203	1495	15.9	12.7	84	10^{*}
V_6	11410	212	1693	17.1	13.2	89	15^{**}
Merlot							
V_1 (Control)	10125	186	1318	12.9	9.7	73	-
V_2	9836	178	1226	13.3	10.1	76	3^{NS}
V_3	10823	193	1462	13.7	10.3	78	5^{NS}
V_4	11510	201	1619	15.3	11.8	84	11^{*}
V_5	11798	196	1618	15.6	11.9	86	13^{*}
V_6	12216	206	1761	16.7	12.5	91	18^{**}
Cabernet Sauvignon							
V_1 (Control)	9751	196	1338	14.1	12.2	82	-
V_2	9598	188	1263	14.3	12.4	83	1^{NS}
V_3	9996	199	1382	14.5	12.7	85	3^{NS}
V_4	10217	205	1466	16.2	13.3	90	8^{*}
V_5	10098	202	1428	16.3	13.4	91	9^{*}
V_6	10832	210	1592	17.9	14.3	94	12^{*}

Muscat Hamburg was the most sensitive variety, followed by the Victoria variety. In these cultivars, the method of copper application played a significantly more important role compared to wine varieties known for their resilience. As the amount of copper applied increased, the percentages of fertile buds also increased, with the most effective treatments observed in plots V_6 and V_5 followed by V_4.

The observed differences among experimental treatments, although minor, were also evident when applied to wine grape varieties, demonstrating the importance of effectively managing copper use, even for varieties that typically have high bud fertility percentages in Romania's winter climate conditions (Table 4). Low winter temperatures, grape output levels, growing practices, and grape harvest timing all had an impact on wood maturity and bud fertility. Excessive grape production, particularly in conjunction with less favorable climatic conditions, resulted in delayed grapes technological maturity, with consequent negative impacts on wood maturation. Therefore, the importance of copper management in respect to both dosage and application time becomes very important (Facco et al., 2023); this ensures that plants can withstand winter with sufficient reserves of cane carbohydrates, thereby facilitating optimal wood maturation and corresponding frost resistance in buds.

In addition to the favorable influence on the wood maturation and the buds fertility, copper had an obvious influence on both the quantity and quality of grape production in all varieties. Upon evaluating the experimental plots based on production levels, a consistent pattern emerged across all five varieties. In experimental plot V_6, which received three copper treatments, was registered the highest grape production, followed by plots V_4 and V_5 with two copper treatments, and plots V_3, V_1, and V_2, to which was applied only one copper treatment each. Regarding the time of application, the lowest production was recorded at plot V_2, to which the copper treatment was applied in the first part of vine development, before flowering, which reduced the growth rate for a short time, and subsequent decrease in production. Delayed application of copper after veraison onset (V_1) is not indicated due to the too short interval for the copper to have its effect. Hence, the most optimal timing for copper application was during the berry leg stage, where a single treatment yielded the most favorable results.

When the treatment schemes were applied twice, the optimal time of application was during the berry leg stage and post-veraison. Evaluating grape quality based on sugar content proved that treatment applied to plot V_6, receiving three treatments, exhibited the highest sugar concentration. Too early application of copper (V_2) or too late (V_3) had the effect of reducing the concentration of sugars, compared to the application of copper during berry leg stage.

Table 5. Treatments influence o grape berry accumulation in vitamins for table grape varieties (Mean 2021-2023 growing seasons)

Experimental plot	Vitamin C, total ascorbic acid (mg)	B_1 -Thiamin (mg)	Vitamin B_6 (mg)	Vitamin A. (UI)	Vitamin E - alpha-tocopherol (mg)
			Victoria		
V_1(Control)	11.739	0.046	0.441	27823	2.299
V_2	11.745	0.043	0.441	27821	2.322
V_3	11.747	0.049	0.453	27873	2.312
V_4	11.833	0.056	0.455	27881	2.306
V_5	11.827	0.062	0.462	27914	2.332
V_6	11.871	0.068	0.465	27914	2.324
			Muscat Hamburg		
V_1(Control)	11.710	0.039	0.448	27723	2.323
V_2	11.731	0.041	0.448	27722	2.341
V_3	11.739	0.042	0.456	27779	2.349
V_4	11.812	0.053	0.458	27893	2.352
V_5	11.817	0.061	0.469	27952	2.356
V_6	11.859	0.065	0.472	27957	2.355

International unit (UI); 1 IU=0.3 micrograms; 1 IU=0.0003 mg; 1 IU of vitamin A: the biological equivalent of 0.3 μg retinol or 0.6 μg β-carotene; RAE mcg (retinol activity equivalent).

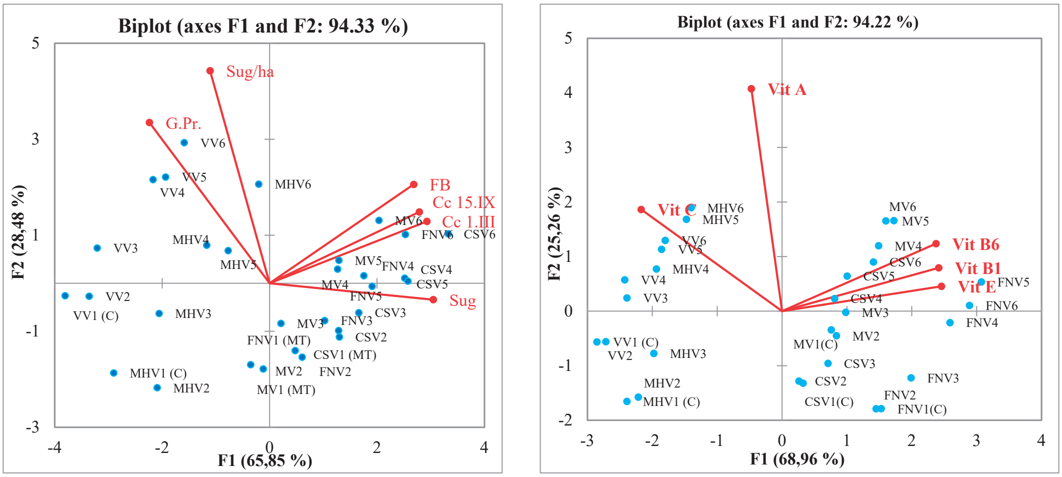
Table 6. Treatments influence o grape berry accumulation in vitamins for wine grape varieties (Mean 2021-2023 growing seasons)

Experimental plot	Vitamin C. total ascorbic acid (mg)	B_1 -Thiamin (mg)	Vitamin B_6 (mg)	Vitamin A. (UI)	Vitamin E - alpha-tocopherol (mg)
			Fetească Neagră		
V_1(Control)	11.289	0.083	0.483	27691	2.412
V_2	11.290	0.082	0.482	27693	2.410
V_3	11.297	0.093	0.489	27723	2.423
V_4	11.321	0.097	0.494	27791	2.475
V_5	11.396	0.107	0.502	27823	2.498
V_6	11.342	0.103	0.498	27804	2.486
			Merlot		
V_1(Control)	11.313	0.079	0.475	27832	2.391
V_2	11.312	0.078	0.476	27821	2.397
V_3	11.356	0.086	0.478	27843	2.399
V_4	11.443	0.091	0.487	27914	2.442
V_5	11.551	0.097	0.493	27923	2.447
V_6	11.543	0.098	0.495	27921	2.449
			Cabernet Sauvignon		
V_1(Control)	11.402	0.083	0.463	27745	2.381
V_2	11.399	0.082	0.462	27751	2.380
V_3	11.421	0.091	0.469	27759	2.389
V_4	11.475	0.096	0.475	27843	2.393
V_5	11.574	0.099	0.478	27852	2.421
V_6	11.576	0.096	0.495	27854	2.420

The vitamin content, the differences among the experimental treatments were relatively low, in all varieties (Tables 5, 6). However, plots receiving copper supplementation, recorded slightly higher values due to improved tolerance to disease and pest and a better photosynthetic efficiency. These parameters were less influenced by the copper doses and application timing compared to the other parameters under consideration.

In similar topic, after two –years of copper treatments application on table grapes, results of Ma et al. (2019), showed vitamin C contents much higher in grape berries than those from the control or Bordeaux mixture treatments.

The PCA diagram was generated to visualize the relationships among the variables and to identify patterns or clusters within the data.



a b

Figure 1. PCA diagram for copper treatments influence on berry quality in table grapes varieties and wine grape varieties (a); PCA diagram for vitamin concentration in table grapes varieties and wine grape varieties (2021-2023 growing seasons) (b) (V_1 – Control; V_2,V_3,V_4,V_5, V_6 – experimental plots; V – Victoria; MH – Muscat Hamburg; FN – Fetească neagră; M – Merlot; CS – Cabernet Sauvignon)

The statistical analysis was performed using Principal Component Analysis (PCA) to assess the influence of copper treatments on berry quality in table grape varieties and wine grape varieties. In the PCA diagram (Figure 1a) it was observed that the first principal component (F1) explained 65.85% of the variability in the dataset, indicating that F1 captured the majority of the variation in berry quality due to copper treatments. Grape production varied across different grape varieties and copper treatments. The Victoria variety exhibited the highest grape production overall, except for the control variant and V_2. However, Merlot, Fetească Neagră, and Cabernet Sauvignon had lower grape production but accumulated higher sugar in berries, particularly after copper application in V_6, V_5, and V_4. Furthermore, after copper application in V_6, V_5, and V_4, Merlot, Fetească Neagră, and Cabernet Sauvignon showed the highest percentage of fertile buds and the highest concentration of canes carbohydrates for both application times (15.XI and 1.III). Muscat Hamburg variety displayed the lowest sugar concentration, fertile buds percentage, and canes carbohydrates concentration overall. These findings suggest that copper treatments have varying effects on grape quality parameters across different grape varieties, with some varieties exhibiting improved berry quality metrics following copper application.

The PCA diagram (Figure 1b) revealed that the first principal component (F1) explained 68.96% of the variability in vitamin accumulation among table grapes. This suggests that F1 captured a significant proportion of the variance in the data related to vitamin accumulation.

Merlot, Cabernet Sauvignon, and Fetească Neagră varieties exhibited the highest accumulation of vitamins B_1, B_6, and E in plots treated with high amounts of copper (V_4, V_5, and V_6). This indicates that these grape varieties may respond positively to copper treatments in terms of vitamin accumulation.

Muscat Hamburg displayed the highest accumulation of vitamin A in plots V_5 and V_6, indicating a potential beneficial effect of copper treatments on this vitamin in this particular grape variety.

Furthermore, Muscat Hamburg also showed significant accumulation of vitamin C in plots V_5 and V_6, while Victoria variety was also rich in vitamin C. This suggests that these grape varieties may have a higher capacity to accumulate vitamin C compared to others under specific copper treatments.

Control experimental plot V_1 and V_2 plot, which received fewer copper treatments, exhibited lower levels of vitamins overall, indicating the importance of copper treatments in enhancing the accumulation of vitamins in grape varieties.

CONCLUSIONS

Copper plays an essential role in grapevine growing, serving both in disease control and various physiological and metabolic functions. The quantity and timing of copper application had significant influence on grape production, its quality, and the overall health of the vines. These factors impact several key aspects, including carbohydrate content of the canes, percentage of wood maturation, fertility of buds, and vine resilience during winter frost conditions. Research findings show a proportional increase in carbohydrate content of one-year-old canes, percentage of wood maturation, and bud fertility with higher levels of copper administered to the vines.

The moment of copper application is also important, to ensure that the treatment is the expected one. Early application before flowering or late application after veraison onset proved to be less effective compared to application at the berry leg stage. Among the monitored parameters, the most effective copper dose of 2.28 kg/hectare applied in three stages (before flowering, at the berry leg stage, and at veraison onset) yielded optimal results across all varieties. The differences between the experimental plots were much more evident in the Victoria and Muscat Hamburg varieties, which are more sensitive to winter frosts, and generally have a poorer annual wood maturation, particularly under conditions of high grape production or late harvesting.

Overall, the PCA analysis provided insights into the relationship between copper treatments and vitamin accumulation in different grape varieties, highlighting potential variations in response to copper treatments among grape varieties and the importance of copper in enhancing vitamin content in grapes.

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CABERNET SAUVIGNON VARIETY BEHAVIOURAL ANALYSIS IN ROMANIAN VINEYARDS AREAS UNDER THE CLIMATE CHANGE INFLUENCES

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Abstract

In the European Union, the change of wine grape variety for economic reasons in vineyards established under reconversion/ restructuring programs can be done after at least 10 years of vineyard exploitation. In the context of climate changes modifications in the microclimates of vine-growing areas are increasingly observed. Extreme climatic phenomena, with an impact on the harvest or the lifespan of grapevines, are becoming more and more aggressive, with quantifiable effects in the wine industry as well. We have carried out an analysis, on various aspects, of the behavior of the 'Cabernet Sauvignon' variety in the vine-growing areas of Romania. For comparison, the years 2018 and 2022 were analyzed, in the terms of cultivated areas and produced and marketed wines. Our analysis suggests that in the near future, in Romania, due to climate change, there will be vineyard areas where this variety will either no longer be cultivated, or it will be used only for the production of rosé wines or bulk red wines and oenological modelling according to consumption trends is neither economical nor justified.

***Key words**: climatic conditions, grapevines, production, trends, wines.*

INTRODUCTION

Wine quality and yield are strongly influenced by climatic conditions and depend on complex interactions between temperature, water availability, plant material and viticultural techniques (Santos et al., 2020). In the case of vineyards in established wine-growing regions, growers have optimized yield and quality by choosing plant material and viticultural techniques according to local climatic conditions, but as the climate changes, these will need to be adjusted (Leeuwen et al., 2019). This is one of the reasons for our study, to present on the basis of sound numerical arguments, the inefficiency for the future of growing international varieties such as 'Cabernet Sauvignon' in Romanian vineyards.

The wine sector needs to take significant adaptation measures in the coming decades, given the undeniable evidence of global warming and extreme drought (Fraga, 2020).

Only a small number of studies have developed multi-scalar and multi-lever approaches to quantify the feasibility and effectiveness of climate change adaptation. In addition, it was found that climate data sources were not explicitly presented in a systematic way that climate uncertainty was hardly taken into account (Naulleau et al., 2021).

Temperature is an important environmental factor that influences almost all aspects of plant growth and development. Grapevines (*Vitis* spp.) are quite sensitive to temperature extremes. In the current century, temperatures are expected to rise continuously, with a negative impact on viticulture. These consequences range from short-term effects on wine quality to long-term problems such as the suitability of certain varieties and the sustainability of viticulture in traditional wine-growing regions (Xenophon et al., 2020). Extreme temperatures affect grapevine physiology, as well as grape quality and production (Bois et al., 2014). ‘Cabernet Sauvignon’ is budding in the last decade of April, once every 20 years a week later or 2-3 days earlier (MADR, 2019). Some studies indicate a good suitability of grape varieties tested for cultivation in different wine growing areas from Romania in order to obtain a high quality wine, but in some years can only get current table wines due to changes in climatic conditions (Bora et al., 2014).

In this context, the paper presents an analysis of the evolution of ‘Cabernet Sauvignon’ production in the main wine-regions of Romania, with correspondence on administrative regions, in order to evidence the evolution of this grapevine variety among 2018-2022, and a projection for the future.

MATERIALS AND METHODS

In order to characterize the evolution of ‘Cabernet Sauvignon’ production, between 2018-2022, some indicators were used.

Table 1. Romanian Weather Stations and their elevation

Wine Regions and Vineyard Center	Meteo Station	County	Elevation (m)
North Lechința	Batoș	Bistrița-Năsăud	409.0
North-West Gerăușa	Satu-Mare	Satu-Mare	126.0
West Diosig	Săcueni	Bihor	130.0
West Șiria	Șiria	Arad	384.0
South-West Recaș	Lugoj	Timiș	124.9
South-West Doclin	Reșița	Caraș-Severin	229.5
South Craiova	Calafat	Dolj	55.0
South Drăgășani	Drăgășani	Olt	159.8
South-East Ostrov	Adamclisi	Constanța	112.0
South-East Constanța	Constanța	Constanța	36.6
East Vaslui	Vaslui	Vaslui	118.7
East Iași	Iași	Iași	61.6
Center Alba Iulia	Alba Iulia	Alba	249.3
Center Valea Călugărească	Ploiești	Prahova	160.5

The wine color and sugar content, the authorized surfaces, total yield in the main Romanian wine-regions, wine production in PDO, IGP and varietals, certified wines, evolution by alcohol volume in wines by colour and sugar content were collected from National Research and Development Institute for Viticulture and Winemaking. Weather data were collected from the National Weather Administration stations for each wine region (Table 1). The period analysed in this study was 2018-2022. Climatic parameters/indices used in the evaluation and viticultural zoning are based on the assessment of the eco-pedoclimatic conditions in the representative wine-growing regions of Romania by the National Research and Development Institute for Viticulture and Winemaking (MADR, 2019; Order no. 594/2004).

The study was based on measurements regarding UV index, precipitations and maximum average temperature during growing season and minimum average temperatures during dormant season, data recorded by meteorological stations.

The data about ‘Cabernet Sauvignon’ parameters, collected from the National Office of Vine and Wine Products have been statistically processed and interpreted, building the trend line and setting up the forecast for the next years.

RESULTS AND DISCUSSIONS

In the last years, climatic conditions have had a significant impact on ‘Cabernet Sauvignon’ production. Extreme weather events such as heatwaves, droughts, and wildfires have affected grape yields and quality in some regions, leading to fluctuations in supply and pricing. Winemakers are increasingly focusing on adapting to these changing conditions through vineyard management techniques and technology.

The areas planted with the 'Cabernet Sauvignon' variety, producing grapes for wines with a Controlled Designation of Origin, are constantly decreasing, by the hundreds, while those producing grapes for wines with a Geographical Indication or varietal wines are increasing by the tens.

Areas that disappear from one year to the next either become wine grape producers for rose or red wines without a geographical indication and without a variety denomination, or are replaced by varietal reconversion. Around 200 hectares have been replanted with varieties other than 'Cabernet Sauvignon' through the vineyard reconversion/restructuring plans (Figure 1).

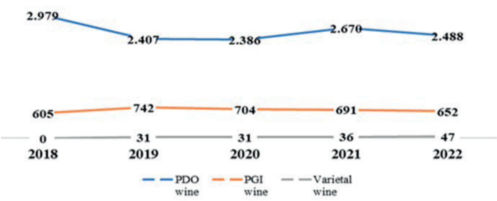


Figure 1. Authorized ‘Cabernet Sauvignon’ surfaces (ha)

The significant increase in the production of 'Cabernet Sauvignon' wine grapes in the South-Eastern and Central areas reveals not only the viticultural potential of these areas, but also microclimatic changes that can enhance the typical qualities of this variety (Figure 2).

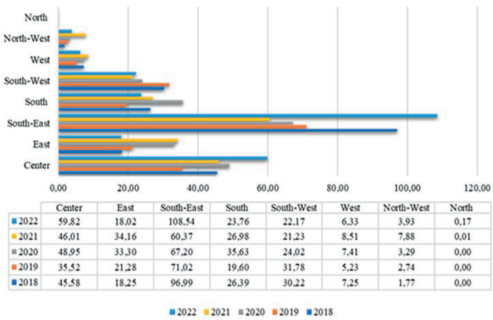


Figure 2. Romanian ‘Cabernet Sauvignon’ wine grape production in Romanian administrative regions (k quintals)

The same downward trend is observed in the quantities of wine produced and declared. Production of PDO wine is decreasing, GI wine production is slightly increasing, and varietal designation wine production is fluctuating, with a general upward trend (in three of the five years studied) (Figure 3). This fluctuation is showed also by the low value of R^2=0.1604 for the PDO ‘Cabernet Sauvignon’ wines during studied period. Regarding PGI wines and Varietal wines, the values of R^2 are a little bit higher than PDO wines, but the values showed also a fluctuation among the five years of studies (Figure 3).

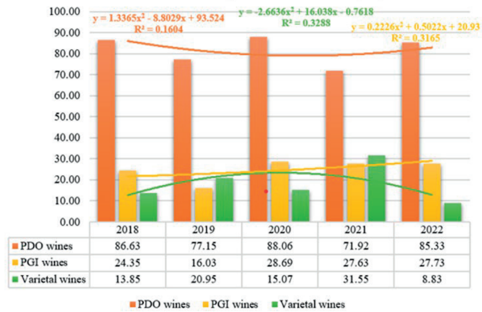


Figure 3. Romanian declared ‘Cabernet Sauvignon’ wine production (k hl)

The extreme increase in the wine-growing areas of Central Romania and the Central-Sub-Carpathian Mountains in the category of wine without a variety denomination, denomination of origin or geographical indication is the result of the choice of production direction oriented towards quantity, to the detriment of quality, obtaining simple, less elaborate wines, implicitly less expensive and easier to sell on the bulk wine market (Figure 4).

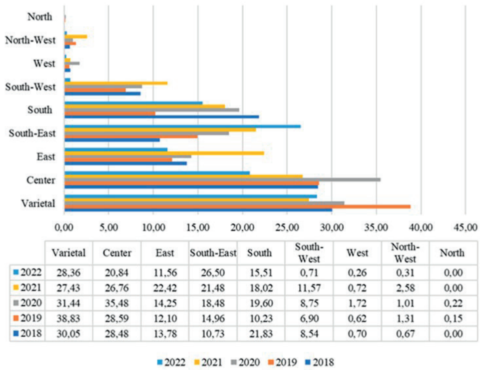


Figure 4. Evaluated ‘Cabernet Sauvignon’ wines for certification (k hl)

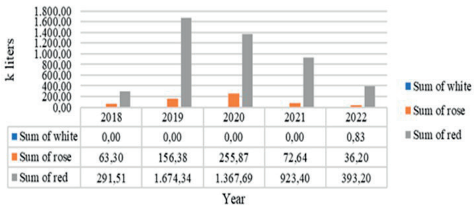
In recent years, regions formerly devoted to white wines have begun to produce interesting red wines, with 'Cabernet Sauvignon' being planted because of both its universality and its plasticity in making different types of wine. Its migration to areas such as the East and North-West shows a growing trend for Romanian red wines in these viticultural areas.

Between 10.36 and 10.99% alcohol by volume, ‘Cabernet Sauvignon’ was only winemaked in 2020 and 2021. Rose and red wines were produced in 2020 and only red wines in 2021 (Figure 5).

	2018	2019	2020	2021	2022
Sum of white	0,00	0,00	0,00	0,00	0,00
Sum of rose	0,00	0,00	0,80	17,00	0,00
Sum of red	0,00	0,00	4,25	0,00	3,98

Figure 5. Wines of Cabernet Sauvignon (k liters), with alcohol content between 10.36-10.99% vol. alc.

Between 11.00 and 11.99% alcohol by volume, 'Cabernet Sauvignon' produced low quantities of rose wines compared to red wines. The years 2019, 2020 and 2021 gave the major quantities of red wines in this alcohol concentration range, with a peak in 2019 of 1,674.34 thousand liters (Figure 6).



	2018	2019	2020	2021	2022
Sum of white	0,00	0,00	0,00	0,00	0,83
Sum of rose	63,30	156,38	255,87	72,64	36,20
Sum of red	291,51	1.674,34	1.367,69	923,40	393,20

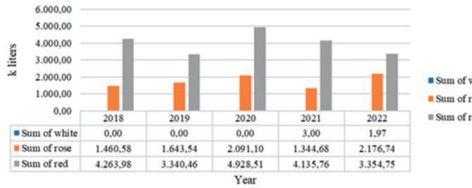
Figure 6. Wines of Cabernet Sauvignon (k liters), with alcohol content between 11.00-11.99% vol. alc.

Between 12.00 and 12.99% alcohol by volume, 'Cabernet Sauvignon' produced the highest quantity of white wine in the period studied, in 2020. Rose wines were obtained at almost the same level as red wines. However, the most red wines were produced from 'Cabernet Sauvignon' in 2021 (Figure 7).

	2018	2019	2020	2021	2022
Sum of white	0,00	5,00	16,50	6,80	5,00
Sum of rose	393,48	773,64	760,47	828,19	718,84
Sum of red	582,66	935,09	1.204,72	2.363,96	1.095,22

Figure 7. Wines Cabernet Sauvignon (k liters), with alcohol content between 12.00-12.99% vol. alc.

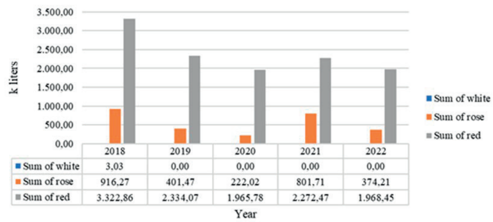
Between 13.00 and 13.99% alcohol by volume, the level of rose wine production stabilises, with no significant differences in quantity from one year to the next. White wines are still made sporadically, in very small quantities. Red wines are the most present, in almost constant quantities in the years studied, over 3,300 thousand litres, compared with the maximum quantity in the 12.00 - 12.99 % vol. range of 2,300 thousand litres (Figure 8).



	2018	2019	2020	2021	2022
Sum of white	0,00	0,00	0,00	3,00	1,97
Sum of rose	1.460,58	1.643,54	2.091,10	1.344,68	2.176,74
Sum of red	4.263,98	3.340,46	4.928,51	4.135,76	3.354,75

Figure 8. Wines of Cabernet Savignon (k liters), with alcohol content between 13.00 – 13.99 % vol. alc.

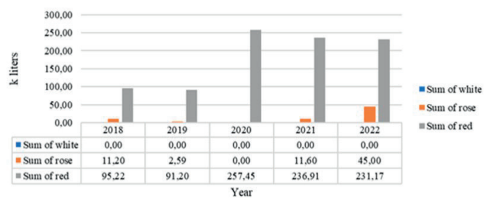
Between 14.00 and 14.99% alcohol by volume, fewer and fewer rose wines are being made. Red wines are the most popular, and their high alcohol content makes them suitable for ageing and long cellaring. The differences are very significant between the quantities of rose and red wine, and no white wines have been produced at all (Figure 9).



	2018	2019	2020	2021	2022
Sum of white	3,03	0,00	0,00	0,00	0,00
Sum of rose	916,27	401,47	222,02	801,71	374,21
Sum of red	3.322,86	2.334,07	1.965,78	2.272,47	1.968,45

Figure 9. Wines of Cabernet Sauvignon (k liters), with alcohol content between 14.00 – 14.99 % vol. alc.

Between 15.00 and 15.99% alcohol by volume, rose wines are sporadic, white wines were not produced at all. In 2020 only red wines made from 'Cabernet Sauvignon' were produced. In the years 2020, 2021 and 2022 there is a quantitative constancy in the production of wine with this high alcohol (Figure 10).



	2018	2019	2020	2021	2022
Sum of white	0,00	0,00	0,00	0,00	0,00
Sum of rose	11,20	2,59	0,00	11,60	45,00
Sum of red	95,22	91,20	257,45	236,91	231,17

Figure 10. Wines of Cabernet Sauvignon (k liters), with alcohol content between 15.00 – 15.99 % vol. alc.

Between 16.00 and 16.99% alcohol by volume, no white or rosé wines were produced. In 2022, no ‘Cabernet Sauvignon’ mono-cepage wines were produced at this alcoholic strength. In 2018 and 2021 almost similar quantities of red wines were produced, around 80 thousand litres, much higher than in 2019 and 2020, when the figures were in the 10-15 thousand litres range (Figure 11).

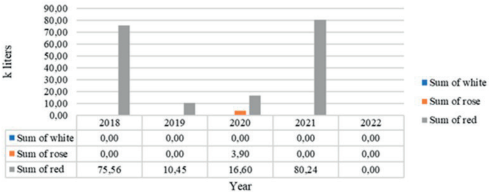


Figure 11. Wines of Cabernet Sauvignon (k liters), with alcohol content between 16.00-16.99% vol. alc.

In wine-growing Romania, ‘Cabernet Sauvignon’ accumulates between 10.39 and 16.55% alcohol by volume. Rose wines produced from this variety are constantly in the 3,000-thousand-liter range from one year to the next. Red wines, at around 9,000 thousand liters in four of the years studied, and decrease significantly in 2022.

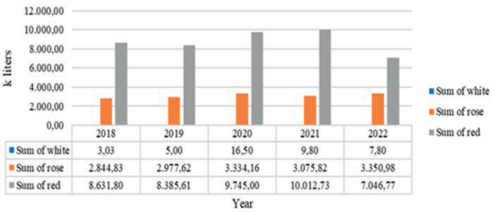


Figure 12. Wines of Cabernet Sauvignon (k liters), with alcohol content between 10.39-16.99% vol. alc.

It is evident that the share of wines of the variety studied is in the dry wine area, with an accent in red wines. However, from a quantitative point of view, rose wines are consistently in the middle of red wines, in the semi-dry range they are even, and in the semi-sweet range, they are almost balanced (Figure 13).

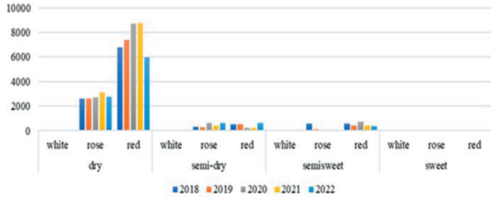


Figure 13. Classification by the colour and sugar content in Cabernet Sauvignon Romanian wines, between 2018-2022 (k liters)

This 2022 is the year in which most coupage and blending with ‘Cabernet Sauvignon’ took place, at the expense of mono-cepage wine (Figure 12).

The highest values of the UV Index were recorded in 2020 and 2022 in the Southern, South-Eastern and Sub-Carpathian Hills Viticultural Regions, with values between 15 and 20. In the South-West, North and Transylvanian Plateau the values remained relatively constant over the period studied, with a minimum of 11 (Lechinta) and a maximum of 16 (Alba Iulia). It is possible that the rapid and enhanced grape pigmentation due to flavonols observed in 2020 and 2022 in vineyards around Craiova or in Dobrogea is a consequence of systematic exposure to extreme ultraviolet radiation on many consecutive days during the veraison period of this wine grape variety (Figure 14).

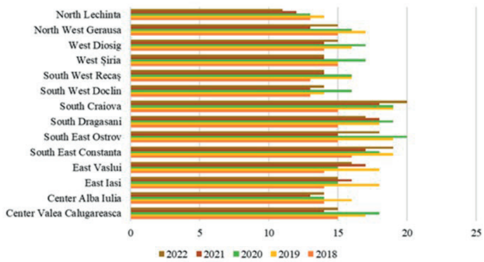


Figure 14. UV Index from different meterological stations, during growing season, between 2018-2022

The most arid regions proved to be the South, South-East and East and in particular the Centre around the Valea Călugărească vineyard.

The precipitation regime during growing season, in 2020 was directly proportional to the hours of sunshine in the areas of the Danube Terraces and Oltenia Sands (Figure 15).

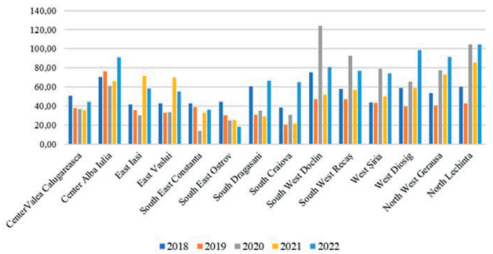


Figure 15. Precipitations from different meteorological stations, during July-September, between 2018-2022

Average minimum temperatures in 2018, during dormant period, were recorded in the North and East (-5ºC), and the highest minimum temperatures were recorded in the West and Southeast (1ºC) (Figure 16).

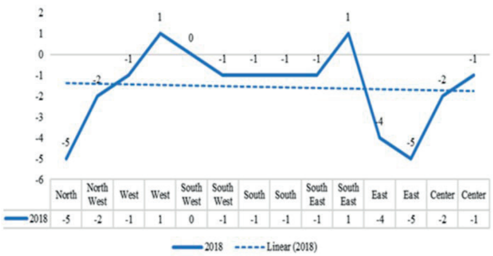


Figure 16. Average minimum temperatures (°C) during dormant period, in 2018

In 2019, the North and East again had the lowest average minimum temperatures, and the warmest cold season was record in the South. Most of the vineyard where 'Cabernet Sauvignon' plantings are located had average lows around (-3ºC) (Figure 17).

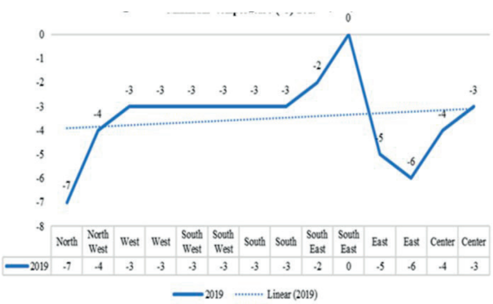


Figure 17. Average minimum temperatures (°C) during dormant period, in 2019

The year 2020 was more contrast, with negative peaks in areas where they were not expected: South-West, Centre (Figure 18).

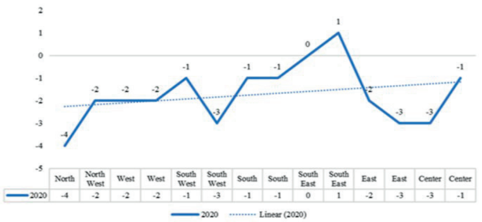


Figure 18. Average minimum temperatures (°C) during dormant period, in 2020

The Southeast becomes the warmest area in the cold season in 2021, with average lows of 2ºC, during which time extremes in the North and East are 1ºC warmer than in previous years (Figure 19).

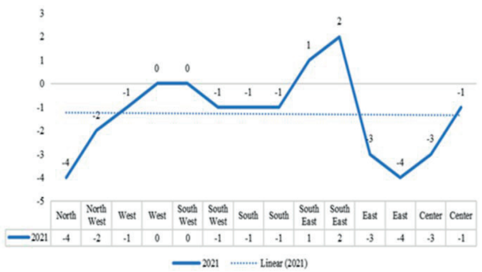


Figure 19. Average minimum temperatures (°C) during dormant period, in 2021

Diametrically opposite to 2018, the year 2022 shows a linear trend of increasing average minimum temperatures from North to South and from East Region to Centre, the lowest being (-7ºC) and the highest 1ºC. In 3 out of 5 years the trend is for increasing average minimum temperatures, with 2020 and 2022 even having steep increases from North to South (Figure 20).

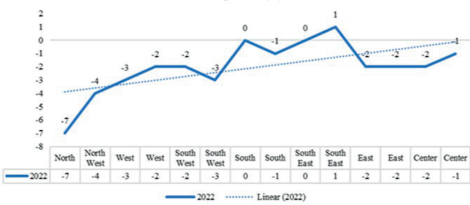


Figure 20. Average minimum temperatures (°C) during dormant period, in 2022

The average maximum temperatures for 2018 fluctuated from 27ºC in the North to 31ºC in the Southeast. Most values were around 29ºC (in 4 viticultural centres), with the same frequency as values of 30ºC, also in 4 viticultural centres (Figure 21).

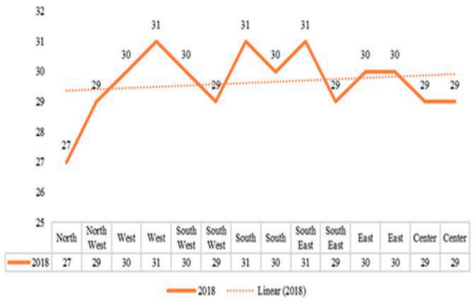


Figure 21. Average maximum temperatures (°C) during growing period, in 2018

In 2019, the average maximum temperatures during growing period (June - September) had the same linear trend as the minimum, with the greatest amplitude in the East, where the average minimum was (-6ºC) and the average maximum 30ºC (Figure 22).

Several vaues of 31ºC and even 32ºC are already observed, which justifies us to consider 2019 as a year with a warm ripening season for ‘Cabernet Sauvignon’ grapes for West and South (Figure 22).

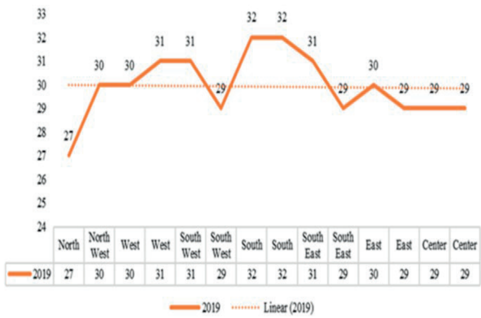


Figure 22. Average maximum temperatures (°C) during growing period, in 2019

The year 2020 was homogeneous, with 29ºC in most wine-growing centres. The East was on trend, but the South-West matched the North at an average high of 26ºC (Figure 23).

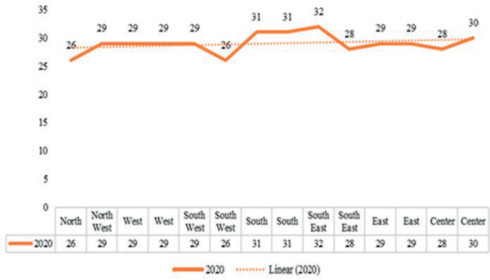


Figure 23. Average maximum temperatures (°C) during growing period, in 2020

In 2021, in all the wine-growing regions of Romania, average maximum temperatures were around 31ºC, as it is shown in Figure 24. No extremes, no average temperatures below 26ºC. The year 2022 was by far the warmest year. A year of extremes, 2022 had average maximum temperatures of 31-33ºC in almost all wine-growing regions. The precipitation-rich areas of the North with 28ºC, the South-West and the Transylvanian Plateau with 30ºC lowered the total annual average by an insignificant amount (Figure 25).

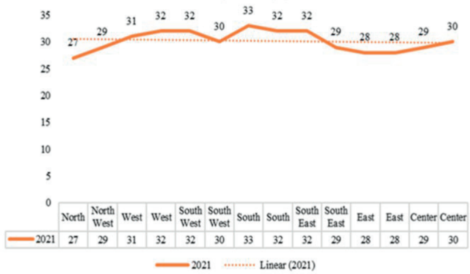


Figure 24. Average maximum temperatures (°C) during growing period, in 2021

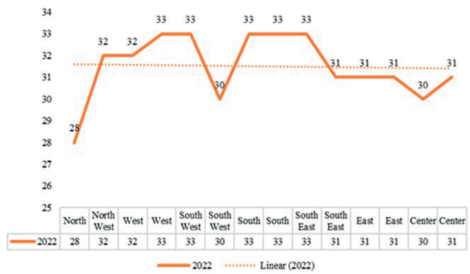


Figure 25. Average maximum temperatures (°C) during growing period, in 2022

All these climatic influences contribute to the main final product of the wine grapes, imprinting, in addition to its genetic inheritance, a large part of the soil characteristics, the benefits of modern technologies and the oenologist's skill. The trend of ‘Cabernet Sauvignon’ in Romania has been quite positive in recent years, reflecting both increased production and growing recognition on the international stage. Romanian winemakers have expanded the cultivation of ‘Cabernet Sauvignon’, especially in regions like Dealu Mare, Drăgășani, and Recaș. These areas benefit from favorable climatic conditions and diverse soil types, which contribute to the unique profiles of the wines produced there. Overall, the trend for ‘Cabernet Sauvignon’ in Romania is one of growth and increasing prestige, supported by a combination of quality production, favorable climatic conditions, and successful international marketing. This has led to a broader appreciation and higher demand for Romanian ‘Cabernet Sauvignon’ both locally and internationally.

CONCLUSIONS

‘Cabernet Sauvignon’ is grown in all wine-growing regions of Romania. In recent decades, the market demand for this wine has led to the selection of areas where typically, premium wines are obtained. Most producers have found deficiencies in the unbalanced accumulation of sugars compared to phenolic compounds and acidity, so more and more ‘Cabernet Sauvignon’ rose wine is being produced, and overripe grapes, with very high sugar content, are used in mixtures with other varieties, or the resulting wines enter blends. Single-vineyard ‘Cabernet Sauvignon’ wines are increasingly rare, dry, medium alcohol, high in anthocyanins and full-bodied. Experiments with ‘Cabernet Sauvignon’ “blanc de noir” wines are expanding in response of consumer demand, who increasingly appreciate thin, fine, low-alcohol, mostly white wines. Further planting of this variety in Romania must take into account all the parameters necessary to obtain typical wines that correctly express its genetic potential and the imprint of the area.

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MONITORING OF A VINEYARD BY FLYING OVER WITH A UAV

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Abstract

With the help of constant visual control and spectral during the period 2021-2022, the development of a vineyard was followed, and the dynamics of climatic factors were followed. Monitoring of climatic parameters (air temperature, soil temperature, atmospheric humidity, leaf humidity, sunshine, wind direction, evapotranspiration, rainfall) was carried out throughout the growing season of the vineyard. As a result of the observations and reports, some conclusions were drawn related to the effectiveness of drone monitoring. The BNDVI indices, which range from 0 to 1, were measured, and soil and leaf moisture parameters were recorded throughout the growing season. During the drone survey, the dynamics of the vegetation index of the vine was tracked in the established field experiment. The results of the two-year research on yield and quality of grapes fully correspond and are linked to the influence of climatic factors during the growing season and the dynamics and course of phenophases. A difference was reported between the two years, both in terms of climate and grape and wine quality. Considering the non-uniform site and sloping terrain of the vineyard, it was concluded that remote monitoring data is an excellent tool for control, tracking and forecasting, but when considering a specific local site, professional visual inspection and the application of additional analyzes and performance of measures related to cultivation technology.

Key words: *grapes, monitoring, drone, vegetation index, yield, quality.*

INTRODUCTION

Drones are still considered a new tool in agriculture, but their proven utility for assessing plant health in the field and their potential for return on investment make them an attractive addition to the precision toolkit. Interest in the use of drones has grown significantly in recent years, Macrina et al. (2020). Their application in areas such as photography, construction, monitoring are only part of the possibilities of use. Unmanned aerial vehicles (UAVs) can perform planned missions without human intervention, reports Azar et al. (2021). Automated drone survey aims to optimize the detection capabilities and the extraction of object shapes and thus increase the autonomy of surveyed fields (Orengo et al., 2021). This high-tech technology allows farmers to collect, store, combine and analyze the data layers performing precise management of fertilization and irrigation An algorithm was developed to detect the position and number of plants in vineyards using RGB drone images with a plant detection accuracy of 87%, reports Bruscolini et al. (2021) This monitoring tool enables winegrowers to keep their vines under control and improve plant health with targeted actions such as irrigation scheduling or specific treatment with plant protection agents and foliar fertilizers.

Chung et al. (2020) systematize the state-of-the-art approaches for drone application optimization, including construction, agriculture, transportation, security, disaster management, etc. They also present developed mathematical models, methods for solving problems. Drones are highly resource-constrained devices and therefore it is not possible to deploy heavy security algorithms on board, explains Hassija et al. (2021). A surface moisture mapping index (SMMI) model based on a modified normalized difference water index and a topographic wetness index is proposed by a team of scientists, reported Tang et al. (2020). The model combines the emission properties of reflectance from moisture-bearing surfaces of an agricultural field and the slope gradient and micro-topographic positions in the field.

In precision viticulture, the characterization of spatial variability in the field is a crucial step for efficient use of natural resources by reducing environmental impact commented Pagliai et al. (2022). Technologies such as unmanned aerial vehicles (UAVs), mobile laser scanners (MLS), multispectral sensors, mobile applications (MA) and structure-of-motion (SfM) techniques enable the characterization of this variability with little effort. The authors report UAV flight testing, MLS scanning over the vineyard and MA acquisition over 48 georeferenced vines. The obtained results give them reason to state that the analysed instruments are able to correctly distinguish zones with different characteristics.

Since 2010, farmers have been using remote sensing data from unmanned aerial vehicles that have high spatiotemporal resolution to determine the condition of their crops and how their fields are changing, Di Gennaro et al. (2023). According to the authors, imaging sensors such as multispectral and RGB cameras are the most widely used tool in vineyards to characterize vegetative crown development and detect the presence of missing vines along rows. A combination of photogrammetric techniques and spatial analysis tools underpins a methodology for identifying missing vines that works with 92.72% accuracy.

NDVI is widely used to estimate leaf chlorophyll content and photosynthetic activity of plants using aerial images obtained from unmanned aerial vehicles (UAVs) or from satellites (Matese et al., 2015; Campos et al., 2021; 2023).

UAVs have surveillance advantages that are characterized by high flexibility in flight planning, low operating costs, and high spatial ground resolution of captured images at different heights and with different resolutions. One of the disadvantages is that they are difficult to apply in the presence of clouds. NDVI can only refer to the crown of the vine (its leaf mass), and when NDVI is reported with satellite images it represents an average value between the NDVI for the vine and the inter-row distances covered by weeds or soil in each pixel (Khaliq et al., 2019).

These visualization inaccuracies are the basis for the results being indicative and indicative of trends. UAV monitoring is necessary to specify the terrain with its strong and weak points, but not to predict the condition of the vines, as well as the expected yield from them. Remote surveys have been done in perennials for NDVI and the evaluation of leaf vegetative mass of the vine (Rey-Caram`es et al., 2015; Caruso et al., 2017) yield and fruit quality (Lamb et al., 2004; Matese et al., 2021).

Despite the widespread use of NDVI in precision viticulture, there are no studies aimed at distinguishing the specific impact of leaf area and leaf chlorophyll concentration on the resulting NDVI vegetation index. Such information can be crucial when there is a water or nutrient deficit (Caruso et al., 2023)

Data from drone coverage of perennial crops is an addition that can give guidance on the overall condition of the plot, take into account problems related to waterlogging or drying of the soil surface (overall or locally), unfavorable soil conditions in local areas, as well as areas with lack of/or nutritional macro or micro elements from a given cultivated area.

The purpose of the study is to study the terrain with remote monitoring to study the advantages and disadvantages of the vineyard, considering the non-uniform plot and sloping terrain.

MATERIALS AND METHODS

During the period 2021-2022, monitoring of the permanent plantations was carried out by flying over with unmanned aerial vehicles (UAVs). Satellite imagery is for the entire vegetation and UAV imagery is in the "seed pouring" phase.

In the monitored vineyard, climate indicators (air temperature, soil temperature, atmospheric humidity, leaf humidity, sunshine, wind direction, evapotranspiration, rainfall) were monitored throughout the growing season, as well as two-year irrigations with drone to track the dynamics of the vine vegetation index. As a result of the observations and reports, some conclusions were drawn related to the effectiveness of drone monitoring. The BNDVI indices, which range from 0 to 1, were measured, as were the limits of soil and leaf moisture throughout the growing season.

In the vineyard there are marked vines of the Syrah variety, to which normalization of the bunches and defoliation in the area around the bunches have been applied. The observed vines of each variant have a load of 28 winter eyes.

There are 30 pieces of each variant formed. vines: lime 1. Control - without green prunings; var. 2. Bunch rationing - 15 bunches left per vine in the "pea" phase; var. 3. Norming and

defoliation - 15 bunches per vine are left in the "pea" phase and defoliation in the area of bunches in the "shattering" phase.
The yield by variants was reported and the mass values of one bunch were averaged by variants. The results of the experiment were processed by means of one-factor analysis of variance. Comparative evaluation was performed using the Duncan Test to assess differences at the 0.05 level of statistical significance.
Of the technological indicators for grapes, the following were measured:
- grape sugars, % - Dujardin hydrometer;
- titratable acids (TC), g/l - titration with 0.1n NaOH under bromothymol blue indicator.

RESULTS AND DISCUSSIONS

The study was conducted in the region of Stara Zagora, Bulgaria with geographical coordinates are 42°33′ North latitude and 25°53′ East GMT (GPS).
The region refers to the European-continental climatic region, Transitional-continental subregion, which includes the region of Eastern Central Bulgaria covering the Thracian lowland.
The region is characterized by a continental warm temperate climate with an average annual rainfall of 565.1 mm and an average annual temperature of 15.1°C.

Figure 1. The top image area "a" shows the area surveyed by the UAV, and area "b" shows the monitored area where the vineyard is located

The years of the survey are characterized as very warm. Vegetation in 2021 starts in the middle of March in 2021, and in 2022 at the end of March (Figure 1). The months of July and August coincide with the "pea" phase and the beginning of the ripening of the grapes, which reach their final size and begin to ripen. The need for rainfall and optimum temperature during this period are crucial for the quality and quantity of the yield obtained. During this period, the average daily temperature averaged over ten days reaches almost 30^0C. The temperature of the soil also increases, which makes it difficult for the root system to function when there is a lack of soil moisture. The amounts of precipitation during this period of the growing season are minimal. These temperature conditions combined with the minimum amounts of precipitation in July, August and September and the low atmospheric humidity make it difficult for the physiological processes in plants to proceed normally, as a result of which partial leaf fall is observed. The loss of part of the leaf mass delayed the technological maturity of the grapes.
In 2022, the vegetation started during the last ten days of March (Figure 1) and the values of the average day-night temperature in the following months were 3-4°C lower than the previous year, which also affected the soil temperature. The air temperature and the

amount of precipitation is more favorable in 2022 (Figures 2-4).

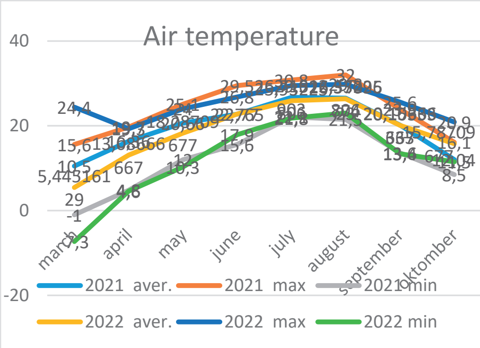


Figure 2. Air temperature (min., max., average)

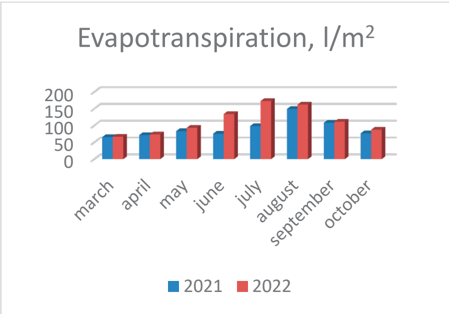


Figure 3. Evapotranspiration, l/m^2

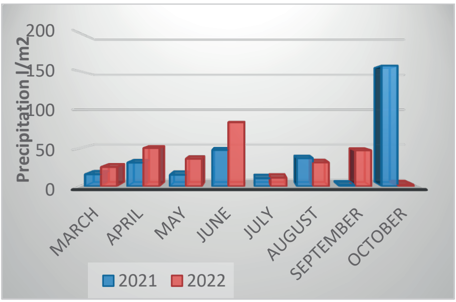
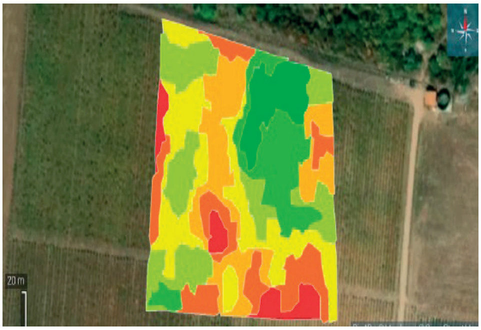


Figure 4. Precipitation, l/m^2

Through remote monitoring, a rapid and non-destructive analysis of the state of the vineyard was carried out. The series of photographic images taken during the two years of the study allow models to be made for yield prediction, water stress management, phenological development, etc. Figure 4 shows the distribution of moisture in the surface soil horizon at a specific moment. The monitoring allows to trace the available moisture in the soil at any moment of the vegetation. In this particular case, the observation shows the moisture in July 2022, the period of active vegetation when the vines need available moisture for their growth and development. From the image it can be seen that a large part of the vineyard is experiencing a water deficit. With a lack of moisture, the assimilation of water and the minerals dissolved in it is difficult.

The uneven distribution of moisture in the monitored massif also leads to differences in the development and productivity of individual plants relative to the area of the field. As a result of the uneven moistening of the soil horizon and the unfavorable vegetation in 2021/2022, uneven ripening of the bunches was observed and differences and fluctuations in the mass of the bunches after reaching technological maturity were found.



a)

Prescription table

Zone	Average value	Area [ha]	Rate [units/ha]	Amount [units]
	0.25	951.92	0.00	0.00
	0.29	0.25	0.00	0.00
	0.32	0.29	0.00	0.00
	0.35	0.36	0.00	0.00
	0.38	0.32	0.00	0.00
	0.42	0.23	0.00	0.00
	0.47	0.13	0.00	0.00
Total:		1.68		0.00

b)

Figure 5. Distribution of moisture in the surface soil layer in July 2022

Plants have been found to capture visible light for the process of photosynthesis. On the other hand, near-infrared (NIR) photons do not have enough energy for photosynthesis, but they carry a lot of heat that is reflected by plants and can be captured by cameras. This reflection mechanism breaks down when the leaf dies. Near infrared sensors take advantage of this property by monitoring the difference between NIR reflectance and visible light reflectance. This calculation is known as the normalized difference vegetation index (NDVI).

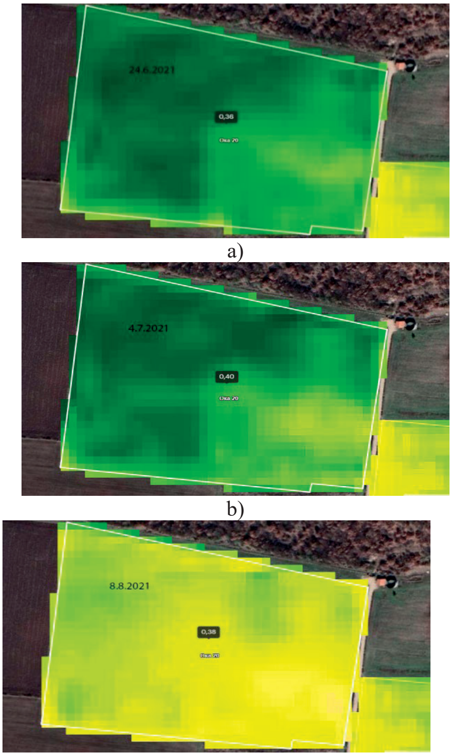


Figure 6. Development of the vegetation index NDVI in 2021

High NDVI means high plant density and low NDVI indicates problem areas in the field. Through NDVI, areas of the field where crops are growing better can be clearly distinguished from those where they are not, allowing zones to be created where the correct amount of fertilizer can be applied to each location in the field.
The NDVI index is related to many plant properties. By tracking changes in the arrays, various changes that affect crop productivity can be identified. Areas with permanent water deficit or waterlogging can be located, the health status of plants can be monitored.
By means of the vegetation index NDVI, the health status of the plants, the phenological development and the biomass of the plants are determined. It is standardized and has values between -1 (absence of any vegetation) and +1 (abundant vegetation). Differences in illumination and the influence of land suitability can be compensated for:

$$NDVI = (NIR - Red) / (NIR + Red)$$

In Figure 6 traces the development of the NDVI index in 2021. The series of photographs show an increase in the index from 0.38 measured in June to 0.40 measured in early July. The rise in values coincides with the period of intense vine growth. While in August, a decrease in the index was already recorded, which is due to the decrease in the photosynthetic activity of the green parts of the vine and an increase in transpiration, which is further stimulated by the lack of moisture for the roots of the vine.
Meivel et al. (2021) are of the opinion that real-time monitoring associated with NIR imaging enables the tracking of plants and soil conditions, as well as the vegetation index responsible for vegetation growth, etc.
In the second year, remote aerial monitoring was carried out using an unmanned aerial system (drone) equipped with a specialized camera allowing the generation of the vegetation index NDVI (measuring the possibilities of absorption and reflection of incoming light by vegetation, its photosynthetic capacity and biomass concentration). The camera's multispectral and solar sensors capture the amount of light that is absorbed and reflected by the vines. The UAVs used have a high spatio-temporal resolution. To carry out accurate surveys and evaluations of vineyards, according to Ferro et al. (2023) it is important to choose the appropriate sensor or platform because the algorithms used in post-processing depend on the type of data collected.
In the second year, during the observations, weeding in the inter-rows was not recorded, while in the first year, when measuring and photographing the vine array, the entire array was observed as a whole.
Each photo taken by the hyperspectral camera is accompanied by a legend and index values are indicated (Figure 7). The NDVI Vegetation Index - can also reveal the presence of weeds, pests, water shortages and other problems, giving the grower the information needed to identify and quantify the problems, and then how best to deal with them.

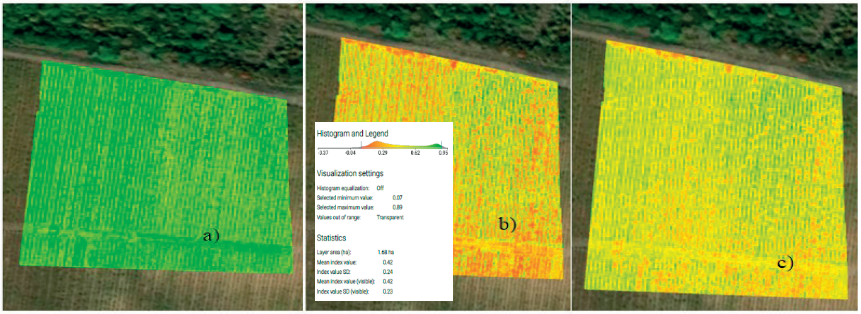


Figure 7. Development of the vegetation index NDVI in 2022.

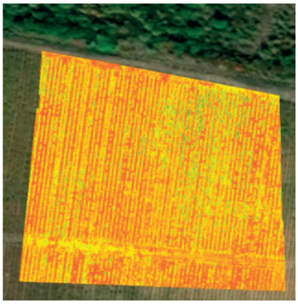


Figure 8. Determination of the Modified Chlorophyll Absorption in Reflectance Index (MCARI)

Figure 9. Determination of the Normalized Difference Water Index (NDWI)

The performance of high-resolution imagery was evaluated by considering the well-known relationship between the Normalized Difference Vegetation Index (NDVI) and crop vigor, reported Khaliq et al. (2019). The advantage of drone monitoring is that other indices can be determined, such as the Green Normalized Difference Vegetation Index (GNDVI) for evaluating photosynthetic activity. This index is more sensitive to crop chlorophyll than NDVI. The more intense the green, the more developed the vegetative mass.

MCARI is an index that responds to leaf chlorophyll concentration and ground reflectance. High index values mean low chlorophyll concentrations. Low chlorophyll indicates nutrient deficient plants, pest infestation.

The Normalized Difference Water Index (NDWI) is another index that measures plant moisture content in near real time. Information on the presence of water stress is an important point in the management of water resources.

The high-tech instrumentation used in precision agriculture enables rapid and non-destructive analysis of a large set of data. In the present study, several surveys were conducted, as a result of which the indices were established. In practice, it is necessary to carry out monitoring during the entire period of crop development. Through the capabilities of autonomous vineyard monitoring techniques, the series of photographic images and videos captured by the drone camera can be used for forecasting, crop yield modeling, disease prediction, stress management. On the basis of the obtained results, preventive measures can be taken and optimal practices can be applied to obtain high yields. According to Di Gennaro et al. (2023) the development of a methodology represents an effective decision support for the proper management of missing vines, which is essential to preserve the productive capacity of the vines and, more importantly, to ensure economic returns to the farmer.

In precision agriculture, the characterization of spatial variability in the field is a step towards optimal use of resources and minimization of negative environmental impacts.

Table 1. Comparative evaluation of indicators for yield, sugars and titratable acids according to the pruning option for Syrah variety

Year	Variants	Yield of 1 vine, g	Mass of 1 bunch, g	Mass of 100 grains, g	Sugars %	Titratable acids, g/l
2021	Control - without green prunings	3074±22[a]	106±5[b]	112±3[b]	189±1[c]	6.45±0.12[a]
	Normalized	2055±22[c]	137±3[a]	117±3[ab]	203±1[b]	5,94±0,07[b]
	Normalized and defoliated	2145±18[b]	143±4[a]	124±4[a]	209±1[a]	5.65±0.10[b]
	Average value	2425±163	129±6	118±2	200±3	6.02±0.13
	P-Value	0.000	0.002	0.094	0.000	0.004
2022	Control - without green prunings	2185±9[a]	104±5	131±5	228±1[a]	7.00±0.04[a]
	Normalized	1650±25[c]	110±4	128±4	226±1[a]	6.72±0.04[b]
	Normalized and defoliated	1785±13[b]	119±5	127±3	219±1[b]	6.88±0.03[a]
	Average value	1873±81	111±3	129±2	225±1	6.87±0.04
	P-Value	0.000	0.140	0.766	0.002	0.006

[a, b, c] - evidence of differences at a statistical significance level of 0.05.

To study the influence of the pruning variant on the productivity of the grapes, as well as the content of sugars and titratable acids, a one-factor analysis of variance was applied for each of the years of study. Comparative evaluation was conducted using the Duncan Test to assess differences at the 0.05 level of statistical significance. Both the average values of each indicator for the corresponding type of pruning, as well as the standard error, giving information about the degree of variation of the characteristic, were calculated.

Given the results in Table 1, it should be considered that in 2021 the type of applied pruning has a statistically significant effect on all the studied indicators, and in 2022 - only on yield from one vine, content of sugars and titratable acids.

In 2021, the highest yields were found in the vines without green pruning (3074 g), and the lowest - in normal pruning. The normalized and defoliated version of pruning has a positive effect on the mass of grapes (143 g) and per hundred grains (124 g), as well as on the content of sugars (209 g/dm^3). The richest in titratable acids are the vines without green prunings (6.45 g/dm^3).

In 2022, it turns out again that the most productive are the vines without green prunings, whose yields reach 2185 g. Significantly less productive are normalized and defoliated (1785 g), and the lowest productive - normalized (1650 g). Grapes from vines without green prunings (228 g/dm^3) and normalized (226 g/dm^3) are the richest in sugars. The highest content of titratable acids was found in grapes without green prunings (7.00 g/dm^3), followed by normalized and defoliated (6.88 g/dm^3) and normalized (6.72 g/dm^3).

Linear regression is a statistical method for constructing a linear relationship between a set of independent variables and dependent variables. Through regression analysis, the nature of the relationship between the studied indicators is presented. The coefficient of determination (R^2 = 0.8927) was calculated when analyzing the relationship between yield and mass of 100 grains. A strong positive correlation dependence was established.

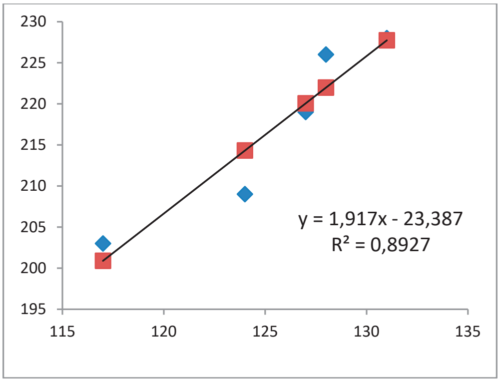


Figure 10. Linear regression model between sugars and 100 grains mass

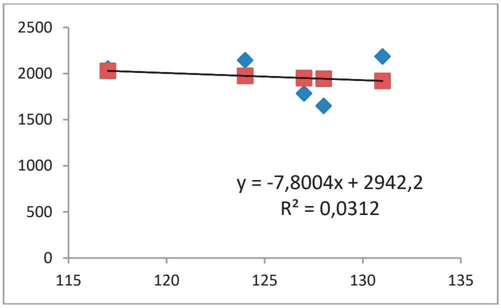


Figure 11. Linear regression model between yield and weight per 100 grains

From the linear model in Figure 11, it can be seen that there is a weak correlation between yield and mass per 100 grains. The calculated coefficient of determination (R^2 = 0.0312). A strong positive correlation was found between the mass of one bunch and sugars, with a coefficient of determination R^2 = 0.895.

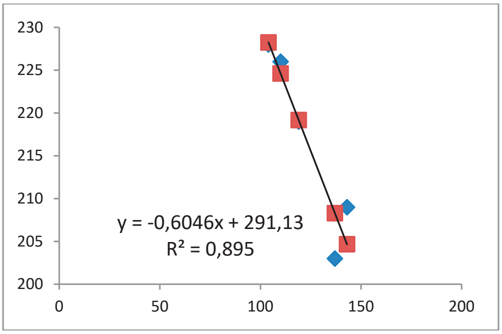


Figure 12. Linear regression model between mass of 1 bunch and sugars

The results showed that the remote monitoring of perennial crops provides indicative data, taking into account the trend of some indicators tracked during the vegetation period. When registering a low vegetation index during the growing season, for example, it is necessary to look for the reasons, to take soil samples, to make an irrigation or any other measure that is necessary to ensure the health status of the vines.

CONCLUSIONS

As a result of the conducted remote monitoring, it can be concluded that the development and growth of the vegetative mass of the vine corresponds to the recorded vegetation index of the vine for the phenophase in which the monitoring was conducted.

The influence of climatic factors greatly affects the development, growth, yield and physiology of the vine. Vegetation indices are a complex assessment of the whole terrain, not just the plant itself, and with UAVs a difference in the captured geometric image is taken into account, which affects the NDVI values.

Linear regression models show a strong positive correlation between the mass of one bunch and sugars, with a coefficient of determination R^2 = 0.895, as well as between the yield and the mass of 100 grains (R^2 = 0.8927).

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PHYSICO-CHEMICAL COMPOSITION OF DIFFERENT GRAPE VARIETIES FROM PIETROASA VINEYARD

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Abstract

The objective of this study was to evaluate the quality aspects of grapes harvested prior to the commencement of the 2023 winemaking campaign at Pietroasa Winery. Grape samples from eight distinct varieties - Busuioacă de Bohotin, Tămâioasă Românească, Riesling Italian, Alb Aromat, Fetească Regală, Fetească Neagră, Merlot, and Cabernet Sauvignon - were collected during the harvest season at Pietroasa. This paper details the analysis of key quality indicators, including fruit weight, shape index (SI), firmness, total soluble solids (Brix), total titratable acidity (TTA), pH, dry matter content (DM), total polyphenols content (TPC), and antioxidant activity (AA), all of which are critical to assessing wine quality. It synthesizes current research on grape quality, highlighting the significant antioxidant capacity attributed to polyphenol content and suggesting avenues for future research on wine production at Pietroasa Winery. In conclusion, the study reaffirms the suitability of the Pietroasa region for vinification, attributed to its terroir which ensures the production of grapes of high quality, resulting in wines with desirable and distinctive aromatic profiles.

Key words: *quality, grape, wine, polyphenols, antioxidant activity*

INTRODUCTION

Grapes are among the most cultivated plants on Earth, largely due to their direct human consumption as fresh fruits, raisins, and juices, as well as for wine production. They thrive globally across various climates due to their remarkable adaptability. Their adaptability to different climatic conditions and soils, along with their significant cultural and economic importance, place them at the forefront of global agricultural crops. Additionally, the genetic diversity of grapevines plays a crucial role in their widespread adaptability and cultivation success. This diversity allows grapevines to flourish in a broad range of environmental conditions, from temperate to arid climates and enables the production of a wide variety of grape types, each with unique flavors and uses. However, this genetic wealth also poses significant challenges in terms of conservation and sustainable cultivation practices. Efforts to understand and harness grapevine genetic resources can lead to the development of new grape varieties that are more resistant to diseases, pests, and climate variability, ensuring the long-term viability of grape cultivation for economic and cultural purposes (Valente et al., 2022; Smith et al., 2015; Sabir, 2018)

According to FAOSTAT, worldwide, grape production in the last reported 5 years ranged between 70 and 80 million tons (Figure 1), resulting in the production of 25-29 million tons of wine.

In Europe, grape production in the last reported 5 years ranged between 25 and 30 million tons (Figure 2), resulting in the production of 15-18 million tons of wine. Regarding Romania's situation, in the last 5 reported years, grape production was around 1 million tons, resulting in the production of 0.38-0.53 million tons of wine. An exception to this was in 2018 when wine production was only 0.12 million tons (Figure 3).

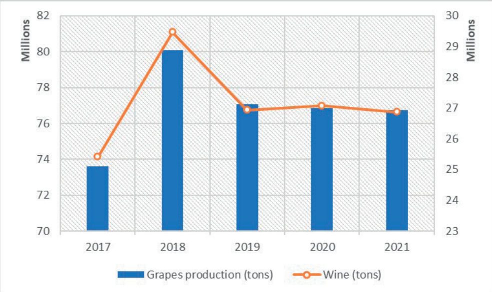

Figure 1. Grape and wine production - worldwide

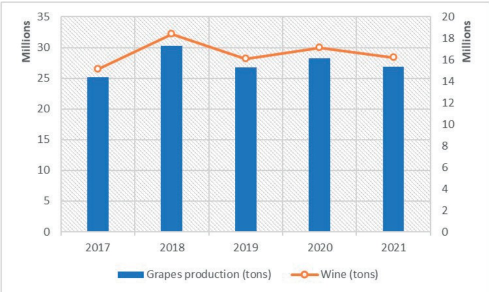

Figure 2. Grape and wine production – Europe

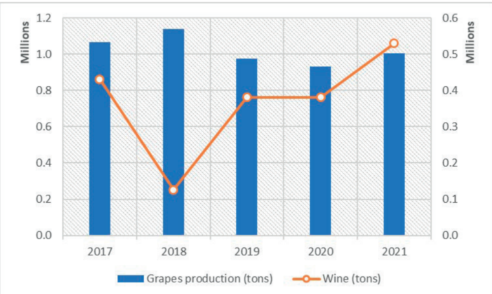

Figure 3. Grape and wine production - Romania

Grapes vary greatly in size, color, sugar content, and acidity, directly influencing the wine's aromatic and organoleptic profile (Steiner et al., 2021; Koundouras, 2018; Pérez et al., 2007). In this regard, analyzing and evaluating the quality characteristics of grapes are crucial for understanding their potential in the winemaking process. By determining these characteristics, a detailed picture of the quality and maturity of the grapes can be obtained, which will later be reflected in the quality of the resulting wines (Matthews et al., 2007; Gutiérrez-Escobar et al., 2021). Thus, carefully selecting grape varieties is crucial in winemaking to ensure the desired quality and sensory characteristics of wines (Grainger & Tattershall, 2016; Poni et al., 2018). Climatic factors have a significant effect on grape development. Increasing temperatures and altered precipitation patterns have been shown to influence the phenology and quality traits of *Vitis vinifera*, emphasizing the need for adaptive strategies in viticulture to sustain wine quality in changing climatic conditions (Van Leeuwen et al., 2019). Temperature plays a pivotal role in the accumulation of total soluble solids and affects total titratable acidity (TTA) and pH levels, where cooler conditions preserve acidity, contributing to the balance and taste profile of the wine. Also, drought conditions can increase dry matter and concentrate flavors, while moderate stress enhances total phenolic content (TPC) and antioxidant activity (AA), essential for wine's color, flavor, and health benefits (Biasi et al., 2019; Cozzolino et al., 2011; Soar et al., 2008; Grifoni et al., 2006). The grape variety's characteristics manifest in finished wines depend on many factors, the most important of which is the terroir soil and microclimate within the vineyard - viticultural management practices and the chosen winemaking technique (Goldammer, 2018; Trubek et al., 2010; Stevenson, 2005). An intriguing region from the perspective of terroir is the Pietroasa area in Buzău County, renowned for its terroir conditions that are conducive to viticulture. This includes volcanic soils and a microclimate that plays a significant role in cultivating high-quality grapes. These conditions are perfect for producing wines that are distinct and embody unique characteristics. Phenolic compounds, including tannins, flavonols, and anthocyanins that determine berry color, are produced and stored mainly in the skins and seeds of berries (Mollaamin, 2023; Li and Sun, 2017; Chira et al., 2011; Yilmaz and Toledo, 2003; Xu et al., 2011). The role of these compounds in enhancing the nutritional value of grape products and their potential health benefits, particularly as antioxidants, is a subject of ongoing research (Sabra et al., 2021).
These compounds are synthesized in the berry and concentrated in the fruit skin, and seeds when present (Teixeira et al., 2013).
The contributions of phenolic compounds to the nutritive value of grape products, and their

potential health benefits as antioxidants, are currently active areas of investigation. (Dookozlia, 2020; Pirvu et al., 2011).

Despite the critical importance of understanding the physico-chemical properties of grape varieties for wine production, there exists a notable gap in the scientific literature addressing this subject, particularly within the unique context of the Pietroasa winery. This paper aims to assess the quality parameters (fruit weight, shape index, firmness, total soluble solids, total titratable acidity, pH, dry matter content, total phenolic content, and antioxidant activity) of eight grape samples from Pietroasa Winery to enhance a better understanding of their value in winemaking.

MATERIALS AND METHODS

Grape samples from eight different varieties were collected from the Pietroasa Winery vineyards during the 2022-2023 growing season: Busuioacă de Bohotin, Tămâioasă Românească, Riesling Italian, Alb Aromat, Fetească Regală, Fetească Neagră, Merlot, and Cabernet Sauvignon. Each grape variety was carefully selected to represent a diverse range of characteristics and flavors. Upon collection, the grapes were transported to the laboratory for analysis. Various parameters including fruit weight, shape index (SI), firmness, total soluble solids (Brix), total titratable acidity (TTA), pH, dry matter content (DM), Total phenolic content (TPC), and antioxidant activity (AA) were determined using standardized analytical methods.

Fruit weight

To determine the fruit weight, a representative sample of grapes from each variety was selected and separated from the stems. The grape berries were then weighed using a precision scale. Five replicates were performed, and the final value was represented by the mean of these measurements, in grams.

Shape index (SI)

For shape index determination, five grape berries were individually measured for their length (L) and width (W) using a digital caliper. The Shape Index (SI) was then calculated as the ratio of the length to the width (SI = L/W). This calculation provides a numerical value representing the elongation or roundness of the grape berry: a value less than 1 indicates more or less flattened fruits, a value greater than 1 indicates more or less elongated fruits, and a value equal to 1 indicates spherical fruits (Brewer et al., 2006).

Firmness

For firmness determination, a Turoni 53205 model was employed to measure the compressive force required to penetrate the grape berry. Each grape berry was carefully positioned on the analyzer's platform, and a standardized piston (3 mm) was used to apply a controlled force perpendicular to the berry's surface. The force required to penetrate the berry by a predetermined distance was recorded as the firmness value (Petre et al., 2021). This process was repeated 5 times, and the average firmness value was calculated and expressed as N (Newton).

Total soluble solids (Brix)

For total soluble solids (Brix) determinations, a hand refractometer was utilized to measure the sugar content in the grape juice. A small sample of grape juice was extracted from each grape variety and placed onto the prism of the refractometer (Petre et al., 2021). The refractometer measures the bending of light passing through the juice, which is directly correlated with the sugar concentration. The Brix value, expressed in degrees, represents the percentage of soluble solids in grapes.

Total titratable acidity (TTA) and pH

For total titratable acidity determination (TTA), the titration method was conducted using the TitroLine Easy apparatus. Initially, a homogeneous ground grape sample was titrated with a standardized solution of 0.1N NaOH using the method described by Petre et al., 2023. The titration process continued until a final 8.1 pH was reached. Additionally, the initial pH was recorded. The volume of 0.1 N NaOH solution required to reach this endpoint was recorded by the instrument. The TTA value, expressed as g tartaric acid/100 g of fresh fruit, was calculated based on the volume and concentration of the 0.1N NaOH solution used. The average TTA value was calculated based on the results of these three replicates.

Dry matter content (DM)
For the dry matter (DM) determination, a gravimetric method was employed. Specifically, 1 gram of homogeneous fresh sample was weighed into pre-weighed laboratory crucibles and then dried in a laboratory oven at 105°C, until a constant weight was achieved (Iliescu et al., 2019). The difference in mass before and after drying represented the dry matter, expressed as a percentage of the initial sample mass. This process was repeated for each grape variety, with three replicates for each.

Total polyphenol content (TPC)
The extraction of polyphenols was based on the method described by Barbulescu et al., 2022. Therefore, 1 gram of each grape sample was initially triturated with 10 mL of 70% MeOH, and the resulting mixture was left to incubate overnight in darkness at ambient temperature. Following this maceration period, the samples were homogenized on an orbital shaker for one hour. After the homogenization, the extraction process continued with stirring for an hour, 500 rpm, followed by centrifugation at 7000 rpm, 4°C, for 5 minutes. The supernatant was collected in 50 mL tubes, and the residual material underwent two successive extractions until a final extract volume of 30 mL was obtained. A standard solution of gallic acid was prepared to generate a standard curve ($y = 0.012x + 0.003$, $R^2 = 0.9982$). From the grape extract obtained, 0.5 mL of the extract with 2.5 mL of Folin-Ciocalteu reagent, followed by a 2-minute incubation at room temperature, approximately 21°C. In the subsequent step, 2 mL of a 7.5% sodium carbonate (Na_2CO_3) solution is added to this mixture, which is then incubated for an additional 15 minutes at 50°C. After the incubation, the absorbance was measured at a wavelength of 750 nm using a Specord 210 Plus UV-VIS spectrophotometer (Analytik Jena, Jena, Germany). The results were expressed as mg of gallic acid equivalents per gram of fresh weight (mg GAE/100 g fresh weight).

Antioxidant activity (AA)
Antioxidant activity (AA) was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Stan et al., 2020). This process entailed combining 0.2 mL of the extract with 2 mL of a 0.2 mM solution of DPPH in methanol, followed by a 30-minute incubation in darkness with homogenization. Subsequently, the absorbance of the resulting samples was measured at a wavelength of 515 nm. The results were expressed as mg Trolox equivalent/100 g FW (fresh weight) using a Trolox calibration curve ($y = 0.0238x + 0.0884$, $R^2 = 0.9985$).

Statistical analysis
Standard deviation was employed as the statistical analysis technique for all samples, quantifying the variation or dispersion from the mean value, based on either five or three replicates per sample.

RESULTS AND DISCUSSIONS

This study aimed to analyze 8 grape samples from Busuioacă de Bohotin, Tămâioasă Românească, Riesling Italian, Alb Aromat, Fetească Regală, Fetească Neagră, Merlot, and Cabernet Sauvignon varieties harvested from Pietroasa Winery. These analyses are crucial for assessing the qualitative aspects of the grapes, as they directly impact the potential quality and characteristics of the wines that can be further produced.

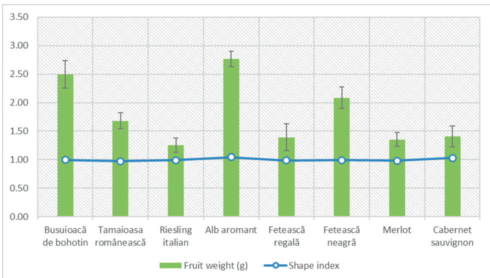

Figure 4. Fruit weight and shape index for analyzed samples

The fruit weight varied among the analyzed samples (Figure 4). The highest average grape weight was observed for the Alb Aromat variety, at 2.77 g, while the lowest average grape weight was recorded for the Riesling Italian variety, at 1.26 g. Regarding the shape index, the samples exhibited values ranging between 0.97 and 1.05, which are characteristic of spherical-shaped grapes. This indicates a consistent shape across the samples, with most grapes exhibiting a round or spherical morphology.

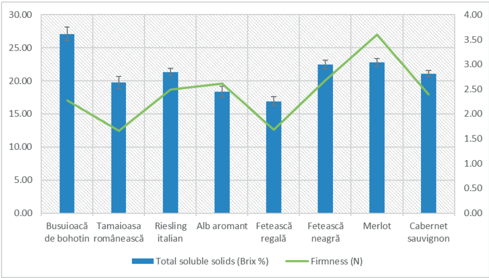

Figure 5. Brix and firmness values of the analyzed samples

For the Brix values (Figure 5) the highest value was recorded for the Busuioacă de Bohotin variety, reaching 27.10%, while the lowest value was observed for the Fetească Regală variety, at 16.92%. The values obtained for the Merlot (22.78%) and Cabernet Sauvignon (21.10%) varieties are confirmed by Karoglan et al. in 2014, with 23.20% Brix for Merlot and 21.20% Brix for the Cabernet Sauvignon variety. A value of 20.5 % was also mentioned by Stănuș et al., in 2019 for the Fetească Regală variety. Regarding firmness, the highest value was measured for the Merlot variety, reaching 3.60 N, while the lowest firmness was observed for the Tămâioasă Românească variety, at 1.66 N.
These results reveal significant variability in sugar concentration (Brix) and firmness among the grape samples analyzed. The wide range of Brix values indicates variations in sugar levels across the samples, which can have implications for the potential alcohol content and taste profile of the wines produced from these grapes.
Additionally, the variation in firmness suggests differences in berry structure, which can influence extraction during winemaking processes and ultimately impact the texture and mouthfeel of the resulting wines.
The data presented by Beauchet et al. (2020) reflected that sugar content from grapes and total acidity were strongly linked to Soil Annual Practices, (respectively, 26.60% of the contribution for sugar content and 19.61% for total acidity) and Structure Perennial Practices (20.07% for sugar content and 19.88% for total acidity). By the statistical analysis, they identified the main viticultural practices of the soil and climate variables related to the grape quality at harvest. The choice of harvest dates and locations is usually planned based on the color development of the grape bunches (Pothen & Nuske, 2016).

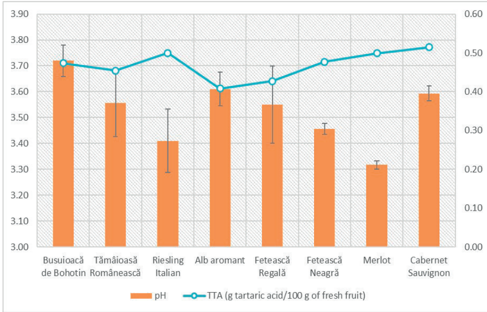

Figure 6. Total titratable acidity and pH of the analyzed samples

Variations in Total Titratable Acidity (TTA) and pH play a crucial role in shaping the sensory characteristics and overall quality of wines derived from different grape varieties (Pedneault et al., 2013). Higher acidity levels, typically indicated by lower pH values, contribute to wine freshness and longevity, while lower acidity levels, reflected by higher pH values, may lead to wines with a softer mouthfeel and smoother taste profile (Waterhouse et al., 2016).
In our analyzed grape samples, notable variations were observed in both TTA and pH levels (Figure 6). For TTA, the Cabernet Sauvignon variety exhibited the highest acidity levels, with a TTA of 0.51 g tartaric acid/100 g of fresh fruit, whereas the Alb Aromat variety displayed the lowest acidity levels, with a TTA of 0.41 g tartaric acid/100 g of fresh fruit. As for pH, the Busuioacă de Bohotin variety has the higher pH (3.72) while the Merlot variety exhibited the lowest value of pH (3.32).
Similar values were obtained also by Leila et al., in 2008 for the TTA and pH of the Cabernet Sauvignon, 0.67-0.85 g tartaric acid/100 g fresh weight for TTA and a pH between 3.49 and 3.77.
The highest dry matter content was recorded for the Busuioacă de Bohotin variety, reaching 28.44%, and the lowest was observed for the Fetească Regală variety, with a value of 18.07% (Figure 7). Dry matter content is an important parameter to know because a higher concentration of solid material in the grapes can influence factors such as mouthfeel, body, and potential alcohol content in the resulting wines. Conversely, a lower concentration of solid material in grapes may result in wines with a

lighter body and potentially lower alcohol content.

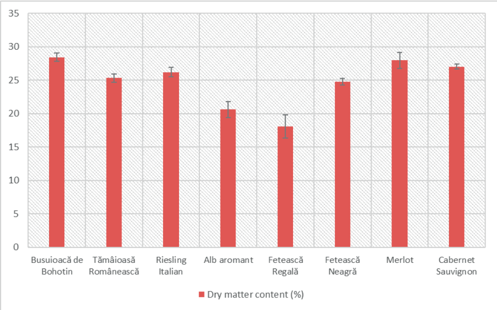

Figure 7. The dry matter content (%) of the analyzed samples

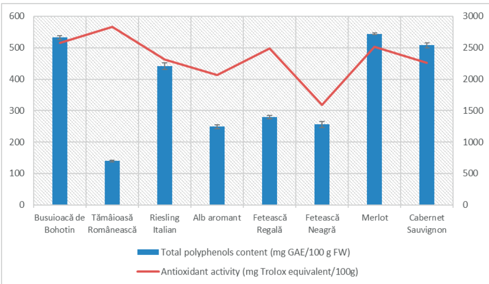

Figure 8. Total polyphenols content (TPC) and Antioxidant activity (AA) content of the analyzed samples

TPC and AA serve as crucial parameters, offering valuable insights into the antioxidant potential, health-promoting characteristics, and aging capabilities of wines derived from grapes. For the analyzed samples (Figure 8), in terms of TPC, the lowest value was recorded for the Tămâioasă Românească variety, with a value of 139.70 mg GAE/100 g sample, while the highest value was observed for the Merlot variety, reaching 543.73 mg GAE/100 g sample. Regarding AA, the lowest value was found for the Fetească Neagră variety, with a value of 1591.91 mg Trolox/100 g sample, while the highest value was observed for the Tămâioasă Românească variety, with a value of 2828.52 Trolox/100 g sample.

CONCLUSIONS

The results obtained in this study showed variations among the analyzed grape varieties, highlighting notable differences in antioxidant potential and health-promoting characteristics of the wines. Merlot stands out for its highest phenolic content, while Tămâioasă Românească excels in antioxidant activity.

The analyzed samples demonstrated consistency in the shape index, showing an almost spherical morphology across the grape varieties. However, there were notable differences in TTA and pH among the varieties, with Cabernet Sauvignon exhibiting higher acidity, and Merlot showing lower pH values, which impact their sensory profiles and aging potential.The outcomes of our study contribute to a deeper comprehension of the physico-chemical attributes of eight grape varieties, including fruit weight, shape index (SI), firmness, total soluble solids (Brix), total titratable acidity (TTA), pH, dry matter content (DM), total polyphenol content (TPC), and antioxidant activity (AA), which are crucial in the wine production process.

The Pietroasa region is traditionally recognized for producing distinctive wines, thanks to its terroir, which influences grape quality and contributes to the wine's unique and appealing aroma.

ACKNOWLEDGEMENTS

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IDENTIFICATION OF NEW YEAST ISOLATES FROM THE ŞTEFĂNEŞTI VINEYARD THROUGH PCR ITS-RFLP TECHNIQUE

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Abstract

The Ştefăneşti-Argeş wine region is known for its unique terroir and traditional practices in viticulture and winemaking. Yeasts play a significant role in shaping wines' characteristics and fermentation processes. The surface of grape berries is a natural habitat for various microorganisms, mainly represented by yeasts, lactic acid bacteria, and acetic acid bacteria, known as the epiphytic microbiota. The aim of this work was the isolate, identify, and characterize some yeast strains from different grape varieties: five internationally well-recognized varieties for white and red wines grown in the INCDBH Stefanesti vineyard. The isolates selected in pure culture proved to belong to both Saccharomyces and non-Saccharomyces genera, an aspect confirmed by microscopic and molecular analyses. For correct identification, the morphological characteristics were complemented by molecular analyses by PCR amplification of the ITS1-5.8S-ITS2 region.

Key words*: grapes, wine, local strains, PCR, Ștefănești vineyard.*

INTRODUCTION

Yeasts are eukaryotic, unicellular microorganisms belonging to two different phyla (*Ascomycota* and *Basidiomycota)* which reproduce asexually by mitosis and develop predominantly by vegetative budding or fusion (Kurtzman C.P. & Fell J.W., 1998). Yeasts are free-living organisms present in diverse terrestrial, aquatic, and marine environments on all continents, forming associations with numerous species of plants, fungi, and insects (Kurtzman et al., 2011; Chavez et al., 2024).

The study of yeast taxonomy has been an important objective for numerous research efforts, both nationally and internationally (Gayon et al., 2006; González, 2007) but, the reference work was done by Lodder (1970). Numerous yeast species play a very important role in various industries and human activities, such as the baker's yeast *Saccharomyces cerevisiae*, used in baking, brewing, winemaking, and biotechnology. The classification into *Saccharomyces* and *non-Saccharomyces* was established following various studies carried out on their diversity and the frequency of yeasts on the surface of grape bunches and wine production (Grangeteau et al., 2017; Abdo et al., 2020). The frequency and type of yeasts present on grapes are closely related to the health status of the grapes and the vegetative stage (phenophase) of berries development (Dumitrache et al., 2020). A lower frequency and diversity of yeasts was observed in unripe grapes compared to ripe grapes, which showed a significantly higher abundance of yeasts with an extensive variety of species and genera. (Barata et al., 2008; Grangeteau et al., 2017). *Non-Saccharomyces* yeasts grow better in the preliminary stages of the fermentation when the ethanol concentration is still low, being later replaced by *Saccharomyces*, which are more ethanol tolerant and competitive for growth in high sugar media (Fleet & Heard, 1993; Sabate et al., 2002). Traditionally, strain identification depends on morphological and physiological characteristics (Dumitrache et al., 2020). These classic methods require about 50-100 tests to accurately determine the species of most yeasts. (Lin & Fung, 1987; Sabate et al., 2002). The progress of biotechnology, together with molecular studies, has opened new opportunities and possibilities for the faster identification of numerous samples (Sabate et al., 2002). The analysis at the DNA level

allows the evaluation and identification of a larger sample of samples in a much shorter time. One of the most effective methods is RFLP (restriction fragment length polymorphism) using the ITS-5.8S region (Esteve-Zarzoso et al., 1999; Fernández-Espinar et al., 2000; Dumitrache et al., 2020).
This research aims to identify and characterize native yeast strains from the Ştefăneşti vineyard, with potential application in the process of producing wine.

MATERIALS AND METHODS

Grape samples and yeast isolation

The grapes were harvested aseptically from the INCDBH Stefanesti-Arges vineyard. Were selected some grape varieties very appreciated for the production of white and red wines ('Fetească regală'-FR, 'Tămâioasă românească'-TR, 'Sauvignon'-Sauv, 'Cabernet sauvignon'-CS, 'Burgund' - Burg.).
The procedure for isolating microorganisms, regardless of their nature, involves applying a series of techniques designed to facilitate the sampling and separation of the microorganisms of interest from their natural environment, followed by their inoculation in specific culture media under controlled laboratory conditions (*in vitro*).
Healthy grapes were crushed in a sterile mortar and mixed a the pestle, and the resulting mixture was allowed to stand for approximately 25 minutes under laminar flow to facilitate samples infusion.
The must samples were processed using serial decimal dilutions, according to the Domerq (1956) methodology. The crushed extracts were inoculated with automatic pipettes on Petri dishes with Potato-Dextrose-Agar (PDA/ch) and Yeast Extract-Peptone-Dextrose (YPD/ch), both culture media supplemented with chloramphenicol to inhibit bacterial growth. After inoculation, Petri dishes were incubated for 48-72 hours at 27°C in an incubator controlled by the thermostat (CL 53, Pol-EKO).

Morphological characterization

Yeast strains were isolated by cultivating them on a selective medium YPD and YPG at 27°C for 48-72 hours. The strains that were newly isolated were morphologically characterized using the "OPTIKA" binocular digital microscope, and the visualization of the obtained results was possible using the B-290TB tablet following the Pintilie (2011) protocol for yeast analysis.
Yeasts were then classified based on colony characteristics and microscopic observations according to the methodologies updated by Kurtzman et al. (2011) and Boekhout et al. (2016).

DNA isolation

DNA extraction from yeast isolates was performed using the ZR Fungal/Bacterial MiniPrep™ kit (Zymo Research, USA) following the producer's protocol.
The extracted total DNA samples were checked for their quantity and quality using a BioPhotometer plus spectrophotometer (Eppendorf, USA). Extracted DNA was stored at -20°C before processing.

PCR technique for amplifying the ITS region

The ITS1-5.8S-ITS2 region was obtained by amplifying each yeast isolate using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3')
universal primers as described by White et al. (1990).
PCR reactions were performed using the Mango Taq™ DNA polymerase kit from Meridian Bioline in a total volume of 50 µl.
The methodology of these reactions was based on the protocols used by Dumitrache et al. (2020) and Manolescu et al. (2022), in similar research. The amplification process was performed with the Techne TC-512 thermal cycler.

Restriction digestion

According to the ITS-RFLP methodology described by Esteve-Zarzoso et al. (1999), the digestion of the ITS-PCR products was performed with the restriction enzymes HhaI, HaeIII and HinfI (Thermo Scientific, USA). Each digestion reaction had a total volume of 20 µl, containing 2 µl of 10X digestion buffer, 1 U of the corresponding enzyme, 10 µl of PCR product, and ultrapure water to reach the final volume.
The enzymes were incubated at 37°C for 90 minutes, following the instructions for preparing the reaction mixture.

Electrophoresis and DNA products

After ITS-PCR amplification and digested PCR products, for migration in agarose gel (1% and 2% with TAE buffer and stained with ethidium bromide). Electrophoretic profiles were visualized under UV light using the Gene Flash Syngene Bio Imaging system.

A Quick-Load Purple 100 bp DNA ladder was used to determine the sizes of separated DNA fragments.

According to Esteve-Zarzoso et al. (1999), Sabate et al. (2002), and Baffi et al. (2010), restriction fragment profiles were analyzed for the electrophoretic patterns of different yeast species.

RESULTS AND DISCUSSIONS

Morphological characterization

The microbial contamination associated with freshly squeezed must be estimated at 10^6 CFU/ml. The semi-selective PDA/ch medium facilitated the growth of yeasts, molds, and some chloramphenicol-resistant bacteria (Figure 1-left), so too few yeasts could be isolated in pure culture.

The first strains with morphology similar to yeasts were observed on the YPG/ch selective culture medium, thus obtaining the first pure cultures (Figure 1 - center). This method led to the isolation of five different strains of yeast (Figure 1 - right).

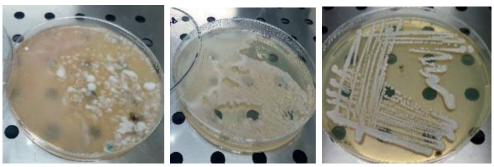

Figure 1. Yeast cultures at various stages of isolation and purification; **left.** in mixed cultures on PDA/ch; **center.** cultures with distinct colonies on YPD/ch and **right.** pure cultures of yeast on YPG medium

This approach is considered more efficient than the direct isolation of yeasts from intact grapes Valero et al. (2007); Manolescu et al. (2022). The reduced presence of fermentative *Saccharomyces* on intact grape skins, in contrast to the higher abundance of other microbial colonizers like non-Saccharomyces yeasts and filamentous fungal spores, likely accounts for this difference (Ükelgi, 2011).

Microscopy studies showed that all yeast strains used in the identification procedure were from pure cultures. The morphological aspect of the cells varied from strain to strain, with different shapes and sizes (Figure 2). Some yeast isolates showed a cream pigmentation, and others red-brown, with smooth or opaque surfaces, and with ovoid cell morphology until apiculation (lemon shape). The spheroidal and ellipsoidal shape cells were also observed for other isolated strains with a milky white to light brown (buttery) appearance with regular borders. The yeast cell size for each isolate is presented in Table 1.

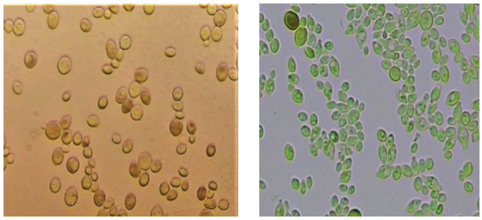

Figure 2. Microscopic observations of yeast cells derived from pure cultures (l**eft** - *Saccharomyces cerevisiae* and **right** - *Hanseniaspora uvarum)*

Table 1. Average dimensions of yeast cells grown on the PDA/ch medium after three days of incubation

No. code	**Yeast Isolate Code**	**Cell Dimensions**	
		Average Length (L) µm	**Average Width (l) µm**
D1	Isolate 1 FR	5,60±0,46	5,27±0,31
D2	Izolate 2 TR	5,34±0,37	4,92±0,29
D3	Izolate 3 Burg.	6,30±0,36	5,01±0,33
D4	Izolate 4 CS	5,68±0,32	4,30±0,35
D5	Izolate 5 Sauv.	5,60±0,31	4,86±0,34

Based on our results and microscopic characteristics of the cells, the following yeast species were estimated for the five analyzed isolates: Isolate 1 FR (D1), with dimensions of 5.60 µm x 5.27 µm, corresponds to *Saccharomyces cerevisiae* (Figure 2 - left); Isolate 2 TR (D2), with dimensions of 5.34 µm x 4.92 µm, is associated with *Pichia pastoris*; Isolated 3 Burg. (D3), which measures 6.30 µm x 5.01 µm, aligns with *Hanseniaspora uvarum* (Figure 2 - right); Isolate 4 CS (D4), measuring 5.68 µm x 4.30 µm, can be identified as *Kluyveromyces lactis;* and finally Isolate 5 Sauv (D5), measuring 5.60 µm x 4.86 µm, is associated with *Debaryomyces hansenii*.

With classic microscopy techniques, sizes from 4 µm for haploid cells to 6 µm for diploid cells were highlighted, these results being by those obtained by Milo & Phillips (2015). The observations regarding these morphological dimensions are recorded in the case of single cells, but also for mother cells that have a single bud, as in the case of yeasts from the Saccharomyces genus.
This identification is frequently supplemented by molecular biology analyses involving digestion with restriction enzymes to detect the ITS1-5,8S -ITS2 region, and to determine the polymorphism among strains (White et al., 1990; Esteve-Zarzoso et al., 1999; Sabate şi colab., 2002; Baffi et al., 2010).

DNA concentration and purity
Following the DNA extraction, the obtained results showed that the applied method was efficient in obtaining a DNA extract with a concentration of 7.1 to 15.8 ng/µl, and of good purity for PCR reactions. These results are presented in table 2.

Table 2. DNA concentration and purity

Isolate code	Concentration <ng/µl>	Rapport A260/A280	Rapport A260/A230
D1 – FR	15,7	1,73	0,81
D2 – TR	12,9	1,80	0,99
D3 – Burg.	15,0	1,76	0,48
D4 – CS	7,1	1,77	0,55
D5 – Sauv.	15,8	1,66	0,00

Analyzing the A260/A280 ratio, which provides information on RNA contamination in the final DNA solutions, it was certain that the obtained genetic material exhibited very good purity. The A260/A280 values ranged between 1.66 and 1.80, indicating high-quality DNA, despite relatively low concentrations in ng/µl.

PCR amplification and ITS-RFLP profiling of the ITS1-5.8S-ITS2 region
The ITS1-5.8S-ITS2 region was amplified via PCR, and the resulting products exhibited five distinct bands of varying sizes: 380 bp, 650 bp, 740 bp, 750 bp, and 840 bp, corresponding to the five isolates (Figure 3). According to White et al. (1990); Arlorio et al. (1999), and Esteve-Zarzoso et al. (1999), these band sizes are characteristic of isolates from the genus *Saccharomyces*, *Pichia*, *Hanseniaspora*, *Kluyveromyces*, and *Debaryomyces*.
The genetic variability of the yeast isolates introduced into the study was analyzed using 3 restriction enzymes: *Hae*III, *Hinf*I and *Hha*I. By using each of these, in distinct reactions.

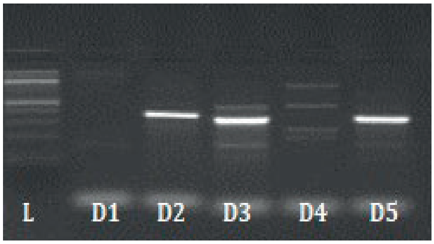

Figure 3. Electrophoretic profile of the ITS1-5.8S-ITS2 region of yeasts obtained using the ITS 1/4 primer set
Legend:* ***L* *= DNA ladder;* ***D 1÷5*** *= code for isolated yeasts*

The genetic variability of the yeast isolates included in the study was investigated using three restriction enzymes: *HaeIII, HinfI* and *HhaI*.
The electrophoretic profiles obtained in distinct reactions for each of these enzymes revealed differences between the analyzed yeast strains. The sizes of the PCR products and restriction fragments are summarized in Table 3.

Table 3. RFLP analysis for the identification of isolated yeasts

Strains identification	Analyzed strains	Produced by PCR (pb)	Obtained restriction fragments (pb)		
			HaeIII	*HinfI*	*HhaI*
Saccharomyces cerevisiae	D1	840	320 + 230 + 180 + 130	360 + 120	360 + 330+130
Pichia pastoris	D2	380	380	360	250+130
Hanseniaspora uvarum	D3	750	750	320+310+105	350 + 200 + 180
Kluyveromyces lactis	D4	740	655 + 80	290+ 180+ 120+80+65	360 + 230+140
Debaryomyces hansenii	D5	650	420+ 150+90	325 + 325	300+250

The comparative analyzes of the electrophoretic profiles obtained for each yeast isolate showed the differences at the molecular level among them Based on this analysis, isolates that showed different restriction profiles were grouped into different categories according to their profiles for ITS1-5.8S-ITS2 region, previously described in reference studies (White et al., 1990; Esteve-Zarzoso et al., 1999; Sabate et al., 2002; Baffi et al., 2010). The five yeasts strains newly isolated from grapes belong to the among the species listed in Table 3.

Saccharomyces cerevisiae, the yeast predominantly involved in alcoholic fermentation, was isolated from the grapes of a single white wine variety, 'Fetească regală'. According to specialized literature, S. cerevisiae is not frequently detected on grapes (Combina et al., 2005), this observation being a notable one. Its presence was rarely detected during ripening or on the surface of damaged grapes (Renouf et al., 2007; Dumitrache et al., 2020). The other four strains belong to the *non-Saccharomyces* group of yeasts. For this reason, our studies will continue aiming to identify the role of these yeasts in the wine fermentation process.

CONCLUSIONS

The study successfully isolated and characterized five distinct yeast strains from grape samples, integrating both morphological and molecular techniques. Microscopic analysis revealed diverse cell shapes and sizes, aiding initial species identification. ITS1-5.8S-ITS2 region amplification by PCR, coupled with restriction enzyme analysis using *HaeIII, HinfI*, and *HhaI*, confirmed species such as *Saccharomyces cerevisiae*, *Pichia pastoris*, *Hanseniaspora uvarum*, *Kluyveromyces lactis*, and *Debaryomyces hansenii*.

This combined approach not only demonstrated the effectiveness of yeast identification but also its importance for winemaking. The identification of fermentative *Saccharomyces* species alongside *non-Saccharomyces* yeasts, which can influence the aromatic complexity of wine, emphasizes the relevance of yeast biodiversity in viticulture and enology. The study reinforces the role of molecular techniques in the precise identification of microbial communities, important for wine fermentation and for improving the special aroma and taste of winemaking products.

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PERFORMANCES EVALUATION OF SOME WINE GRAPE CULTIVARS GROWN IN NORTH-WEST OF ROMANIA

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Abstract

The historic vineyards of Satu Mare County, mentioned since feudal times, were the focus of research in the Bogdand village area from 2021 to 2022. Various wine grape cultivars including 'Fetească regală', 'Sauvignon Blanc', 'Italian Riesling', 'Traminer rose', 'Muscat Ottonel', 'Tămâioasă românească', 'Pinot noir', 'Burgund mare', 'Cabernet Sauvignon', and 'Merlot' were studied. The research encompassed cultivation techniques, soil tillage frequency, pest and disease treatments, and quantitative and qualitative production analyses, including organoleptic evaluations. Despite challenges like droughts and heavy rainfall in 2022, meticulous management yielded balanced sugar-to-acidity ratios and high-quality grapes, notably in the 'Cabernet Sauvignon' and 'Traminer rose' varieties. Recommendations from an initial agrochemical study were implemented in subsequent years to address reported issues. Climatic variations influenced technological applications. National programs aimed at revitalizing Romanian viticulture through European funds have proven successful. The unique terroir and local culinary tradition elevate the profile of these wines, labeled "I.G. Hills of Sătmarului."

Key words: *grape varieties, Satu Mare County, vineyards, wines.*

INTRODUCTION

Vineyards and wine nurseries in Satu Mare County account for roughly 2% of Romania's wine-growing territory (Malaescu et al., 2022). The majority of today's viticultural plantings are based on Satu Mare County's well-documented viticultural history (Bogdan et al., 2021). In 1975, more than 4,300 hectares of vineyards were grown in Satu Mare County (Ștefan et al., 2017). At that time, however, large areas were planted with interspecific hybrids. (Cichi et al., 2022). In recent years, with the assistance of reconversion programmes, over 1,000 hectares of noble vines were planted, and also several vineyards were also established with red varieties, which were not previously common in this area (Heizer et al., 2023). The area of yielding vineyards in Satu Mare County was 3271 ha in 1990, but it decreased by around one-third during the next two decades (Dobrei A.G. et al., 2016). According to data provided by the National Institute of Statistics (NIS), the total number of yielding vineyards was 2990 ha in 2022. Wine tourism has gained popular support in Satu Mare County, with local authorities engaging in the promotion of the "Sătmărean Wine Road" project, which contributes to the rural environment's growth from both a social and economic perspective (Pop et al., 2023).
The objective of the research was to evaluate the performance of ten distinct wine grape cultivars over two consecutive years to account for potential year-to-year variability in weather conditions and growing seasons.

MATERIALS AND METHODS

The study was carried out in Bogdand area (47°25'0" North, 22°56'0" East), Satu Mare County, on ten wine grape cultivars during 2021 and 2022 growing seasons, and monitored the stages of vineyard management, the type and frequency of soil tillage, treatments used to prevent and control diseases and pests, analysis of grape yield, and organoleptic analysis of the wines. The following white grape cultivars: 'Fetească regală', 'Sauvignon Blanc', 'Italian Riesling', 'Traminer roz', 'Muscat Ottonel', 'Tămâioasă românească', some of which are aromatic, are among those cultivated in the vineyards from Bogdand area, Satu Mare County. The grape cultivars for red/rosé wines were 'Pinot Noir', 'Burgund mare', 'Cabernet Sauvignon' and 'Merlot'. The vineyards were 10 years old, with a planting distance of 2.7 m between rows and 0.9 m between vines in the row, with a planting density of 4115 vines/ha. The vines were Double Guyot training. Experimental unit was grouped into blocks based on soil management, herbicide treatments and applied fertilizers. Within each block, treatments were randomly distributed and each treatment was replicated three times within each block.

Spring ploughing was done at 15 cm depth for many years, from the first to the third decade of March. The initial hoeing was typically done each year between the first three days and the last decade of March, depending on weather conditions. The second hoeing was done in June to suppress weeds, followed by the third in August. Only the initial hoeing was completed during the second growing season (2022), as the soil was very hard due to the drought. The first herbicide treatment was applied in April, typically within the first two decades of the month, in 40 cm wide strips, and the second treatment occurred during the latter half of June. During the 2022 growing season, a combination of Chikara (containing flazasulfuron 25%) and Glygold (containing glyphosate) was applied at a dose of 0.07 kg/ha (Chikara + 1.0 liter/ha – Glygold) for the first treatment, while Taifun 360 SL was utilized at a rate of 5 liters per 150 liters of water per hectare for the second treatment. The agrochemical analysis performed during the vineyard establishment suggested multiple options for improving some of the negative items identified, as well as measures to increase the output. During the ten years of grape harvesting, the following recommendations were considered: ammonium nitrate (NH_4NO_3) application was forbidden in this vineyard; $Co(NH_2)$-urea has a residual acidifying effect per kg of N, similar to anhydrous ammonia; to neutralize the acidity, was necessary to apply 1.8 kg of Ca_2CO_3/kg of N. Because the ammonia resulting from hydrolysis has a harmful effect on young vines the urea was not applied at planting. Acidity characterized 87% of the charted surface (pH < 6). Calcareous amendments were not applied to the plots. Limitation of the acidification process was achieved by using nitro-phosphate fertilizers. On vineyards soils, acidification was prevented by using ammonium nitrate (NH_4N0_3) and calcium carbonate ($CaCO_3$) mixture (one part of ammonium nitrate and 0.3-0.7 parts of calcium carbonate). This fertilizer compensates for the base losses caused by ammonium nitrate. The implementation of soil maintenance, pruning, disease and pest control treatments were carried out at different stages, from one year to another, in direct correlation with the climatic conditions. At the spring beginning, after the winter pruning, the remaining canes were not chopped, but were collected from the rows by raking and kept for kindling on fire to avoid frosts in the winter or early spring. The same technology was applied for all cultivars. Yield was determined by weighing the grapes from each plot at harvest. The grape quality was also established at harvest by analyzing the sugar content (g/L) in the juice, by using the refractometer (Portable Digital Refractometer, RFT-PD35F, Infitek Co., Ltd.). The total acidity (g/L H_2SO_4) of grape juice was determined by titration as indicated by a pH meter (HI99111, electrode pH FC10483, Hanna Instruments Ltd.).

Data collected in the first year served as a baseline, while data from the second year provided additional insights into the grape varietals' long-term performance consistency. The sensory characteristics of the wines from the second growing season were determined through sensory analysis. The recorded data were statistically processed and interpreted. Table 1 and Figure 1 provide data about the harvested area as well as the distribution of cultivars in each plot. Plots 12 and 14 were planted with the 'Fetească regală' cultivar on 5.91 hectares area. 'Sauvignon Blanc' was planted in plots 13 and 15, covering 6.87 hectares area. In plot 11, two distinct cultivars were grown: 'Italian Riesling' on 4.80 ha and 'Tămâioasă Românească' on 4.50 ha. Plot no. 10 was divided into 'Muscat Ottonel' (5.17 ha) and 'Traminer rose' (5.00 ha). The 'Burgund Mare' cultivar occupied plot 9, which covered 1.54 ha. 'Cabernet Sauvignon' occupied plots 4, 5, and 6, for a total area of 13.5 ha.

Plots 1, 2, and 3 were planned with the ‘Merlot’ cultivar, on a total area of 7.07 ha. ‘Pinot Noir’ was cultivated across plots 7 and 8, with 10.64 ha.

Table 1. The area of grapevine cultivars

Cultivars	The area cultivated with each cultivar (ha)
‘Fetească regală’	1.37
	4.54
‘Sauvignon Blanc’	3.22
	3.65
‘Riesling Italian’	4.80
‘Muscat Ottonel’	5.17
‘Tămâioasă românească’	4.50
‘Traminer roz’	5.00
‘Burgund mare’	1.54
‘Cabernet Sauvignon’	5.15
	4.95
	3.40
‘Merlot’	4.40
	1.31
	1.36
‘Pinot noir’	5.04
	5.60

Bogdand area had an average temperature of 10.54°C in 2021 and received 742.8 mm of precipitation (Figure 2). In 2022 growing season, was registered an average temperature of 11.42°C and total precipitation falling to 633.2 mm. The weather data from 2021 and 2022 show both similarities and contrasts in Bogdand's climatic conditions. Despite modest fluctuations, overall temperature patterns remained similar, with slightly higher temperatures reported in 2022. This could show an ongoing progress towards warmer circumstances, possibly affected by larger climatic issues like global warming. However, precipitation levels differed significantly between the two years. During the 2021-2022 growing seasons, various treatments were applied at different stages of grapevine development for controlling diseases and pests, for assuring crop health and quality (Tables 2 and 3) to all cultivars.

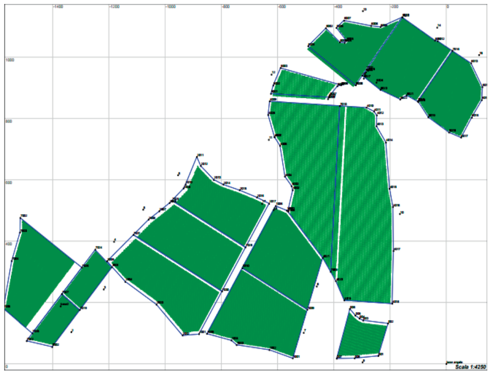

Figure 1. Plots map in Bogdand area

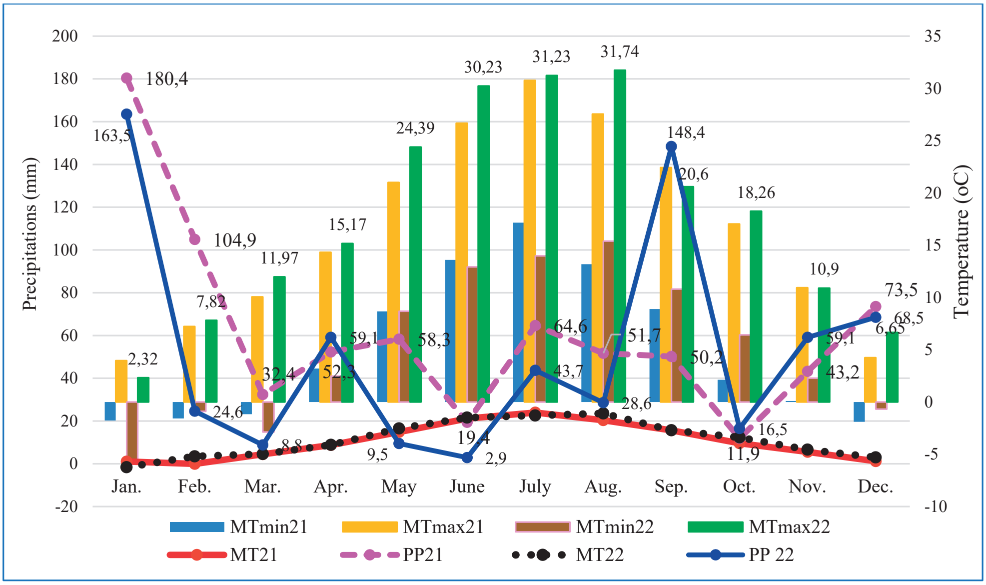

Figure 2. Climate data during 2021-2022 growing seasons in Bogdand area Satu Mare County: MTmin21 - Mean of minimum temperature 2021; MTmax21 - Mean of maximum temperature 2021; MTmin22 - Mean of minimum temperature 2022; MTmax22 - Mean of maximum temperature 2022; MT21 - Mean temperature 2021; MT22 - Mean temperature 2022; PP21 - precipitations 2021; PP22 - precipitations 2022

Table 2. The record of treatments for diseases and pest control during 2021 growing season

No.	Date	Growing stage	Disease or Pest	Treatment and concentration
1	20-30 April	Blooming	Mites Powdery mildew	Nissorun 10 WP, 0.5 kg/ha Thiovit Jet 80 WG (80% sulphur), 3 kg/ha
2	10-25 May	Shoots (10-25 cm)	Downy mildew Powdery mildew	Dithane M45, 0.2% Systhane Forte, 0.01%
3	25 May-10 June	Before flowering	Downy mildew Powdery mildew Moths	Profiler 71 WG, 2.5 kg/ha Talendo® Extra, 0.2 l/ha Sumi Alpha 5EC, 0.2 l/ha
4	10-20 July	After flowering	Downy mildew Powdery mildew, Grape black rot Moths	Profiler 71 WG, 2.5 kg/ha Flint Max 75 WG - 0.16 kg/ha in 1000 l water Sumi Alpha 5EC, 0.2 l/ha
5	20-30 June	Berry development	Downy mildew Powdery mildew Grape black rot Moths	Mikal Flash - 3 kg/ha Flint Max 75 WG - 0.16 kg/ha in 1000 l water Sumi Alpha 5EC, 0.2 l/ha
6	20 July-10 August	Veraison	Downy mildew Powdery mildew gray mold	CopFort, 3 l/ha Thiovit Jet 80WG, 3 kg/ha Pyrus 400 SC, 2.5 l/ha
7	15-30 October	After harvest	Downy mildew	Bordeaux mixture WDG, 0.50%

Table 3.The record of treatments for diseases and pest control during 2022 growing season

No.	Date	Growing stage	Disease or Pest	Treatment and concentration
1	20-30 April	Blooming	Mites Powdery mildew	Nissorun 10 WP, 0.5 kg/ha Thiovit Jet 80 WG (80% sulphur), 3 kg/ha
2	10-25 May	Shoots (10-25 cm)	Downy mildew Powdery mildew	Curzate® F, 0.4 l/ha Talendo®, 0.225 l/ha
3	25 May-10 June	Before flowering	Downy mildew Powdery mildew Moths	Orondis Ultra pack, 0.5 l/ha Dinaly, 0.65 l/ha Wizard - 0.2 l/ha
4	10-20 June	After flowering	Downy mildew Powdery mildew, Grape black rot Moths	Profiler 71 WG, 2.5 kg/ha Flint Max 75 WG - 0.16 kg/ha in 1000 l water Sumi Alpha 5EC, 0.2 l/ha
5	20-30 July	Berry development	Downy mildew Powdery mildew Grape black rot Moths	Zorvec™ Zelavin® Bria 0.2 l/ha+1.25 kg/ha Flint Max 75 WG - 0.16 kg/ha in 1000 l water Wizard - 0.2 l/ha
6	20 July-10 August	Veraison	Downy mildew Powdery mildew gray mold	Cabrio Top, 2 kg/ha Thiovit Jet 80WG, 3 kg/ha Pyrus 400 SC, 2.5 l/ha
7	10-25 October	After harvest	Downy mildew	Bordeaux mixture WDG, 0.50%

Sensory analysis in wines typically follows a standardized protocol to ensure consistency and reliability in evaluations. A group of nine experienced and trained panellists were selected to participate in a sensory analysis of wines. Carefully selected wines were stored appropriately to preserve their integrity. The tasting room was meticulously prepared to eliminate strong odours and distractions, with neutral lighting and controlled temperature ensuring an optimal environment for tasting. Panellists were provided with evaluation sheets containing standardized criteria for assessing various sensory attributes, including colour/clarity, aroma, taste, and overall impression. They swirled the wine in their glasses to release aromas and evaluated the intensity, complexity, and character of the aroma, noting descriptors such as fruity, floral or herbal. Factors such as sweetness, acidity, tannin levels, and flavour intensity were evaluated, with descriptors including fruity, savoury, citrus, or mineral notes. Panellists provided an overall assessment of the wine, considering its balance, complexity, harmony, and potential for aging.

Statistical analysis

Statistical analysis was conducted using XLSTAT (version 2020.1.3; Addinsoft; Paris; France) software for conducting correlation analysis, principal component analysis (PCA), analysis of variance (ANOVA) and t-test to explore the relationships among the variables (sugars content, acidity, maturation index, and alcohol concentration).

Pearson correlation coefficients to assess the linear relationships between variables were calculated. The relationships among the variables were examined at a significance level of $p < 0.05$, providing valuable insights into the interplay between sugars content, acidity, maturation index, and alcohol concentration in winemaking processes.

RESULTS AND DISCUSSIONS

The evaluation of grape quality was done after the analysis of some parameters important for winemaking (e.g. sugars, acidity, ripeness and the alcohol potential of the juice) which indicate the level of ripeness, determining the optimal time of harvesting (Rotaru et al., 2010). The evaluation of the quality parameters is necessary to guide the winemakers in making decisions during the harvest and the winemaking process, for the production of high-quality wines (Dejeu et al., 2008). During 2021 growing season, the most productive varieties were 'Feteasca regală' (10 tons/ha), followed by 'Italian Riesling', 'Burgund mare', 'Merlot' (9 tons/ha), 'Cabernet Sauvignon' (8 tons/ha), 'Sauvignon blanc' (7 tons/ha), and 'Pinot noir' (7 tons/ha). 'Traminer rose', 'Muscat Ottonel', and 'Tămâioasă Românească' cultivars yielded less, at around 6 tons per ha. Qualitatively, in 2021, the cultivars of 'Muscat Ottonel', 'Traminer rose', 'Cabernet Sauvignon', and 'Merlot' had an optimal maturity index, while the other cultivars had a small imbalance in the sugar-to-acidity ratio. In 2021, the cultivars of 'Cabernet Sauvignon' (225 g/L sugars) and 'Traminer rose' (220 g/L sugars) accumulated the highest sugars in the berries, while 'Sauvignon blanc' (190 g/L sugar) and 'Muscat Ottonel' (192 g/L sugars) accumulated the least amounts of sugars. Nistor et al. (2021) found in 'Traminer rose', an amount of sugars between 20.1 and 23.3 ^{o}Brix. The alcoholic potential in 2021, between 11.17 and 13.23% alcohol by volume offer the possibility of producing higher-quality wines for all ten cultivars (Table 4). According to the statistical analysis for the mean of the control varieties, the 'Traminer rose' cultivar had significant positive results, while the 'Cabernet Sauvignon' cultivar is very significant positive.

Table 4. Grape quality in the 2021 growing season

Cultivar	Sugars (g/L)	Total acidity (g/L H_2SO_4)	Maturity indices	Potential alcohol (vol. alc. %)
'Fetească regală '	200	5.8	34.48	11.76
'Sauvignon Blanc'	190	5.3	35.84	11.17
'Italian Riesling'	205	5.2	39.42	12.05
'Muscat Ottonel'	192	4.8	40.00	11.29
'Tămâioasă românească'	198	5.0	39.60	11.64
'Traminer rose'	220	4.7	46.80	12.94
'Burgund mare'	195	5.6	34.82	11.47
'Cabernet Sauvignon'	225	5.1	44.11	13.23
'Merlot'	200	5.0	40.00	11.76
'Pinot noir'	205	5.2	39.42	12.05
T	t=56.11	t=48.54	t=32.15	t=56.06
P value (two tailed)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant (alpha≤ 0.05)	Yes	Yes	Yes	Yes

In the 2022 growing season, there was a severe drought in the vineyards from Bogdand, area, especially from March 1st to September 4th, 2022, when the recorded precipitation was only 47 mm/m^2. Abundant rainfall for two weeks, with measurements indicating a volume over 100 mm/m^2 fell between September 4th and September 18th. As a result of a challenging climatic year, the grape production in 2022 was low, ranging between 4-5 tons/ha for most varieties, with the exception of the 'Cabernet Sauvignon', with 7 tons/ha recorded. The grape harvest for winemaking was left to overripe, with maturity indices averaging 46.13, ensuring that all cultivars had a balanced sugar-to-acidity ratio.

All ten grape varieties investigated in 2022 for grape quality had high levels of sugar in the berries, with at least 210 g/L in the 'Sauvignon Blanc' and 'Muscat Ottonel' cultivars and up to 250 g/L in the 'Traminer rose' and 'Cabernet Sauvignon' (Table 5).

The alcoholic potential, with an average of 11.93% alcohol by volume, for all cultivars, suggests the possibility of producing higher-quality wines. Water availability is crucial for grapevine growth, especially during critical stages like blooming and fruit set (Martínez-Lüscher et al., 2016).

Growing season 2021 had more precipitations, and offered adequate moisture for grapevine growth, given the overall amounts recorded. However, severe rainfall raised the danger of fungal diseases including mildew and botrytis, and affected grape quality and yield.

The slightly decreased precipitation in 2022 reduced disease pressure, resulting in healthier grapevines and better fruit quality.

Table 5. Grape quality for the 2022 growing season

Cultivar	Sugars (g/L)	Total acidity (g/L H_2SO_4)	Maturity index	Potential alcohol (vol. alc. %)
'Fetească regală'	230	5.5	41.81	13.52
'Sauvignon Blanc'	210	5.1	41.17	12.35
'Italian Riesling'	230	4.9	46.93	13.52
'Muscat Ottonel'	210	4.5	46.66	12.35
'Tămâioasă românească'	230	4.8	47.91	13.52
'Traminer rose'	250	4.5	55.55	14.70
'Burgund mare'	220	5.4	40.74	12.94
'Cabernet Sauvignon'	250	4.9	51.02	14.70
'Merlot'	220	4.7	46.80	12.94
'Pinot noir'	220	4.9	44.89	12.94
t	t=50.62	t=46.31	t=31.85	t=50.71
P value (two tailed)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Significant (alpha≤ 0.05)	Yes	Yes	Yes	Yes

The correlation analysis among the variables (sugars, acidity, maturation index, and alcohol volume) provides valuable insights into their relationships and potential impacts on wine characteristics (Table 6).

Table 6. Pearson correlations between berry quality parameters

Variables	Sugars	Acidity	Maturation Index	Vol. alc. %
Sugars	**1**	-0.317	0.795	1.000
Acidity	-0.317	**1**	-0.824	-0.317
Maturation Index	0.795	-0.824	**1**	0.795
Vol. alc. %	1.000	-0.317	0.795	**1**

The strong positive correlation between sugars and berries maturity indicates that wine can achieve higher complexity, taste and flavours. Lower acidity resulted in rounder and smoother wine but with too much sugar which influenced wine balance. The high negative correlation (0.824) between total acidity and maturation index in grape berries for cultivars studied is a natural consequence of the physiological changes explained by acidity decreasing and increase in sugar content; one of the changes is the breakdown of organic acids which contribute to the total acidity of the berries. Usually, acidity adds structure and brightness to wine, because an excessive acidity overshadow wine flavours and alcohol perception; the balanced correlation (-0.317) between sugar and acidity ensured the tasting harmony of wine. In the PCA diagram (Figure 3), F1 accounted for 76.78% and F2, 23.16% of the variation, which is evident that these components play a significant role in modelling the observations. ‘Cabernet Sauvignon’ stood out with the highest volume of alcohol and sugar concentrations, indicating the potential for full-bodied and robust wines. Instead, ‘Burgund Mare’ and ‘Fetească Regală’ presented a high level of acidity, highlighting the potential for refreshing wines. Grapes from the ‘Traminer rose’ variety presented the best ripeness index, suggesting the development of more intense aromas. Besides from its alcohol content, wine is a sophisticated blend of organoleptic characteristics (such as colour, taste, flavour, and aroma (Liang et al., 2021; Dobrei, 2016), Table 7.

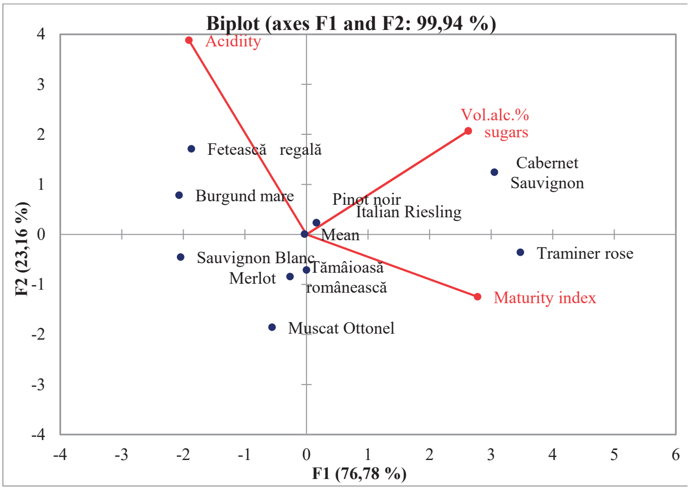

Figure 3. PCA diagram for the quality parameters in the grape varieties

Table 7. The organoleptic items description of the wines from 2022 growing season

Wines	Organoleptic characteristics of wines		
	Colour/Clarity	Aroma	Taste
‘Fetească regală’	Yellow-green/ Clear	subtle floral aroma, yellow melon scent, quince, citrus notes	semi-dry, light, fruity, fresh, high acidity perfectly balanced by sweetness, long aftertaste
‘Sauvignon Blanc’	yellow straw with green hues/Clear	elderflower and summer fruits, peaches, green apples, figs	semi-dry, lively acidity, pleasant, elderflower notes
‘Italian Riesling’	pale greenish-white / Clear	grassy and mineral notes, citrus aromas	dry, fresh, mineral, long aftertaste finish with watermelon notes
‘Muscat Ottonel’	pale yellowish-green/Clear	floral and fruity fragrance, with hints of rose, muscat, and honeysuckle, notes of green apricots	semi-sweet, fresh, discreet, lemongrass notes, sweet-bitter aftertaste
‘Tămâioasă românească’	yellowish-green with golden reflections/Clear	muscat, basil, and honey	semi-sweet, long aftertaste, acacia flower aromas
‘Traminer rose’	golden-yellow / Clear	expressive, delicate, aromas of rose petals, strawberries, grapefruit	semi-dry, aromatic, fruity, full-bodied, moderate acidity, citrus aromas, and rose sweetness
‘Burgund mare’	ruby-red with lilac reflections/Clear	forest red fruits - blueberries and fresh raspberries, hints of violets	semi-dry, balanced, with fruitiness, medium-bodied, persistent aftertaste
‘Cabernet Sauvignon’	dark red colour/ Clear	blackberries, blueberries, black currants, and sour cherries	dry, intense, blackcurrants, slightly astringent, hints of tobacco
‘Cabernet Sauvignon rose’	intense salmon pink/Clear	summer red fruits and dried herbs	semi-dry, fresh, with well-integrated alcohol, balanced acidity, sweetish aftertaste.
‘Merlot’	ruby red/Clear	vibrant aromas of red fruits, very ripe cherries, blackberries, and blueberries	semi-dry, harmonious, fruity, with aromas of plums, cranberries, and ripe cherries
‘Pinot noir’	pale ruby-red/Clear	aromas of cherries and sour cherries, strawberries, and red currants	dry, elegant, fresh, pleasant tannins, lasting aftertaste
‘Pinot noir rose’	rose, strawberry shade/Clear	raspberry and strawberry.	semi-dry, versatile, full-bodied, hints of cranberries and blackcurrants
Blending of ‘Riesling Italian’ + ‘Tămâioasă românească’	golden-yellow/ Clear	rustic aroma, notes of wildflowers, hay, and green apples.	semi-dry, fresh, balanced, fruity, with hints of honey and muscat, lasting aftertaste

Understanding these characteristics is critical to enjoying the complexities and variability found in each glass (Lick et al., 2017). By comparing the colours, certain common traits between the different varieties were found. Every variety shared the same quality of clarity, giving each one a transparent, clear look that amplifies its visual attractiveness. Every wine shared the same quality of clarity, giving each one a transparent, clear look that amplifies its visual attractiveness.

The colours, however, differ greatly, ranging from the rich ruby-reds of Burgund Mare and Cabernet Sauvignon wines to the vivid yellows and greens of Fetească Regală and Muscat Ottonel wines.

The terroir and winemaking technique of each grape variety are reflected in the distinct character and character that each colour expresses. Considering these variations, the wines all have a similar level of elegance and purity. When analysing the aroma profiles of the wines, several patterns emerge. Many wines contain floral notes, which offer refinement and delicacy to the bouquet. The aromatic spectrum of fruits, which range from citrus and stone fruit, to berries and tropical fruits, provide a feeling of freshness and vitality. Herbal and mineral notes offer complexity and depth, improving the whole sensory experience. While each grape variety has its own distinct aroma profile, they all share a thread of complexity, balance, and attractiveness. Many of the analysed wines have flavours that range from fruity and flowery to mineral and savoury, and they all show a good balance between sweetness and acidity. Certain wines, like Muscat Ottonel, offer a more delicate and nuanced flavour profile, while others, like 'Cabernet Sauvignon', offer strong and intense flavours. Despite these differences, all the wines share a common thread of complexity and balance.

CONCLUSIONS

The comparative analysis of Bogdand weather data for 2021 and 2022 demonstrates the strong impact of weather fluctuation on grapevine quality and productivity. While growing season 2021 provided more favourable conditions for grape growing than 2022, minor variations in temperature and precipitation have altered grape ripening processes and overall fruit quality.

Understanding the complex interplay between climatic conditions and grapevine physiology is critical for vineyard management strategies designed to improving grape quality and output in Bogdand and other viticultural regions. The 2022 growing season in the Bogdand area brought substantial challenges due to a prolonged drought followed by plentiful rains, which impacted grape production and harvesting. Despite low yields, accurate management produced balanced sugar-to-acidity ratios and high-quality grapes, particularly in 'Traminer rose' and 'Cabernet Sauvignon'. While water supply remained critical for grapevine growth, the balance of precipitation and disease pressure finally contributed to stronger vines and better fruit quality. This demonstrates vineyards' resilience and adaptation in circumstances of significant climatic conditions. Each variety's flavour profile ranges from fruity and flowery to mineral and savoury, with a balance of sweetness and acidity. While some wines have delicate nuances, some have strong flavours, yet they all share an identifiable characteristic of complexity and balance. Overall, this exploration emphasises the variety and diversity of wines.

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CLIMATE CHANGE TREND AND EFFECTS ON VINE CULTIVATION IN DEALU MARE VINEYARD

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Abstract

The measurement and quantification of climate variability was carried out on the basis of the 27 climate indices (16 indices based on temperature and 11 precipitation indices) defined by the Climate Change Detection and Indices (ETCCDI), which mainly focus on both cold and hot extremes of daily minimum temperature (TN), maximum daily temperature (TX), precipitation (PR) and, also percentile-based thresholds. Compared to climatology 1990-2009, in 2010-2022 the frequency of cold nights decreases and the frequency of warm nights increases. Percentile-based indices measuring the frequency of "cold days" (TX10p) has decreased and "warm days" (TX90p) has increased. The fixed maximum temperature events, freezing days (TX < 0°C) and summer days (TX > 25°C), show decreasing and increasing trends, respectively by 22 days (2.09 days/year), (in line with the general warming trend), but these are generally statistically significant. The effects of climate change are manifested on the development of vegetative phenophases (budding, flowering, leaf, grape ripening) and the evolution of grape production and its quality.

Key words: *ETCCDI indices, freezing days, summer days, vegetative phenophases.*

INTRODUCTION

In the Last Report of the IPCC (Intergovernmental Panel on Climate Change) it is mentioned that "the warming of the climate system is unequivocal, as it emerges from the observations on the increase in air and ocean temperatures, the massive melting of glaciers and on a global increase in the average level of the seas." (IPCC, 2007a, 2007b). The average annual air temperature increased in the period 1989-2021 by 1.3°C, compared to the average 1936-1988 (10.8°C), a value that exceeds the average global warming of 0.85°C in the last 100 years, according to the report AR 5 (IPCC, 2013).

The increase in the concentration of CO_2 in the atmosphere, the higher temperatures, the changes in the annual and seasonal precipitation regimes and the change in the frequency of extreme phenomena will affect the volume, quality and stability of grape production.

The production of grapes and its quality were influenced by the heating during the night and in the spring, where the reduction in the frequency of frosts determined an earlier start of the vine vegetation and implicitly a longer vegetation period.

Using a series of models to simulate climate conditions it can be concluded that the climate warming trend observed in wine growing regions all over the world will continue.

Wigley et al. (1983) suggest that in Europe due to climate warming the duration of the vine growing season will increase, along with this increase the quality of wines in certain wine growing regions.

By using the climate model HadCM3 (Hadley Center Climate Model) and the A2 model (Pope et al., 2000) regarding CO_2 emissions, (Jones, 2005a, 2005b; Lobell et al., 2006; Gordon, 2007) predicted an increase in average temperatures during the growing season by 1.3°C in the northern hemisphere and only 0.9°C in the southern hemisphere.

Examining the degree of change projected for the period 2000-2049, significant changes are expected in each wine-growing region with values ranging from 0.2 to 0.6°C/decade.

The warming of the climate in the wine-growing regions of Europe has induced changes in the phenology of the varieties, namely an earlier development by 6-25 days of the vegetative phenophases, especially the fallow phenophases veraison and the ripening of the grapes.

Croitoru et al. (2016) showed that increasing trends are stronger for heat wave indices defined on the basis of maximum temperature (at least three consecutive days when the maximum temperature exceeds the 90th percentile) compared to those obtained on the basis of minimum temperature (at least three days consecutive when the minimum temperature exceeds the 90th percentile).

Other examples of using the identification methodology with thresholds defined by percentiles are indices that can quantify thermal extremes are those calculated according to the procedure recommended by the Expert Team on Climate Change Detection and Indices (ETCCDI) (Alexander et al. 2006; Zhang, et al., 2006). Global observed changes in daily climate extremes of temperature and precipitation.

Based on the climate data recorded in the period 1956-2006 in the Cotnari vineyard, a slight warming trend of the climate was evident due to the increase of both minimum and maximum daily temperatures (Chiriac, 2007).

Similar to climate changes at the global level, changes in the regime of extreme thermal values were also highlighted in our country, namely: the increase in the annual frequency of tropical days (daily maximum >30°C) and the decrease in the annual frequency of winter days (daily maximum <0°C), the significant increase in the average summer minimum temperature and the winter and summer maximum temperature, the increase in duration of sunshine. The phenomena of temperature increase became more acute after the year 2000, the winter of 2006-2007 being the warmest winter.

MATERIALS AND METHODS

Climate data used in this analysis are for the Research Institute for Viticulture and Enology Valea Călugărească station for 1990 to 2022. The data consist of daily observations of the minimum and maximum temperature (TN, TX) and daily precipitation (PR).

The indices recommended by ETCCDI (http://cccma.seos.uvic.ca/ETCCDI/list_27_indices.shtml) are calculated based on daily observations of maximum and minimum temperatures and precipitation. According to the Climate Change Detection and Indices (ETCCDI) methodology, 27 climate indices were studied (16 indices based on temperature and 11 on precipitation). The indices for the station points were calculated using the CLIMPACT software.

The 16 indices based on temperature are: Summer days (SU25)-annual count when daily maximum TX>25°C (days); Ice days (ID0) - annual count when daily maximum TX<0°C (days); Tropical nights (TR20)-annual count when daily minimum TN>20°C (days); Frost days (FD0) - annual count when daily minimum TN<0°C (days); Max Tmax (TXx)-annual maximum value of daily maximum temperature (°C); Min Tmax (TXn)-annual minimum value of daily maximum temperature (°C); Max Tmin (TNx)-annual maximum value of daily minimum temperature (°C); Min Tmin (TNn)-annual minimum value of daily minimum temperature (°C); Warm days (TX90p)-percentage of the days when TX>90th percentile; Cool days (TX10p)-percentage of the days when TX<10th percentile; Cool nights (TN10p)-percentage of the days when TN<10th percentile;Warm nights (TN90p) percentage of the days when TN>90th percentile; Growing season length (GSL), days; Diurnal temperature range (DTR)-annual mean diference between TX and TN (°C); Warm spell duration indicator (WSDI)-annual count at least 6 days consecutive when TX>90th percentile (days); Cold spell duration indicator (CSDI)- annual count at least 6 days consecutive when TN<10th percentile (days).

The indices based on precipitation are: Max 1 day precipitation amount (RX1day), mm; Max 5 days precipitation amount (RX5day), mm; Simple daily intensity index (SDII)-PRCP>1.0mm; Number of heavy precipitation days (R10)-annual count of days when PRCP≥10 mm (days); Number of heavy precipitation days (R20)-annual count of days when PRCP≥20 mm (days); Number of heavy precipitation days (R25)-annual count of days when PRCP≥25 mm (days); Consecutive dry days (CDD)-maximum number of days with RR<1mm (days); Consecutive wet days (CWD)-maximum number of days with

RR≥1mm (days); Very wet days (R95p) annual total PRCP when RR>95th percentile; Annual total wet-dry precipitations (PRCPTOT), mm.

RESULTS AND DISCUSSIONS

Extreme temperature frequency indices showed that the frequency of cold nights (TN10p) and cold days (TX10p) decreased significantly during 2010-2022, with a decrease rate of 3.38% and 4.80%, respectively ($p < 0.05$), while the frequency of hot nights (TN90p) and hot days (TX90p) increased significantly, with an increase rate of 2.11% and 4.86%, respectively ($p < 0.05$) (Figure 1).

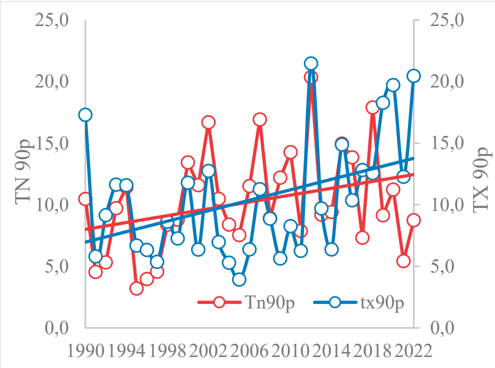

Figure 1. Frequency of warm nights (TN90p) and warm days (TX90p) from 1990-2022

The data obtained are consistent with the data obtained at the global level (Donat et al., 2013) indicating that the tendency of nocturnal extremes (TN10p and TN90p) were higher than those for daytime extremes (TX10p and TX90p).

Regarding the indices of thermal extremes that define frost days expressed by the number of days per year with the minimum temperature below 0°C (FDO) and the number of days per year with the maximum temperature below 0°C (IDO), the tendency is to decrease with 5.17 days/year, respectively 8.9 days/year ($p<0.05$) (Figure 2).

While the cold indices (FDO and IDO) showed decreasing trends, the warm indices (SU) showed increasing trends.

The indices of summer days (SU) recorded increasing trends, with rates of 1.72 days/year in the period 2010-2022 ($p < 0.05$) (Figure 3).

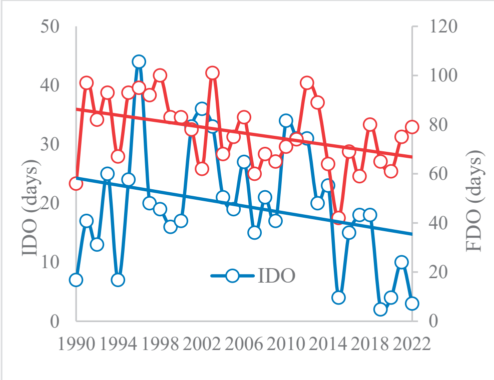

Figure 2. The annual Ice days (IDO) and Frost days (FD0) from 1990-2022

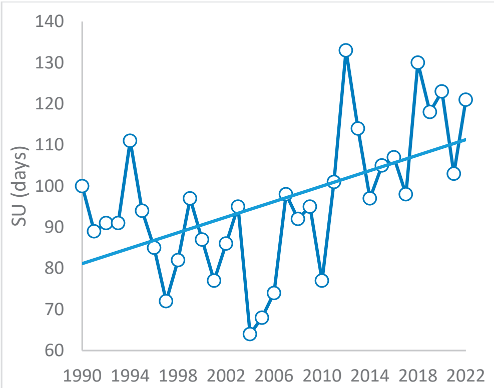

Figure 3. The daily maximum TX>25°C (SU) from 1990-2022

The warm spell duration index (WSDI) showed a significant increasing trend with a rate of 15.2 days per 2010-2022 period ($p < 0.05$) (Figure 4).

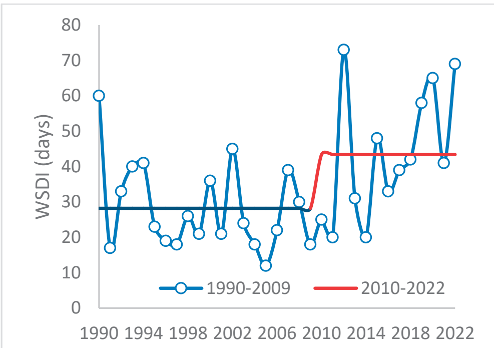

Figure 4. The warm spell duration indicator (WSDI) from 1990-2022

The cold spell duration indicator (CSDI)-annual count at least 6 days consecutive when TN<10th percentile showed a decrease, with a rate of 12.6 days/2010-2022 period ($p < 0.05$).
Among the indices based on precipitation Maximum 1-day precipitation (Rx1day) showed decreasing and obvious inter-decadal changes with increasing rates of 3.59 and respectively maximum 5-day precipitation (Rx5day) showed increasing trends 3.56 mm/2010-2022 period ($p<0.01$).
The Rx1day index had higher values in the period 1990-2009 and showed statistical significance ($p<0.05$), and the Rx5day index in the same period had lower values and did not present statistical significance (Figure 5).

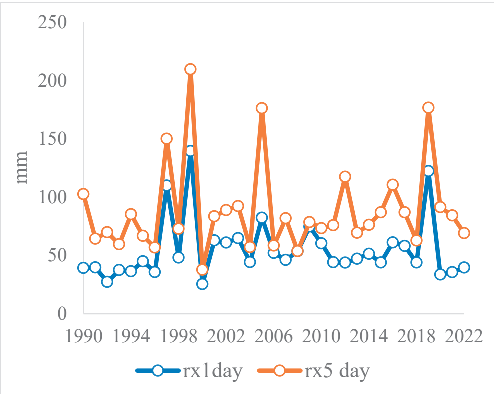

Figure 5. The rx1 day and rx5 day from 1990-2022

Methods for identifying signals in the configurations of precipitation-derived indices are also based on thresholds using specified values of precipitation amounts and thresholds using percentiles of the distribution of precipitation values.
Compared to the precipitation from the period 1990-2009, for the period 2010-2022, the trend is increasing with a rate of 1.64 mm/year, and for the precipitation during the vegetation period, the uneven distribution and the reduction of 2.27 mm/year can be observed (Figure 6).
Based on the results of annual trends in precipitation indices, an increasing trend of 0.21 mm/year was observed for the period 2010-2022, which may suggest that the annual number of days with heavy precipitation, number of days with dry and wet periods increased, compared to 1990-2009.

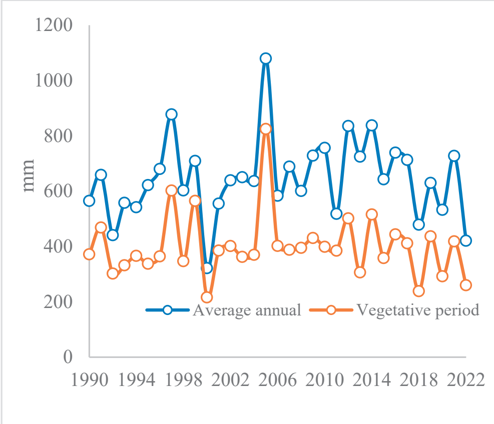

Figure 6. The annual amount and during the vegetation period of precipitation from the period 1990-2022

The indices R95p (Very Wet Days) and R99p (Extremely Wet Days) showed decreasing trends of 1.49 during 2010-2022, respectively, the increasing trend of 1.33 in R99p, data according to the literature (Donat et al., 2013a, 2013b; Zhou et al., 2016).
The maximum number of consecutive dry days (CDD) with RR<1mm and the maximum consecutive wet days with RR≥1mm (CWDrecorded in the period 2010-2022, also increasing values of 3.2 days, respectively 0.39 days (Figure 7).

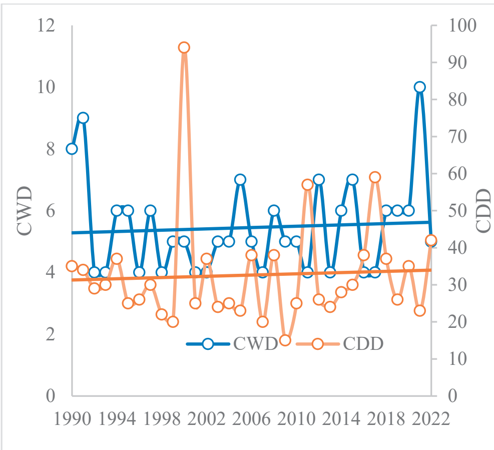

Figure 7. The CDD and CWN from 1990-2022

Due to the high temperature regime in the months of April and May, the flowering of the vines started earlier, by approximately 10-14 days compared to the normal period.

In general, flowering started almost simultaneously in all vinifera varieties, with differences of only 1-3 days between varieties.

The very high temperatures recorded in the air (which exceeded 35°C and even 40°C in the months of July and August) but especially those recorded on the surface of the soil which often exceeded 50°C and even 60°C determined a strong evaporation of water from the soil, leading to the phenomenon of pedological drought in the months of August and September.

The long period of drought associated with a high thermal regime and an air hygroscopicity of less than 40%, installed during the period of intense growth of shoots and grapes determined a drastic reduction in the growth of shoots, a pronounced debilitation of the buds and an early termination of grape growth (the weight of the grape, in most varieties, is lower by 40-50%). These changes in the wine-growing climate determined an advance development of the vegetative phenophases, especially of the fallow phenophases and the ripening of the grapes (by approximately 1-5 days), which influenced especially tfe quality of the grape production

Regarding the quality of grape production, the changes in the wine-growing climate led to an increase in the accumulation of sugars in the grapes, against the background of a drastic reduction in total acidity. This caused the glucoacidimetric index to take on high values, far above the optimal values for obtaining typical and high quality wines.

CONCLUSIONS

Annual increasing trends were recorded namely: TNn, TNx, TXx, ID, and SU, while decreasing trends were observed in three indices (ie, TXx and FD).

The frequency indices of nocturnal extremes (TN10p and TN90p) were higher than those for daytime extremes (TX10p and TX90p), ($p<0.05$).

While all indices suggest a constant heating of both minimum and maximum temperatures, a decrease in TXn may suggest that the coldest daytime temperature has decreased annually. In conclusion, we can say that the warming trend of the viticultural climate manifested by the recording of excessive temperatures since April and until the ripening of the grapes led to an advance triggering of all the vegetative phenophases and to a standardization of the moment of their triggering in the vinifera varieties.The grape production has been influenced by climate change. High temperatures and precipitation in lower quantities have affected grape production and vegetative growth is lower.

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RELIMINARY RESEARCH ON THE GRAFTING AFFINITY OF SOME NEW GRAPEVINE CULTIVARS CREATED AT RDSVO ODOBESTI ON DROUGHT-RESISTANT ROOTSTOKCS

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Abstract

The global warming of the climate, a phenomenon that has significantly characterized the last decades, has considerably influenced the evolution of the thermal and water regime annually and during the growing season in the viticultural ecosystem of the Odobeşti vineyard, the atmospheric and pedological drought characterizing the last four years of viticulture. In this context, the use of drought-resistant rootstocks for grafting is one of the solutions to counteract this extreme phenomenon that is increasingly present in the wine-growing areas of southern Moldova. In the present paper, preliminary results are presented regarding the grafting affinity of three new vine varieties created at RDSVO Odobești ('Putna', 'Măgura' and 'Vrancea'), on three rootstocks with drought tolerance obtained by Romanian viticultural research ('Drăgășani 70 M.', 'Crăciunel 71 Bl.', 'Ruggeri 140 Vl.'). For comparison, the rootstock 'Berlandieri x Riparia Sel.Oppenheim 4 –4 Bl.' was used, with the widest use for grafting in the Odobești wine-growing area. The preliminary results obtained show a good and very good grafting affinity on 'Ruggeri 140 Vl.' rootstock. for all three varieties studied.

Key words: *rootstock, grafting affinity, drought.*

INTRODUCTION

One of the basic conditions that must be taken into account when choosing rootstock varieties is their affinity with *Vitis vinifera* cultivars. A good affinity between grafts and rootstocks can guarantee obtaining grafted vines with complete concretion, long life and increased yield.

The broad spectrum of influence under which the affinity between grafts and rootstocks is manifested has led to its subdivision into morphological affinity and physiological affinity (Dalmasso, 1950), grafting affinity and production affinity (Zimmerman, 1959). Grafting affinity is an essential condition in the production of good quality viticultural planting material, while production affinity is the basic requirement for the establishment of new vine plantations, with superior technological potential. Scientific research carried out over time has demonstrated once again that in the case of grapevine grafting, there is a mutual influence of the grafted partners (Southey, 1992; Tița, 1998; Stoian et al., 2004; Vršič et al., 2004; Hamdan et al., 2010; Korkutal et al., 2011; Köse et al., 2014; Grigolo et al., 2021; Ungureanu et al., 2021). Thus, the rootstock exerts a strong influence on growth (Constantinescu et al., 1966; Grecu, 1980), fertility (Huglin, 1958), resistance to drought, at minimum temperatures, productivity (Martin et al., 1973; Țardea and Rotaru, 2003), as well as the oenological potential (Taran et al., 2017).

The errors committed as a result of the incorrect choice of rootstock varieties for grafting vinifera varieties cannot be corrected and as a result, productivity decreases, the resistance of the stumps to chlorosis, drought and frost is reduced, which later causes the appearance of voids and the premature disappearance of wine plantations (Ungureanu et al., 2021). In the context of current climate changes that have led to the significant manifestation of the drought phenomenon in many wine-growing areas (Cichi, 2006; Gladstones, 2011; Roehrdanz and Hannah, 2016; Van Leeuwen and Darriet, 2016; Santos et al., 2020; Pușcalău et al., 2021), the use of rootstocks with drought resistance is increasingly required (Mărculescu et al., 2006; Bekar, 2019). Establishing the most appropriate varieties of rootstock for grafting the newly created vinifera cultivars is one of the main objectives for the establishment of new wine plantations, justifying the current research on establishing the affinity of grafting and the affinity of production of these cultivars on different cultivars of rootstock.

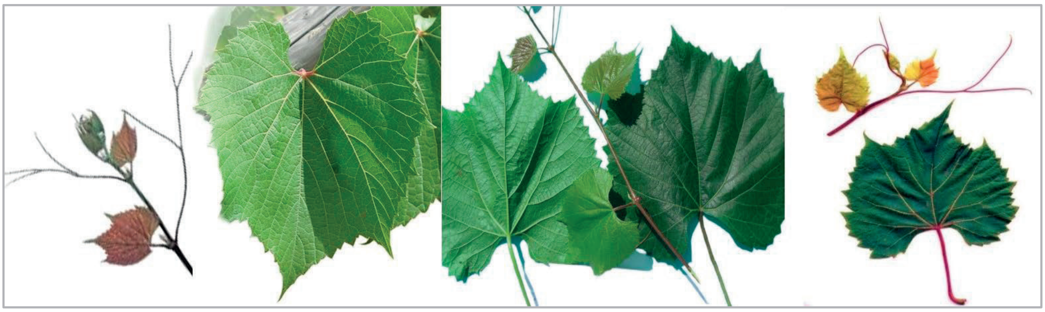
MATERIALS AND METHODS

The research took place at RDSVO Odobești in the period 2022-2023 and included the organization of experimental variants in which two main factors were taken into account: grafted *V. vinifera* cultivars and rootstock cultivars. For this purpose, three new vinifera cultivars created at RDSVO Odobești were used: ′Vrancea′ - a cultivar for white wines, obtained from crossing the hybrid combination (′Traminer′ x ′Armaș′) with the ′Fetească regală′ cultivar, approved in 2018, ′Măgura′ - a tinctorial cultivar for red wines, obtained from the crossing of the cultivars ′Băbească neagră′ x (′Merlot′ x ′Alicante Bouschet′), approved in 2014 and ′Putna′ - a cultivar for table grapes, obtained from the crossing of the cultivar ′Ceauș′ with the cultivar ′Muscat de Alexandria′, approved in 2014 (Figure 1).

The vine cultivars mentioned were grafted on three rootstock clones with drought resistance obtained in Romania: ′Berlandieri x Riparia - Sel. Drăgășani 70 M.′ supplied by RDSVO Drăgășani, ′Teleki 8 B - Sel. Crăciunel 71 Bl.′ supplied by RDSVO Blaj and ′Ruggeri 140 - Sel. 59 Vl.′ provided by RDIVO Valea Călugărească (Figure 2). The rootstock ′Berlandieri x Riparia Sel.Oppenheim 4 - clone 4 Bl′ was used as a control.

a) b) c)

Figure 1. The *V. vinifera* cultivars studied: a) ′Putna′; b) ′Măgura′; c) ′Vrancea′



a) b) c)

Figure 2. Rootstock cultivars studied: a) ′Sel. Drăgășani 70 M′; b) ′Sel. Crăciunel 71 Bl.′; c) ′Ruggeri 140 - Sel. 59 Vl.′

From the combination of the three *Vitis vinifera* graft cultivars with the three rootstock cultivars, 12 experimental graft/rootstock variants resulted, each with three repetitions.
The forcing of the grafted cuttings was carried out by the method with total stratification with sawdust and external heating. The planting of cuttings in the vine nursery was done in linear beds of soil made in the spring, on which a drip irrigation hose was placed.
To determine the grafting affinity, after forcing and hardening the grafted cuttings, the formation of the circular callus at the grafting point, the growth of the shoots from the grafts and the formation of roots at the base of the rootstock were observed in particular. After harvesting the vines from the vine nursery, the yield and quality of the planting material was monitored for each variant of the experiment.
The obtained data were statistically processed for analysis of variance using the FoxPro/LAN 2.0 statistical analysis program.

RESULTS AND DISCUSSIONS

Through the determinations made after forcing and tempering the grafted cuttings, the experimental data presented in Table 1 (Figure 3) were obtained. They show the fact that the percentage of grafted cuttings with circular callus at the grafting point, in all the vinifera cultivars studied, was higher in the case of their grafting on rootstocks ′Sel. Drăgășani 70 M.′ (95.6%) and ′Ruggeri 140 - Sel. 59 Vl.′ (93.3%).

Figure 3. Aspects of the process of grafting and forcing grafted vines

The cuttings grafted on the rootstock ′Sel. Crăciunel 71 Bl.′, showed a lower callus index (83.5%), a value close to the control rootstock ′Berlandieri x Riparia Sel.Oppenheim 4 - 4 Bl.′ (78.8%). After tempering, before planting in the school of vines, for all the vinifera varieties studied, the average percentage of cuttings with shoots from grafts was higher in the case of using the rootstock ′Ruggeri 140 - Sel. 59 Vl.′ (84.7%), followed by ′Sel. Drăgășani 70 M.′ (81.9%), and smaller on the rootstock ′Sel. Crăciunel 71 Bl.′ (73.4%), closer to the value recorded by the control rootstock (68.6%).
In terms of root formation at the base of the grafted cuttings after hardening before planting in the vine nursery, the obtained data demonstrate that the type of rootstock influences both the duration of the root formation period and the number of rooted cuttings.

Table 1. Data obtained from forging and hardening grafted cuttings

Grape cultivar	Rootstock cultivar: Sel. Drăgășani 70 M.	Sel. Crăciunel 71 Bl.	Sel. Ruggeri 140 - 59 Vl	Sel.Opp. 4 - 4 Bl. (control)
Cuttings with circular callus at grafting point (%)				
′Putna′	95.65	92.27	97.22	85.02
′Vrancea′	97.10	76.98	91.66	67.64
′Măgura′	94.06	81.45	91.16	83.84
Average	95.60	83.56	93.34	78.83
Cuttings with shoots from grafts started to grow (%)				
′Putna′	83.85	78.49	91.66	80.34
′Vrancea′	91.30	76.98	91.66	59.80
′Măgura′	70.55	64.72	70.71	65.66
Average	81.90	73.40	84.67	68.60
Cuttings with roots at the base of the rootstock, after hardening (%)				
′Putna′	64.62	47.69	68.68	44.84
′Vrancea′	57.97	45.45	62.87	32.35
′Măgura′	60.29	41.41	64.71	39.39
Average	60.96	44.85	65.42	38.86

For all the *vinifera* cultivars studied, the number of cuttings with roots varied depending on the cultivar of rootstock used for grafting.
The percentage of grafted cuttings with roots at the base, before planting in the vine nursery, in the three vinifera cultivars studied, was higher at the variants in which the rootstock 'Ruggeri 140 - Sel 59 Vl.' was used for grafting (average value 65.42%) and rootstock 'Sel. Drăgășani 70 M.' (average value 60.96). If the was used for grafting the rootstock 'Sel. Crăciunel 71 Bl.', 44.85% of cuttings with roots were registered. The lowest number of cuttings with roots, in all the vinifera cultivars studied, was recorded in the cuttings grafted on the control rootstock 'Sel. Oppenheim. 4 - 4 Bl.' (38.86%).

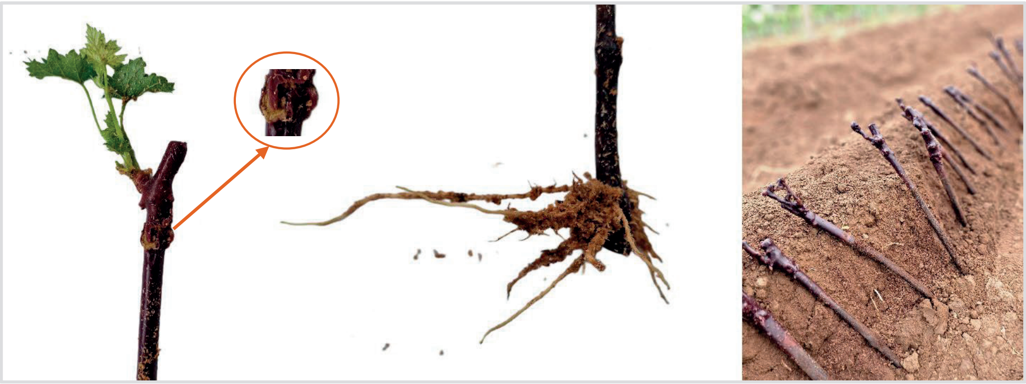


Figure 4. Aspects after forcing and hardening of grafted vines and planting in the vine nursery

Statistical interpretation of the experimental data obtained after forcing and hardening the grafted cuttings, shows distinctly significant differences regarding cuttings with circular callus at the grafting point for the 'Vrancea' grape cultivar grafted on 'Sel. Drăgășani 70 M.' and 'Ruggeri 140 - Sel. 59 Vl.' rootstocks, compared to the control rootstock (Table 2).

Table 2. Cuttings with circular callus for 'Vrancea' grape cultivar

Variant	Cuttings with circular callus at grafting point (%)			
	% vines with circular callus	%	Difference (%)	Significant
'Vrancea' x 'Sel. Drăgășani 70 M.'	97.10	143.53	29.45	**
'Vrancea' x 'Sel. Crăciunel 71 Bl.'	76.98	113.79	9.33	
'Vrancea' x 'Ruggeri 140 - Sel. 59 Vl.'	91.66	135.49	24.01	**
'Vrancea' x 'Sel. Oppenheim 4-4 Bl.' (control)	67.65	100.00	0.00	-
DL (P 5%)	14.5977			
DL (P 1%)	22.1051			
DL (P 0.1%)	35.5112			

In the case of grafting on the rootstock 'Berlandieri x Riparia - Sel. Drăgășani 70 M.', the Vrancea variety did not show significant differences.
In contrast, the 'Putna' and 'Măgura' grape cultivars did not show significant differences compared to the control regarding the cuttings with circular callus at the grafting point. Regarding cuttings with shoots from grafts started to grow, the 'Vrancea' grape cultivar showed distinctly significant differences compared to the control rootstock in the case of grafting on 'Sel. Drăgășani 70 M.' and 'Ruggeri 140 - Sel 59 Vl.' rootstocks, and significant differences in the case of grafting on rootstock 'Sel. Crăciunel 71 Bl.' (Table 3).
'Putna' and 'Măgura' *vinifera* cultivars did not show significant differences compared to the control rootstock in terms of the percentage of cuttings with shoots from grafts started to grow.

Table 3. Cuttings with shoots from grafts started to grow for ′Vrancea′ grape cultivar

Variant	Cuttings with shoots from grafts started to grow (%)			
	% vines with shoots	%	Diference (± %)	Significant
′Vrancea′ x ′Sel. Drăgășani 70 M.′	91.30	152.68	31.50	**
′Vrancea′ x ′Sel. Crăciunel 71 Bl.′	76.98	128.73	17.18	*
′Vrancea′ x ′Ruggeri 140-Sel. 59 Vl.′ Vl.′	91.66	153.28	31.86	**
′Vrancea′ x ′Sel. Oppenheim 4-4 Bl.′ (control)	59.80	100.00	0.00	-
DL (P 5%)	14.0157			
DL (P 1%)	21.2238			
DL (P 0.1%)	34.0953			

The variance analysis regarding the percentage of cuttings with roots at the base of the rootstock established very significant differences for the ′Vrancea′ grape cultivar in the case of grafting on ′Sel. Drăgășani 70 M.′ and ′Ruggeri 140 - Sel 59 Vl.′ rootstocks, and significant differences in the case of grafting on ′Sel. Crăciunel 71 Bl.′ compared to control rootstock ′Sel. Oppenheim 4 - 4 Bl.′ (Table 4).

Table 4. Cuttings with roots at the base of the rootstock, after hardening for ′Vrancea′ grape cultivar

Variant	Cuttings with roots at the base of the rootstock, after hardening (%)			
	% vine with roots	%	Diference (± %)	Significant
′Vrancea′ x ′Sel. Drăgășani 70 M.′	57.97	179.20	25.62	***
′Vrancea′ x ′Sel. Crăciunel 71 Bl.′	45.45	140.49	13.10	*
′Vrancea′ x ′Ruggeri 140 - Sel. 59 Vl.′ Vl.′	62.87	194.34	30.52	***
′Vrancea′ x ′Sel. Oppenheim 4 - 4 Bl.′ (control)	32.35	100.00	0.00	-
DL (P 5%)	9.9608			
DL (P 1%)	15.0835			
DL (P 0.1%)	24.2312			

For the ′Măgura′ grape cultivar, the analysis of variance established very significant differences for cuttings with roots at the base of the rootstock in the case of grafting on the rootstocks ′Sel. Drăgășani 70 M.′ and ′Ruggeri 140 - Sel. 59 Vl.′, compared to the control rootstock Sel. Oppenheim 4 - 4 Bl.′ (Table 4). The grape cultivar ′Putna′ did not show significant differences compared to the control rootstock in terms of the percentage of cuttings with roots at the base of the rootstock.

Table 5. Cuttings with roots at the base of the rootstock, after hardening for ′Măgura′ grape cultivar

Variant	Cuttings with roots at the base of the rootstock, after hardening (%)			
	% vine with roots	%	Diference (±%)	Significant
′Măgura′ x ′Sel. Drăgășani 70 M.′	60.21	152.86	20.82	***
′Măgura′ x ′Sel. Crăciunel 71 Bl.′	41.41	105.13	2.02	ns
′Măgura′ x ′Ruggeri 140-Sel. 59 Vl.′	64.90	164.76	25.51	***
′Măgura′ x ′Sel. Oppenheim 4-4 Bl.′ (control)	39.39	100.00	0.00	-
DL (P 5%)	6.2634			
DL (P 1%)	9.4846			
DL (P 0.1%)	15.2368			

The basic criterion that determines the establishment of grafting affinity is the yield and quality of the grafted vines (Figure 5). The data presented in Table 6 confirm the fact that in the combined graft/rootstock interaction, the best results for all the *vinifera* cultivars studied were obtained in the case of grafting on the rootstock ′Ruggeri 140 - Sel. 59 Vl.′: ′Putna′ (69.1%); ′Vrancea′ (62.4%); ′Măgura′ (61.9%), followed by the rootstock ′Sel. Drăgășani 70 M.′:

ʹMăguraʹ (48.5%); ʹPutnaʹ (48.3%); ʹVranceaʹ (43.5%). The lowest yields were recorded in the case of the ʹVranceaʹ and ʹPutnaʹ cultivars grafted on the control rootstock ʹSel. Oppenheim 4 - 4 Bl.ʹ (21.5% and 30.8%, respectively), and the ʹMăguraʹ cultivar recorded the lowest yield in the case of grafting on the rootstock ʹSel. Crăciunel 71 Bl.ʹ (23.9%). The table grape cultivar ʹPutnaʹ recorded the highest average value of yield in standard vines (45.9%).

Figure 5. Aspects after forcing and hardening of grafted vines and planting in the vine nursery

Table 6. The yield in standard vines following the classification of vines from native *Vitis vinifera* cultivars grafted on rootstock cultivars with drought resistance (%)

The grape cultivar	The rootstock cultivars				Average/ cultivar
	ʹSel. Drăgășani 70 M.ʹ	ʹSel. Crăciunel 71 Bl.ʹ	ʹRuggeri 140 - 59 Vl.ʹ	ʹSel. Oppenheim 4 - 4ʹ (control)	
ʹPutnaʹ	48.35	35.35	69.08	30.82	45.90
ʹVranceaʹ	43.48	24.68	62.37	21.53	38.02
ʹMăguraʹ	48.55	23.94	61.87	33.33	41.92
Average/rootstock	46.79	27.99	64.44	28.56	41.94

The synthesis of experimental data regarding the influence of rootstock varieties with drought tolerance on the yield results of the grafted planting material for the ʹPutnaʹ table grape cultivar are presented in Table 7.

Table 7. The yield of planting material obtained after classification the vines for the ʹPutnaʹ cultivar

Variant	Yield obtained in the vine nursery %	%	Diference (± %)	Significant
ʹPutnaʹ x ʹSel. Drăgășani 70 M.ʹ	48.35	156.88	17.53	*
ʹPutnaʹ x ʹSel. Crăciunel 71 Bl.ʹ	35.35	114.70	4.53	ns
ʹPutnaʹ x ʹRuggeri 140 - Sel. 59 Vl.ʹ Vl.ʹ	69.08	224.14	38.26	***
ʹPutnaʹ x ʹSel. Oppenheim 4 - 4 Bl.ʹ	30.82	100.00	0.00	-
DL (P 5%)	14.4687			
DL (P 1%)	21.9097			
DL (P 0.1%)	35.1972			

Thus, the statistical interpretation of the experimental data obtained after classification the grafted vines, shows that the rootstock ʹRuggeri 140 - Sel. 59 Vl.ʹ exerted a positive influence in the case of the ʹPutnaʹ cultivar, in the form of a 38.3% increase in grafted planting material, compared to the control. A positive influence on the ʹPutnaʹ cultivar was also exerted by the rootstock ʹSel. Drăgășani 70 M.ʹ, with a

17.5% increase in grafted planting material, compared to control ′Sel. Oppenheim 4 - 4 Bl.′.
In the case of the ′Vrancea′ white wine cultivar, distinctly significant differences were recorded for the rootstock ′Ruggeri 140 - Sel. 59 Vl.′, with an increase of grafted planting material of 40.8%, compared to the control rootstock (′Sel. Oppenheim 4 - 4 Bl.′). Significant differences were also recorded for the rootstock ′Sel. Drăgășani 70 M.′, with an increase of grafted planting material of 17.5%, compared to the control rootstock. (Table 8).

Table 8. The yield of planting material obtained after classification the vines for the ′Vrancea′ cultivar

Variant	Yield obtained in the vine nursery %	%	Diference (± %)	Significant
′Vrancea′ x ′Sel. Drăgășani 70 M.′	43.48	201.95	21.95	*
′Vrancea′ x ′Sel. Crăciunel 71 Bl.′	24.68	114.63	3.15	ns
′Vrancea′ x ′Ruggeri 140 - Sel. 59 Vl.′	62.37	289.69	40.84	**
′Vrancea′ x ′Sel. Oppenheim 4 - 4 Bl.′ (control)	21.53	100.00	0.00	-
DL (P 5%)	17.1550			
DL (P 1%)	25.9776			
DL (P 0.1%)	41.7322			

The statistical interpretation of the experimental data regarding the influence of rootstock varieties with drought tolerance on the yield results of the grafted planting material for the ′Măgura′ tinctorial red wine cultivar are presented in Table 9.

Table 9. The yield of planting material obtained after classification the vines for the ′Măgura′ cultivar

Variant	Yield obtained in the vine nursery %	%	Diference (± %)	Significant
′Măgura′ x ′Sel. Drăgășani 70 M.′	48.55	145.66	15.22	*
′Măgura′ x ′Sel. Crăciunel 71 Bl.′	23.94	71.83	-9.39	ns
′Măgura′ x ′Ruggeri 140 - Sel. 59 Vl.′	61.87	185.63	28.54	***
′Măgura′ x ′Sel. Oppenheim 4 - 4 Bl.′ (control)	33.33	100.00	0.00	-
DL (P 5%)	10.2755			
DL (P 1%)	15.5601			
DL (P 0.1%)	24.9968			

And for the ′Măgura′ cultivar, the data obtained show that the rootstock Ruggeri 140 - Sel 59 Vl. exerted a very significant positive influence, in the form of a 28.5% increase in grafted planting material, compared to the control.
The ′Sel. Drăgășani 70 M.′ rootstock also exerted a positive influence on the ′Măgura′ cultivar, with an increase of grafted planting material of 17.5%, compared to the control ′Berlandierii x Riparia Sel. Oppenheim 4 - 4 Bl.′

CONCLUSIONS

Preliminary research on establishing the grafting affinity of the three vinifera cultivars in interaction with the three rootstocks with drought resistance demonstrated the existence of significant differences compared to the control rootstock.

The ′Vrancea′ cultivar showed distinctly significant differences regarding the percentage of grafted cuttings with circular callus at the grafting point and the percentage of shoots starting to grow from the graft, in the case of grafting on ′Sel. Drăgășani 70 M.′ and ′Ruggeri 140 - Sel. 59 Vl.′ rootstocks.
The cultivars ′Vrancea′ and ′Măgura′ registered very significant differences compared to the control rootstock for the percentage of cuttings with roots at the base of the rootstock in the case of grafting on ′Sel. Drăgășani 70 M.′ and ′Ruggeri 140 - Sel. 59 Vl.′ rootstocks.
The highest yield of standard planting material for all the studied cultivars was recorded in the case of their grafting on ′Ruggeri 140 - Sel. 59 Vl.′ rootstock (average value 64.4%).
The cultivar for table vines ′Putna′ and the cultivar for tinctorial red wines ′Măgura′

recorded very significant and significant differences regarding the yield of standard vines in the case of grafting on the rootstock ′Ruggeri 140 - Sel. 59 Vl.′, respectively on the rootstock ′Sel. Drăgăşani 70 M.′

The cultivar for white wines ′Vrancea′ showed distinctly significant and significant differences in the case of grafting on the rootstock ′Ruggeri 140 - Sel 59 Vl.′, respectively on the rootstock ′Sel. Drăgăşani 70 M.′.

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CHALLENGES CAUSED BY CLIMATE CHANGE IN ORGANIC GRAPE PRODUCTION IN THE SOUTHWESTERN WINE-GROWING REGION

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Abstract

In recent years, the adverse effects of climate change on agricultural productivity have been increased drastically. As a result of this change, in the Southwestern wine-growing region, which has a transitional Mediterranean climate, the possibilities for organic grape production are hampered. Productivity and profitability decrease due to uncontrollable events caused by climatic anomalies. Rainfalls of 119 mm, and 218.2 mm, per m² were recorded during the study period. These rainfalls accumulated during the autumn-winter period in combination with the low temperatures of the atmosphere and the soil create prerequisites for stopping rhizogenesis and suction activity of the root system in the spring, and this reflects on the growth processes of the vines. All this has a direct impact on the accumulation of biomass, which has a direct impact on the duration of the growing season. These components lead to significant changes in the intensity of growth processes in the vine. From here follows the formation of yields with inappropriate technological quality.

Key words: *climate, organic viticulture, phenology, Shiroka Melnishka loza.*

INTRODUCTION

According to statistical data, Southwestern Bulgaria occupies about 30% of the total area of the vineyards in the country (Bulgarian Ministry of Agriculture, 2020). Although organic viticulture still represents a small share of the total wine production in the region, recent years have seen a high growth of interest in this direction. Of the total agricultural area in the country, about 8% is cultivated organically, and approximately 3% of them are vineyards.

Sustainable development and the green economy is a key priority at the international level, especially in the context of climate change and environmental protection. In this regard, the EU Green Deal is an ambitious initiative that aims to transition to sustainable and climate-neutral economic development by 2050. Within this strategy, organic viticulture plays a key role. The EU aims for 25% of agricultural areas to be cultivated organically by 2030, which provides significant opportunities for the development of organic viticulture in Bulgaria.

Climatic conditions have key importance for the growth of vines (Roychev, 2012). According to the research of Lazarevskiy (1946), the temperature threshold at which the vine begins its active vegetation is very close to the temperature at which all biological activity stops, known as "biological zero degree". After conducting research in different but geographically limited areas, Arnaudova & Popov (2010) found that temperature plays a key role in the growth and fruiting of grapevines. As a result, when developing schemes for zoning and optimal distribution of vines, temperature is taken as the main and decisive factor. This highlight the importance of climatic conditions and their impact on the successful development of the viticulture industry. In this regard, the grower must take measures to reduce the risks of climate change and maintain agricultural productivity and the profitability of the agricultural holdings. This seems important for the overall sustainability of the vine and wine sector and agriculture in general (Filippo & Sciancalepore, 2022).

Global warming causes many disturbances in the viticultural ecosystem, grape varieties are forced to change their annual cycle of vegetation, with consequences, most often negative for the quality and quantity of grape production and also for the produced wines (Tănase et al., 2019).

The European Environment Agency indicates that the overall impact of climate change could significantly reduce EU agriculture (up to 16%

revenue loss by 2050), with large regional variations (Fetting, 2020).

Even in regions that do not have a decrease in precipitation, an increase in air temperature will lead to higher evapotranspiration (Seneviratne et al., 2010).

For this reason, the agricultural sector must build capacity to adapt to the increasingly dry and warm conditions brought about by climate change.

In the complex soil-plant relationship, plants play a key role as solar energy converters. Proper tillage of the soil creates favorable conditions for appropriate biological processes to take place, to improve the work of soil microorganisms and nutrients to be in an easily digestible form, to reduce weed vegetation and maintain fertility.

Organic fertilization and comprehensive plant protection are key components in sustainable viticulture, which focus on the healthy and sustainable development of vines. Organic fertilizers are designed to enrich the soil and plants with natural nutrients, thus supporting healthy growth and resistance of vines to stressful conditions (Coll et al., 2011).

This not only reduces the risk of environmental pollution, but also supports the development of biodiversity in the vineyards (Muneret et al., 2019).

Modern research highlights the importance of combining organic fertilization and total plant protection to achieve optimal results in viticulture. Such methods not only improve the quality of the grapes, but also contribute to the long-term sustainability of the vineyards (Provost & Pedneault, 2016; Volanti et al., 2022).

Weed control in organic viticulture requires an integrated approach that combines different methods and practices to ensure healthy vine growth without the usage of herbicides. By planting certain types of grasses or cover crops between the rows of vines, a competitive environment is created for weeds. These plants can compete with weeds for light, water and nutrients, thus reducing their spread (Novara et al., 2018; 2021; Guerra et al., 2022).

Applying organic mulch helps retain soil moisture, provides additional organic matter as it breaks down, and increases soil temperature. (Fraga & Santos, 2018).

Providing nutrients to organically grown vines requires a specific and well-thought-out approach. In the rhizosphere, the zone around the roots of plants, the microflora produces biologically active substances that stimulate plant growth and development, facilitating the exchange of substances. Soil color affects vegetative processes, root growth and reproductive functions. Dark soils help the grapes ripen faster (Keller, 2010). In the Northern regions, when the grapes are positioned further from the earth's surface, there is a lower content of anthocyanins and aromatic substances.

From a practical point of view, there is a clear relationship between soil and air temperatures that directly influence and govern the nutrition of vine plants and the quality of grape production.

The aim of the present study is to determine the influence of “Humat Rost“ on the stress of drought and high temperatures on the vegetative and reproductive characteristics of the vine.

MATERIALS AND METHODS

A vineyard of the the Bulgarian red wine cultivar ‘Shiroka Melnishka’, located in Petrichko-Sandanski basin, was used as the object of this research.

The vines are 30 years old with planting distances 2.5/1.2 m.

The variants, which are included in this research are: V_0 – control; V_1 – 30 cm length of the shoots with control of 10 inflorescences and V_2 – Phase of berry growing with control of 10 bunches.

All variants without the control were treated with “Humat Rost” in dosage of 0.400 mL per decare, four times, 8 days before flowering, after mass flowering, in the phase of berry growth and 15 days after the berry growth phase.

“Humat Rost” is organic product which contains: 78-82% organic matter; 5.5-7.8% humic and fulvic acids; 1.2-4% nitrogen; 2% potassium; 1.9% humic and fulvic hydrocarbons; 1.2% phosphorus; 0.06% magnesium and 0.15% calcium.

The vineyard is grown under non-irrigated conditions.

The research was conducted in 2022-2023.

The phenological observations for each variant were collected from 40 vines, arranged in 4 replicates of 10 vines.

The data for temperature (ºC) and precipitation (mm) was taken from a Meteobot automatic weather station located 1 km from the vineyard. The correlations between the studied variables are obtained by regression analysis in MS Excel.

RESULTS AND DISCUSSIONS

The orography directly affects the formation of the climate in the area. The average altitude of the basin is about 200 m. Its length is 42 km, and its width reaches 13 km.

Petrichko-Sandanski basin has a transitional Mediterranean climate and is one of the warmest in Bulgaria. Here are the highest average annual temperatures for the country. For 2022, the average annual temperature in Sandanski was 14.8°C, with summer temperature between 26.8 -28.1 (Table 1). The maximum temperature measured being 39.6°C, in the month of July (Table 2). The lowest temperature was recorded in the month of January -8.7°C (Table 3).

In the second year of the experiment, differences in seasonal temperature dynamics were found for 2023. The average annual temperature in the area of the Sandanski station is set to be higher, and we assume that this will be due to the warmer winter, spring, summer and autumn.

Table 1. Average air temperatures (ºC) by season in Sandanski

Year	Winter temperature °C	Spring temperature °C	Summer temperature °C	Autumn temperature °C	Total temperature sum	Average annual temperature °C	Amplitude °C
2022	3.2	13.7	26.8	15.7	5292	14.8	28.1
2023	7.0	12.1	28.1	21.0	6937	17.0	34.0

Table 2. Maximum monthly air temperatures (ºC) in Sandanski

Year	Month											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2022	16.8	18.5	22.8	27.7	34.6	35.9	39.6	38.2	33.8	27.2	25.8	7.9
2023	18.2	21.3	23.5	22.8	28.1	34.3	40.8	33.5	30.7	29.1	24.9	17.4

Table 3. Minimal monthly air temperatures (ºC) in Sandanski

Year	Month											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
2022	-8.7	-4.1	-5.3	1.5	7.5	13.3	14.0	12.0	5.9	0.2	0.1	-3.2
2023	-1.2	-6.6	-0.4	1.3	8.9	12.0	13.5	16.1	14.4	5.7	-5.4	-3.4

The average winter temperature in 2023 is 7.0°C, (Table 1), and the maximum temperature measured was 40.8°C, in the month of July (Table 2). The lowest temperature was recorded in the month of February - 6.6°C (Table 3). Weather data also shows also shows one change that is being observed, which is the formation of low temperatures in inappropriate phases of rest in the vine.

Here we must emphasize that a temperature such as -6.6°C is suitable for the period of vernalization at the buds of the vine, which coincides with the dormancy period in the month of January. Lower temperature conditions create conditions for prolonging periods of dormancy, and this prevents the passage into the phase of forced dormancy. These temperatures are not typical for the region of the Petrichko-Sandanski basin, creating prerequisites for intervention and change in the phenological cycles of the grapevine varieties that are grown in this region of the country. This trend was observed throughout the 2023 growing season within the study area and would affect the phenolic maturity and yield potential of the grape. The climate in Sandanski municipality is characterized by large diversity. It is characteristic of the winter months that the average monthly temperatures in the plain and foothills are higher. Therefore, the precipitation in the winter months is mostly rain. Spring comes early in this part of the country. The whole area in summer has the longest sunshine in our country. Precipitation and relative air humidity in the Sandanski region are the lowest in the country. Autumn is relatively mild. It is characterized by sunny days that last until the end of November, and very often throughout the winter. In the distribution of precipitation by season, a trend characteristic of the Mediterranean climate is also noticeable. The most significant amount of precipitation is formed in autumn, winter and spring. At least they are during the summer months, which creates conditions for longer droughts. This trend persists until the beginning of autumn. The annual amount of precipitation for 2022 is only 349 mm in Sandanski, which characterizes the year as extremely dry (Table 4).

Table 4. Precipitation regime in Sandanski for 2022-2023, mm

Year	Average rainfall winter	Average rainfall spring	Average rainfall summer	Average rainfall autumn	Annual rainfall	Month with maximum rainfall	Month with minimal rainfall
2022	78	68	84	119	349	XI	X
2023	126	92	52	196	767	I	IX

The maximum amount of precipitation was recorded in autumn – 119 mm. Compared to the precipitation for the winter-spring period of 2023, 218.2 mm is distinguished by a significant increase, as values compared to the previous year 2022. This increased water condition is positive for the grapevine in the upcoming vegetation, but from a phenological point of view, this huge water content in combination with the low temperatures of the atmosphere and the soil, create conditions for instability around the rhizosphere capsule. Water molecules displace the air via a capillary route by settling around the root hairs. All this leads to a momentary suspension of rhizogenesis and suction activity of the root system, and this reflects on the growth processes.

The data from the conducted phenological observations show that between the individual phases in the experience in the two years, significant differences are demonstrated between them (Table 5).

Table 5. Timings of the phenological phases of development in 2022-2023

Variants	Year	Bud burst	Appearance of 1st leaf	Appearance of 1st inflorescence	Flowering	Pea size berry	Veraison	Maturity	Leaf fall
V_0	2022	14 Apr.	25 Apr.	29 Apr.	04 Jun.	01 Jul.	17 Aug.	24 Sept.	05 Nov.
	2023	18 Apr.	28 Apr.	01 May	09 Jun.	08 Jul.	20 Aug.	-	02 Dec.
V_1	2022	14 Apr.	25 Apr.	29 Apr.	04 Jun.	02 Jul.	17 Aug.	24 Sept.	05 Nov.
	2023	18 Apr.	28 Apr.	04 May	09 Jun.	06 Jul.	18 Aug	-	02 Dec.
V_2	2022	16 Apr.	25 Apr.	29 Apr.	04 Jun.	01 Jul.	17 Aug.	24 Sept.	05 Nov.
	2023	18 Apr.	28 Apr.	04 May	09 Jun.	06 Jul.	20 Aug.	-	02 Dec.

The duration of the budburst phase is 4 days apart, and that of the flowering phase – 9 days in 2022, while in 2023 this was done in 14 days. A difference in the phase of berry growth was observed. For variants V_1 and V_2, it occurred two days earlier. A similar dependence was also observed during berry veraison in the second year of the experiment. As the difference that is formed is 5 days for V_1 and three days for V_2 compared to the control. The results of the phenological observations give us reason to summarize that the reason for the occurrence of the phenophases is due to three main factors, and these are the greater amount of precipitation in the winter-spring period combined with low temperatures, as well as reducing the yield by more than half compared to the vines in the control.

One of the main factors that directly affect the accumulation of biomass are: the different periods of controlling the yields, the availability of food resources and, last but not least, the duration of the growing season. These components lead to significant changes in the intensity of growth processes. This also has an effect on the amount of vine canes that are obtained after the pruning in autumn (Figure 1).

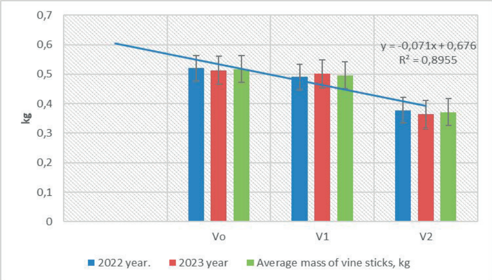

Figure 1. Mass of the vine canes, kg

The data show that the early shoot thinning V_1 has influenced within the experiment a reduction in the mass of the mature cane growth (Figure 2).

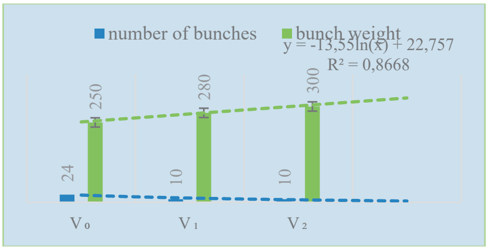

Figure 2. Number and mass of grapes (g)

As the effect was manifested on the average weight of a grape, which is 280 g and outperforms the control on this metric. Controlling the yield in the phase of grain growth in variant V_2 led to a greater mass of grapes of 300 g., which gives us reason to recommend the variants with controlled yield in the production of grapes. In the control, the average mass of the bunch remains the smallest. The data on the quantitative changes of the indicators that characterize the yield of grapes is shown below (Figure 3).

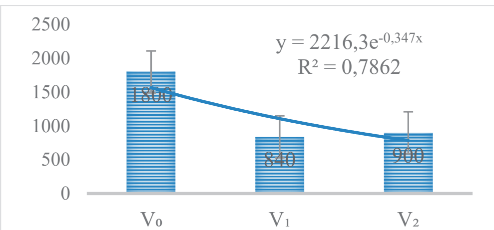

Figure 3. Grape yields, kg/decare

The yield of grapes according to variants is in the range from 840 kg to 1800 kg per decare.
The grape yield was highest at V_0, followed by V_2 as the lowest at and V_1. The yield difference between V_0 and V_1 is significant. In all variants, the yield is formed by spurs. The bunches average mass changes depending on the yield reduction and the phenophase, and also on the application of "Humat Rost". Bunches at V_2 had the largest mass, followed by V_1, and control V_0 had the smallest bunches. An increase in average vine yield leads to an increase in average decare yield, but not in average grape mass, this depends on the phenophase of yield reduction and vine nutrition.
Plant protection products approved for organic production were used to protect the plantation from diseases. They are described in the EU Regulation (2019).
In 2022, 8 plant protection treatments were done on the vines (Table 6).
Least costs to protect the vines from the causative agents of downy mildew and powdery mildew were made in 2023. The main reason for this is that, climatically, the year was rainy and the grape harvest was not reached.
The implemented scheme for the treatment of the vines and the summer pruning operations carried out have had a beneficial effect on the general state of the vineyard and in particular on the vegetative and generative organs of the vines. In the second year of the experiment, an attack from downy mildew and powdery mildew was found, which had a negative effect on the yield of grapes.
The production of organic grapes is only possible if we have a year with a normal distribution of rainfalls. The proposed plant protection scheme will have a positive effect on the vineyard. But in years with cataclysms like 2023, it will be necessary to notify the controlling authority and temporarily put the plantation into a transition period and treat it with synthetic products to protect the grapes from the two economically important vine diseases - downy mildew and powdery mildew.

Table 6. Scheme of treatments

2022	Treatments	2023	Treatments
15 April	Funguran – 0.2% Cosavet – 0.4% Airone – 0.3%	22 April	Funguran – 0.2% Cosavet – 0.4%
09 May	Cosavet – 0.4% Airone – 0.3%	03 May	Funguran – 0.2% Cosavet – 0.4%
20 May	Cosavet – 0.4% Airone – 0.3%	15 May	Airone – 0.3% Cosavet – 0.4%
29 May	Cosavet – 0.4% Airone – 0.3%	23 May	Airone – 0.3% Cosavet – 0.4%
10 June	Cosavet – 0.4% Airone – 0.3%	02 June	Airone – 0.3% Cosavet – 0.4%
23 June	Cosavet – 0.4% Funguran – 0.2%	18 June	Airone – 0.3% Cosavet – 0.4%
06 July	Cosavet – 0.4% Funguran – 0.2%	29 June	Airone – 0.3% Cosavet – 0.4%
20 July	Cosavet – 0.4%	-	-

CONCLUSIONS

The results obtained from the phenological observations give us reason to summarize that the reason for the occurrence of the phenophases is due to three main factors, which are the greater amount of precipitation in the winter and spring period combined with low temperatures, as well as the reduction of the yield by more than half relative to vines in the control. One of the main factors that directly affect the accumulation of biomass is the different periods of yield control in the vines, the availability of food resources and, last but not least, the duration of the growing season. These components lead to significant changes in the intensity of growth processes. An increase in average vine yield leads to an increase in average decare yield, but not in average grape mass, this depends on the phenophase of yield reduction and vine nutrition.

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THE LOCAL GRAPEVINE VARIETIES - A SOURCE OF TYPICITY, AUTHENTICITY, AND ADAPTABILITY, WITHIN THE FRAMEWORK OF SUSTAINABLE VITICULTURAL TECHNOLOGIES

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Abstract

The research was carried out over the 2020-2022 growing seasons and focused on specific local grape cultivars discovered in 2019 in the gardens or vineyards of amateur winegrowers in Alba County. Among these, 12 little-known or unknown local cultivars were studied, grouped based on grape quality and yield, and compared to well-known reference types widely distributed in the area. The local white wines were compared to the control 'Fetească regală', the red wine cultivars to 'Cabernet Sauvignon', and the fresh consumption cultivars to 'Chasselas dore'. The aim was to identifying the growth stages of local cultivars and identifies those that bud or bloom later, in order to mitigate risks associated with increasingly frequent climate variability. Additionally, the research focused on the main ampelographic characteristics, grape production quantity and quality, as well as disease and pest resistance, important indicators in the current context emphasizing cost and pollution reduction, and the production of healthier viticultural products. Most of the analysed cultivars displayed higher resilience against diseases and pests when compared to the control cultivars.

***Key words**: adaptability, grapevine, local cultivars, technology, typicity.*

INTRODUCTION

Viticultural technologies remain an ongoing challenge for winegrowers, who are constantly looking for solutions to adapt to the changing landscape of viticulture and comply with current issues in viticultural (Palliotti et al., 2014). Climate challenges, labour shortages, rising input costs, competition, and the professional market's more severe criteria all call for efficient solutions (Cheng et al., 2014). Within these circumstances, local grape emerge as viable choices due to their resistance and flexibility to the pedoclimatic conditions of their particular viticultural regions (Brunori et al., 2015). Furthermore, they provide the essential characteristics of typicity and authenticity, which are required for success in the dynamic viticultural products market (Dobrei et al., 2015). In the overwhelming majority of cases, local grape varieties and biotypes are much more resistant to diseases and pests than their widely grown equivalents (De Lorenzis et al., 2013). Furthermore, they show higher adaptability to local environmental conditions while requiring significantly less technological input (Dobrei et al., 2019). Such varieties develop with basic farming techniques, requiring fewer disease and pest treatments (Parker et al., 2011). As a result, they have the potential to yield viticultural products that are naturally healthier for human consumption (Marques et al., 2023). Regardless of their benefits, most of the local varieties and biotypes are relatively unknown (Ibrahim & Bayir, 2010). They are primarily grown in their native yards and gardens and have not been tested using more advanced cultivation and winemaking technology (Maraš, et al., 2020). Local grape varieties and biotypes are suitable options for ecological grapevine farming, which is growing in popularity as the future of viticulture (Grigoriou et al., 2020). This trend has become especially reliable in European countries known for their advanced viticultural techniques, such as Spain, Italy, France, and Romania (Tardaguila et al., 2021). However, organic viticulture progresses slowly, because of its shortcomings in efficiently battling diseases and pests using methods and products validated by ecological agricultural practices, particularly for the wide range of grape varietals planted (Wei et al., 2023).

The primary aim of research was to identify growth stages of local cultivars, particularly those exhibiting delayed bud break or flowering, in response to the increasingly climate variability. Consequently, the study aimed to contribute valuable insights into the suitability of local grape cultivar for ecological grapevine farming, aligning with the evolving landscape of viticulture towards sustainability and authenticity.

MATERIALS AND METHODS

This research was done between the 2020 and 2022 growing seasons and focused on several local grape cultivars from *Vitis vinifera* sp., found in the yards, gardens, or tiny vineyards of professional or amateur winegrowers in Alba County. Among the large number of local grapes cultivars identified, 12 cultivars native to the area, little or not at all known, were kept and were the subject of research. The local cultivars 'Pleoapă', 'Precupească', 'Butuc alb', 'Fragă', 'Aromat alb', 'Mare timpuriu' are located in Loman area, Săsciori village (45°51′27″N 23°33′11″E; 45°49′23″N 23°33′33″E). In Șard location with 46°07′13″N 23°31′57″E coordinates (Ighiu village) were identified 'Șard 1' and 'Roșu rezistent' local cultivars. In the same area of Ighiu village (46°09′42″N 23°28′59″E) were found 'Busuioacă de Ighiu', 'Izabelă de Ighiu' and 'Vechi de Ighiu' local cultivars. In Alba Iulia city area was identified 'Ruginiu de Alba' local cultivar (46°4′1″N, 23°34′12″E). For each local cultivar were sampling between 8-15 plants, depending of the vines available in each location. These local cultivars were grouped according to grape characteristics in three categories and analysed in relation *V. vinifera* cultivars (considered as control), widespread and well-known in Alba County's wine-growing areas. The local cultivars for white wines were compared with the control cultivar 'Fetească regală', those for red wines with the control 'Cabernet Sauvignon', and those suited for fresh consumption with the 'Chasselas dore' cultivar.

The study focused on several important characteristics of grapevine growing that are critical for establishing the best management strategy. A significant issue was following the progression of vegetative stages among local grape cultivars with a particular emphasis on identifying those with delayed bud break or flowering. This proactive strategy aims to reduce the risks associated with more common climate changes. The BBCH classification served as the basis for assessing vegetative stages. The study monitored indicators related to winter temperature adaptability, such as the percentage of maturated wood or the proportion of viable buds.

Additional research objectives included fertility and productivity characteristics. These consisted of monitoring the rate of fertile shoots, assessing the number of inflorescences per vine, analysing berry size and bunch weight, evaluating bunch size, the number of bunches per vine and grape yield. Furthermore, the study evaluated the photosynthetic efficiency of local grape cultivars, as well as their disease and pest resistance. The amount of leaf area needed to produce one kilogram of grapes was used to measure photosynthetic efficiency. Meanwhile, local cultivars' resistance to disease and pest was assessed using O.I.V. descriptors.

The statistical analysis was conducted using XLSTAT (by Addinsoft, 2018), a software solution for statistical and data analysis (Version 2018.7.5). The one-way analysis of variance (ANOVA) method was employed to analyse the data. By t-calculation was determined the significance of the difference between the samples mean. Results are expressed as mean and a p-value less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS AND DISCUSSIONS

The progression and length of the growing stages are usually controlled by both the genetics of the variety and the environmental conditions in each growing season and location (Simeonov, 2016). Shoot and inflorescence damage caused by extreme temperatures is becoming more likely in today's unstable climate (Schumacher et al., 2024). As a result, biotypes with late budding or delayed flowering are preferred because they are better adapted to manage the risks associated with late spring frost (Linares Torres et al., 2015). Conversely, grape biotypes that reach maturation more quickly are also of importance, as they require fewer sunlight hours and lower temperatures to avoid excessive sugar accumulation, which may result in wines with an excessive alcohol concentration (Rafique et al., 2023).

Regarding the red wine cultivars, none of the local cultivars exhibited delayed bud break compared to the control cultivar 'Cabernet Sauvignon' (Table 1). The only cultivar that showed a bud break similar to the control was 'Roșu rezistent', while the other cultivars commenced bud break 8-10 days earlier than the control. A similar pattern was observed for the phenological stages of shoot emergence and flowering, with local varieties generally preceding the control. Conversely, local grape cultivars tended to achieve full grape maturity earlier than the control, with the 'Pleoapă' notably maturing approximately a week ahead of the control.

Concerning the local grape cultivars used for white wines, bud breaks occurred 4-8 days later compared to the control cultivar except for the 'Busuioacă de Ighiu'. Cultivars with delayed bud break are less susceptible to frost damage during the early stages of growth. However, the flowering stage occurred 1-6 days earlier in local cultivars compared to the control, posing a higher risk of flower damage due to potential low temperatures during this phase. Despite this, all local cultivars in this category reached full grape maturity 2-6 days before the control cultivar. The sugar accumulation was adequate in these cultivars to produce high-quality wines classified as superior wines while also mitigating the risk of excessively high alcohol content.

Table 1. Local wine grape cultivars phenology (2020-2022)

Local cultivars	Year	Growing stage – BBCH code					
		Budburst BBCH 02	Shoots emerge BBCH 13-14	Flowering BBCH 65-68	Bunch density BBCH 77-79	Veraison BBCH 83-85	Full maturation BBCH 89
Local grape cultivars for red wines							
'Pleoapă'	2020	14.IV	28.IV	2.VI	19.VII	19.VIII	30.IX
	2021	11.IV	24.IV	29.V	12.VII	12.VIII	23.IX
	2022	13.IV	26.IV	30.V	14.VII	15.VIII	25.IX
'Vechi de Ighiu'	2020	14.IV	29.IV	5.VI	22.VII	23.VIII	1.X
	2021	12.IV	26.IV	1.VI	18.VII	17.VIII	25.IX
	2022	13 IV	28.IV	4.VI	21.VII	20VII	28.IX
'Izabelă de Ighiu'	2020	15.IV	29.IV	4.VI	25.VII	22VIII	4.X
	2021	13.IV	26.IV	1.VI	20.VII	18.VIII	26.IX
	2022	14.IV	27.IV	2.VI	22.VII	20VII	28.IX
'Roșu rezistent'	2020	27.IV	2.V	12.VI	29.VI	24VIII	2.X
	2021	24.IV	30.IV	10.VI	25.VII	20.VIII	28.IX
	2022	25.IV	30.IV	11.VI	26.VII	21.VIII	30.IX
'Cabernet Sauvignon' (Control)	2020	29.IV	4.V	16.VI	1.VII	26.VIII	5.X
	2021	27.IV	2.V	14.VI	28.VII	24.VIII	30.IX
	2022	27.IV	2.V	14.VI	29.VII	24.VIII	1.X
Local grape cultivars for white wines							
'Șard I'	2020	21.IV	3.V	6.VI	25.VII	23.VIII	28.IX
	2021	18.IV	30.IV	3.VI	22.VII	18.VIII	23.IX
	2022	19.IV	1.V	4.VI	23.VII	20.VIII	24.IX
'Ruginiu de Alba'	2020	23.IV	2.V	8.VI	29.VII	26.VIII	3.X
	2021	20.IV	30.IV	5.VI	25.VII	21.VIII	28.IX
	2022	21.IV	1.V	6.VI	26.VII	22.VIII	30.IX
'Busuioacă de Ighiu'	2020	15.IV	27.IV	3.VI	26.VII	21.VIII	1.X
	2021	12.IV	23.IV	29.V	22.VII	17.VIII	26.IX
	2022	13.IV	24.IV	1.VI	23.VII	18.VIII	28.IX
'Aromat alb'	2020	21.IV	3.V	7.VI	27.VII	25.VIII	2.X
	2021	17.IV	30.IV	4.VI	24.VII	20.VIII	28.IX
	2022	18.IV	2.V	5.VI	24.VII	21.VIII	30.IX
'Fetească regală' (Control)	2020	15.IV	27.IV	9.VI	26.VII	21.VII	30.IX
	2021	12.IV	22.IV	6.VI	22.VII	15.VIII	23.IX
	2022	14.IV	23.IV	7.VI	23.VII	17.VIII	25.IX

Grape varieties grown for fresh consumption are more sensitive to climate variability, enabling the dynamics of growing stages more important in their growing (Dry et al., 2022). Bud break in these cultivars lasted about 10 days. Notably, local cultivars like as 'Precupească' and 'Mare timpuriu' had a delayed bud break compared to the control variety, allowing vines to tolerate or even avoid damage from late spring frosts in some years (Table 2). Conversely, the local cultivars 'Fragă' and 'Butuc alb' bloomed one to four days before the control cultivar 'Chasselas doré. Shoot growing in all local cultivars started 1-7 days later than in the control variety, which is a positive trait that minimises the danger of shoot vulnerability to potentially lower temperatures towards the end of April. The flowering phenophase showed significant differences amongst the investigated cultivars. Some local cultivars, such as 'Fragă' and 'Butuc alb ', flower several days before the control variety, while others, such as 'Mare timpuriu' and 'Precupească', flower after the control. Varieties with delayed flowering have a lesser risk of inflorescence injury from potentially damaging temperatures at the end of May. In regards to reaching full maturity, the local cultivars for fresh consumption showed variable behaviour, with this phenophase lasting around 20 days from the third to second decade of August to September. This extended time is advantageous since it allows for the availability of fresh grapes for consumption for at least 30-40 days, given the grapes' storage durability on the vine. 'Mare timpuriu' was the only local cultivar to mature before the control variety, while the other biotypes matured after the control.

Table 2. Local table grape cultivars phenology (2020-2022)

Local cultivars	Year	Growing stage-code BBCH					
		Budburst BBCH 02	Shoots emerge BBCH 13-14	Flowering BBCH 65-68	Bunch density BBCH 77-79	Veraison BBCH 83-85	Full maturity BBCH 89
'Mare timpuriu'	2020	20.IV	2.V	8.IV	19.VII	3.VIII	3.IX
	2021	15.IV	29.IV	4.VI	14.VII	29.VII	28.VIII
	2022	17.IV	30.IV	5.VI	15.VII	30.VII	30.VIII
'Precupească'	2020	23.IV	2.V	11.VI	20.VII	6.VIII	18.IX
	2021	18.IV	30.IV	7.VI	16.VII	1.VIII	12.IX
	2022	19.IV	30.IV	8.VI	17.VII	2.VIII	14.IX
'Butuc alb'	2020	16.IV	26.IV	29.V	16.VII	6.VIII	23.IX
	2021	11.IV	22.IV	26.V	11.VII	2.VIII	16.IX
	2022	12.IV	23.IV	26.VI	12.VII	3.VIII	18.IX
'Fragă'	2020	14.IV	27.IV	30.V	16.VII	9.VIII	22.IX
	2021	10.IV	22.IV	26.V	11.VII	4.VIII	16.IX
	2022	11.IV	23.IV	27.VI	13.VII	5.VIII	18.IX
'Chasselas doré' (Control)	2020	17.IV	25.IV	6.VI	23.VII	6.VIII	15.IX
	2021	13.IV	21.IV	2.VI	18.VII	1.VIII	9.IX
	2022	14.IV	21.IV	3.VI	20.VII	2.VIII	11.IX

Table 3. Indicators of winter temperature resistance in local grape cultivars for winemaking (mean for 2020-2022 growing seasons)

Local cultivars	Percentage of wood maturation (%)	Viable buds (%)	
		%	Difference to control
Local grape cultivars for red wines			
'Pleoapă'	89.21	89.71	5.46*
'Vechi de Ighiu'	81.35	79.32	-4.93^{NS}
'Izabelă de Ighiu'	72.61	71.63	-12.62**
'Roșu rezistent'	80.29	73.42	-10.83**
'Cabernet Sauvignon' (Control)	82.33	84.25	-
Local grape cultivars for white wines			
'Șard I'	83.64	86.59	6.33*
'Ruginiu de Alba'	73.61	75.11	-5.15*
'Busuioacă de Ighiu'	75.12	76.28	-3.98^{NS}
'Aromat alb'	76.72	78.11	-2.15^{NS}
'Fetească regală' (Control)	78.53	80.26	-

*t significant at p<.05; **t significant at p<.01; ***t significant at p<.001; NS - not significant

Despite the overall rise in average temperatures observed in many wine-growing regions in recent years, climate variability has resulted in brief intervals of low temperatures, especially during nighttime (-19°C and -20°C). These temperatures fall below the frost resistance threshold of certain local grape varieties. Consequently, it is critical for grapevine cultivars to have fast wood maturity at the beginning of winter, enabling them to resist these cold-weather periods successfully.

In local grape cultivar for red wines, the canes wood maturation percentage yielded positive results. Several biotypes outperformed the control standard in this category, despite 'Cabernet Sauvignon's' reputation for strong wood maturity and frost resistance. The local cultivar 'Pleoapă' performed particularly well, with the highest percentage of wood maturation among the examined cultivars. Furthermore, 'Vechi de Ighiu' and 'Roșu rezistent' demonstrated wood maturation percentages that were nearly identical to the control cultivar whereas 'Izabelă de Ighiu' achieved a satisfactory level, but lower than the control. In terms of bud viability, all local cultivars showed high values, eliminating the need for compensatory measures to control crop load. Notably, 'Pleoapă' appeared as the only native local grape cultivar that recorded higher values than the control.

The local grape cultivars and control cultivar for white wine, recorded notable percentages of wood maturation and viable buds, more than enough to apply a standard winter pruning (Table 3). Compared to 'Cabernet Sauvignon' (Control), a popular cultivar in Romania, the results for these characteristics were comparable or even better than the control, especially for the 'Șard 1' local cultivar. The degree of wood maturation observed was affected by pruning techniques, environmental conditions, and grape variety, whereas the viability of buds was influenced by their location on the vine, the quality of pruning, and the management of diseases and pests.

Tabel 4. The variables for tolerance to low temperatures in local cultivars of grapes for fresh consumption (mean 2020-2022 growing seasons)

Local cultivars	Percentage of wood maturation %	Viable buds (%)	
		%	Difference to control
'Mare timpuriu'	80.13	82.12	7.86*
'Precupească'	70.63	72.19	-2.07^{NS}
'Butuc alb'	63.53	69.28	-4.28^{NS}
'Fragă'	66.12	71.11	-3.15^{NS}
'Chasselas doré' (Control)	71.34	74.26	-

*t significant at p<.05; **t significant at p<.01; ***t significant at p<.001; NS - not significant

Although the local cultivars of grapes for fresh consumption are considered to be less resistant to frost compared to the grape varieties for wine, these vines exhibit sufficient accumulation of reserve substances in the canes (Table 4). This accumulation facilitates adequate maturation of the canes and ensures correspondding viability of the buds. Within this category, a renowned control cultivar ('Chasselas doré') known for its robust wood maturation and tolerance to low temperatures was selected for comparison. Nevertheless, local grape cultivars demonstrated values for these indicators that were relatively close to the control cultivar. The 'Mare timpuriu' notably surpassed the control in both the percentage of matured wood and viable buds, with statistical significance validating these differences. Fertility is critical for the vine, but the advantage is that there are relatively few varieties that were negatively influenced from this viewpoint. Local red wine cultivars had higher percentage of fertile shoots than the control in any variety (Table 5). The 'Roșu rezistent' and 'Pleoapă' cultivars achieved the highest ratings in this respect. In regards to the number of inflorescences per vine, 'Izabelă de Ighiu' was the only cultivar with lower values than the control. In exchange, all of the local white wine grape cultivars recorded lower fertile shoot level than the control, despite the fact that the recorded levels are considered extremely quite favourable. None of the local wine grape cultivars presented issues regarding fertility indicators.

As regards the percentage of fertile shoots, the local grape cultivars for fresh consumption had provided values close to or even superior to the control ('Chasselas doré'), which is regarded one of the most fertile table grape cultivar (Table 6). From this perspective, was observed that cultivars 'Mare timpuriu' and 'Butuc alb', had higher values than the control.

Table 5. Fertility components in local red and white wine cultivars (mean 2020-2022 growing seasons)

Local cultivars	Rate of fertile shoots (%)	Inflorescence per vine	Difference to control (Inflorescence per vine)
Red wine local cultivars			
'Pleoapă'	75.21	20.82	4.17*
'Vechi de Ighiu'	73.14	17.37	0.72NS
'Izabelă de Ighiu'	70.76	14.22	-2.43NS
'Roșu rezistent'	75.94	21.32	4.67*
'Cabernet Sauvignon' (Control)	64.88	16.65	-
White wine local cultivars			
'Șard I'	72.87	12.75	-6.22**
'Ruginiu de Alba'	77.11	13.12	-5.85*
'Busuioacă de Ighiu'	71.89	17.84	-1.13NS
'Aromat alb'	75.32	20.23	1.26NS
'Fetească Regală' (Control)	79.23	18.97	-

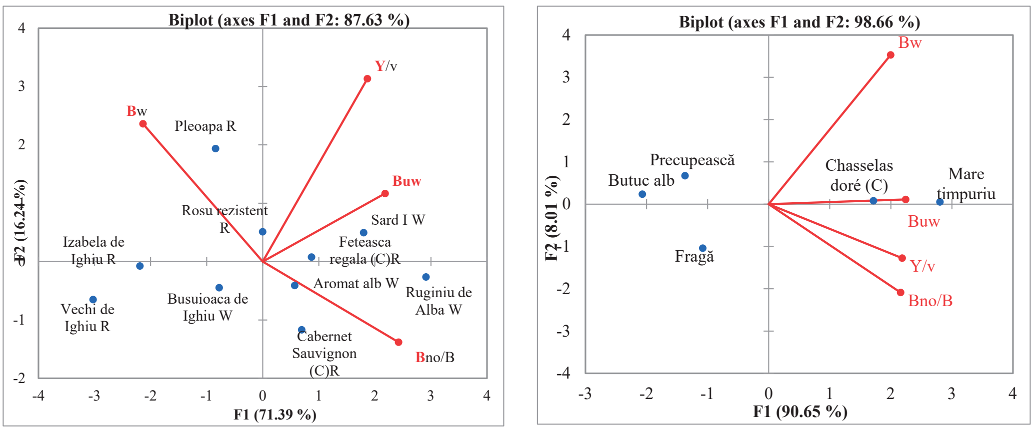
*t significant at p<.05; **t significant at p<.01; ***t significant at p<.001;NS - not significant

The number of inflorescences per plant varies significantly; with the exception of the early 'Mare timpuriu', all of the local cultivars outperformed the control in this regard.

Table 6. Fertility components in local table grape cultivars (mean for 2020-2022 growing seasons)

Local cultivars	Rate of fertile shoots	Inflorescence per vine	Difference to control (Inflorescence per vine)
'Mare timpuriu'	79.11	11.43	-2.32NS
'Precupească'	69.43	31.87	18.12***
'Butuc alb'	77.65	30.34	16.59***
'Fragă'	67.21	28.32	14.57***
'Chasselas doré' (C)	73.41	13.75	-

*t significant at p<.05; **t significant at p<.01; ***t significant at p<.001; NS-not significant



a b

Figure 1. PCA diagram for grape yield components in wine grape cultivars (a) and grapes for fresh consumption (b); Bw - berry weight; Bno/B - berry number per bunch; Buw - Bunch weight; Y/v (g) - grape yield/vine (kg)

The PCA diagram presented in Figure 1 1a illustrates the qualitative characteristics of local cultivars and control cultivars for white and red wine. The principal component F1 contributes significantly to the variability, accounting for 71.39% of the total variability observed in the data. Upon examining the data, it is evident that certain local grape cultivars stand out in terms of berry weight. Specifically, the red wine local cultivars 'Pleopă' and 'Roșu rezistent' exhibited the highest berry weight among the studied cultivars. In contrast, the white wine local cultivars 'Aromat alb' and 'Ruginiu de Alba' displayed the lowest berry weight.

As regards the yield per vine, differences were observed among the studied cultivars. The red wine local cultivars 'Izabelă de Ighiu' and 'Vechi de Ighiu' were found to have the lowest yield per vine. Therefore, the white wine local cultivar 'Sard 1' exhibited the highest weight of bunches, resulting in the highest yield per vine among the studied varieties.

The PCA diagram in Figure 1b compares the qualitative characteristics of table local grape cultivars to the control cultivar 'Chasselas doré'. Additionally, the 'Mare timpuriu' local grape cultivar and the control 'Chasselas doré' cultivar performed significantly better in terms of berry and bunch weight. Therefore a result, they had a higher yield per vine, which was most likely due to the larger amount of berries in each bunch. In contrast, the 'Fragă' local grape cultivar had the lowest values across all of the qualitative parameters evaluated during the study. This analysis emphasises the variability in qualitative characteristics observed across table grapes local cultivars and the control cultivar. The distinctive performance of the 'Mare timpuriu' local cultivar and 'Chasselas doré' in terms of berry and bunch weight indicates that they may be suitable for table grape cultivation. In contrast, the 'Fragă' local cultivar's poor performance emphasizes the need of taking certain qualitative qualities into account when choosing grape types for growing. Such findings are invaluable in guiding viticultural activities and increasing yield and quality in grape production.

The photosynthetic efficiency reflects the variety's adaptation to the sunlight resources of the growing area, and it was calculated using the amount of leaf area required to yield one kilogram of grapes (Domingues Neto et al., 2023). Although the control cultivars 'Cabernet Sauvignon' and ‘Fetească regală’ have long been included in the varietal assortment in the studied locations, the local cultivars tested approached or even exceeded their photosynthetic efficiency (Table 7).

With the exception of the 'Vechi de Ighiu' cultivar, all other local red wine grape cultivars required smaller leaf areas to produce one kilogram of grapes compared with the control cultivar. 'Pleoapă' was the most efficient local cultivar, followed by 'Roșu rezistent' and 'Izabelă de Ighiu', with differences from the control statistically significant. In the group of white wine cultivars, the situation was different, with the control variety outperforming all of the local cultivars in terms of photosynthetic efficiency, with the exception of 'Ruginiu de Alba'.

Table 7. Parameters of photosynthetic efficiency in local grape cultivars for wine (mean 2020-2022 growing seasons)

Local cultivars	Leaf area (m^2/vine)	Grape yield (kg/vine)	Leaf area (m^2/kg grapes)	Difference to control Leaf area (m^2/kg grapes)
Red wine local cultivars				
Pleoapă	8.21	1.92	4.27	-1.02*
Vechi de Ighiu	6.18	1.04	5.94	0.65*
Izabelă de Ighiu	5.35	1.18	4.53	-0.76*
Roșu rezistent	7.95	1.82	4.36	-0.93*
'Cabernet Sauvignon' (C)	7.89	1.49	5.29	-
White wine local cultivars				
Șard I	6.75	1.76	3.83	0.01^{NS}
Ruginiu de Alba	6.43	1.79	3.59	-0.23^{NS}
Busuioacă de Ighiu	5.85	1.44	4.06	0.24^{NS}
Aromat alb	7.36	1.71	4.3	0.48*
'Fetească Regală' (C)	6.93	1.81	3.82	-

*t significant at p<.05; **t significant at p<.01; ***t significant at p<.001; NS - not significant

Photosynthetic efficiencies observed were generally higher in the group of local grape cultivars for fresh consumption than in wine grape cultivars, which are assuming the higher sugar accumulation in wine grape cultivars (Table 8). When compared to the control cultivar, the local cultivars 'Fragă' and 'Precupească' required less leaf area to produce a kilogram of grapes. However, 'Butuc alb' and 'Mare timpuriu' local cultivars necessitated a larger leaf area for the same purpose.

The local red wine cultivars displayed good disease resistance, with only the 'Vechi de Ighiu' and 'Pleoapă' exhibiting moderate resistance to Botrytis (Table 9).

Table 8. Parameters of photosynthetic efficiency in local table grape cultivars (mean 2020-2022 growing seasons)

Local cultivars	Leaf area (m^2/vine)	Grape yield (kg/vine)	Leaf area (m^2/kg grapes)	Difference to control Leaf area (m^2/kg grapes)
'Mare timpuriu'	9.05	2.34	3.86	0.04^{NS}
'Precupească'	6.53	1.75	3.73	-0.09^{NS}
'Butuc alb'	7.15	1.52	4.70	0.88*
'Fragă'	6.75	1.90	3.55	-0.27*
'Chasselas doré' (C)	8.45	2.21	3.82	-

*t significant at p<.05; **t significant at p<.01; ***t significant at p<.001

Table 9. Disease resistance among local grape cultivars for winemaking

Local cultivars	Disease	O.I.V. Code	Local cultivars resistance	Level of resistance
Resistance to biotic factors				
Local cultivars for red wines				
'Pleoapă'	Downy mildew (*Plasmopara viticola*)	451	good	8
	Powdery mildew (*Erysiphe necator*)	454	good	8
	Botrytis cinerea (*Gray mold)*	457	medium	6
'Vechi de Ighiu'	Downy mildew (*Plasmopara viticola*)	451	good	8
	Powdery mildew (*Erysiphe necator*)	454	good	7
	Botrytis cinerea (*Gray mold)*	457	medium	6
'Izabelă de Ighiu'	Downy mildew (*Plasmopara viticola*)	451	good	8
	Powdery mildew (*Erysiphe necator*)	454	good	8
	Botrytis cinerea (*Gray mold)*	457	good	7
'Roșu rezistent'	Downy mildew (*Plasmopara viticola*)	451	good	8
	Powdery mildew (*Erysiphe necator*)	454	good	8
	Botrytis cinerea (*Gray mold)*	457	good	8
'Cabernet Sauvignon' (Control)	Downy mildew (*Plasmopara viticola*)	451	weak	2
	Powdery mildew (*Erysiphe necator*)	454	medium	5
	Botrytis cinerea (*Gray mold)*	457	good	7
Local cultivars for red wines				
'Șard I'	Downy mildew (*Plasmopara viticola*)	451	good	7
	Powdery mildew (*Erysiphe necator*)	454	medium	6
	Botrytis cinerea (*Gray mold)*	457	weak	2
'Ruginiu de Alba	Downy mildew (*Plasmopara viticola*)	451	very good	9
	Powdery mildew (*Erysiphe necator*)	454	good	7
	Botrytis cinerea (*Gray mold)*	457	good	7
'Busuioacă de Ighiu'	Downy mildew (*Plasmopara viticola*)	451	good	7
	Powdery mildew (*Erysiphe necator*)	454	good	7
	Botrytis cinerea (*Gray mold)*	457	medium	5
'Aromat alb'	Downy mildew (*Plasmopara viticola*)	451	good	8
	Powdery mildew (*Erysiphe necator*)	454	good	8
	Botrytis cinerea (*Gray mold)*	457	medium	6
'Fetească regală' (Control)	Downy mildew (*Plasmopara viticola*)	451	medium	7
	Powdery mildew (*Erysiphe necator*)	454	medium	7
	Botrytis cinerea (*Gray mold)*	457	weak	2

This susceptibility was largely attributed to the exceptionally rainy conditions that characterized the 2020 growing season. Following the application of the standard treatment regimen, disease resistance was markedly elevated in local white wine grape cultivars compared to the 'Fetească regală' control cultivar, which underwent a conventional treatment protocol. Among the local cultivars, 'Șard 1' demonstrated the highest sensitivity to Botrytis, particularly evident during the 2020 growing season.

Table 10. Disease resistance among local table grape cultivars

Local cultivars	Disease	O.I.V. Code	Local cultivars resistance	Level of resistance
Resistance to biotic factors				
'Mare timpuriu'	Downy mildew (*Plasmopara viticola*)	451	medium	6
	Powdery mildew (*Erysiphe necator*)	454	good	7
	Botrytis cinerea (*Gray mold)*	457	medium	5
'Precupească'	Downy mildew (*Plasmopara viticola*)	451	medium	6
	Powdery mildew (*Erysiphe necator*)	454	medium	6
	Botrytis cinerea (*Gray mold)*	457	weak	2
'Butuc alb'	Downy mildew (*Plasmopara viticola*)	451	medium	5
	Powdery mildew (*Erysiphe necator*)	454	medium	6
	Botrytis cinerea (*Gray mold)*	457	medium	5
'Fragă'	Downy mildew (*Plasmopara viticola*)	451	medium	6
	Powdery mildew (*Erysiphe necator*)	454	good	8
	Botrytis cinerea (*Gray mold)*	457	good	7
'Chasselas doré' (Control)	Downy mildew (*Plasmopara viticola*)	451	very weak	1
	Powdery mildew (*Erysiphe necator*)	454	medium	6
	Botrytis cinerea (*Gray mold)*	457	medium	6

Although table grape varieties are thought to be more vulnerable to disease attack, the local grape cultivars showed relatively strong resilience, even if disease attacks were more visible compared to the wine grape cultivars (Table 10). 'Precupească' was the only native cultivar with higher vulnerability to the Botrytis attack.

CONCLUSIONS

The unfavourable climatic circumstances of 2020 had a detrimental impact on grape quality and yield, since outputs were very low due to the significant precipitation in June, July, and August, deviating substantially from the previous two years of research. The local cultivars performed similarly to the control varieties in all indicators, despite the fact that the chosen controls were some of the most valuable and cultivated cultivars from Alba County's wine-growing areas. A significant number of the local cultivars registered delays of a few days compared to the control for the budburst and flowering ('Șard 1', 'Ruginiu de Alba', 'Aromat alb', and 'Precupească'), which is an advantage especially in unfavourable years, with climate variability, when late spring frosts can significantly damage the shoots or even the inflorescences. The resistance to disease was notably superior in local cultivars compared to the control, with significantly fewer signs of disease attack evident even in the absence of complex treatment. In local table grapes cultivars, due to the fresh consumption, it is considered that good resistance to diseases is a major advantage, so that consumers can benefit from healthy grapes, with minimal exposure to phytosanitary treatments, which fall under in the standards of sustainable viticulture. Although, there were very few local grapes cultivars ('Pleoapă', 'Roșu rezistent', 'Mare timpuriu') that surpassed the controls for grape yield, due to their rusticity and the possibilities of offering authentic wine products, these local grapes cultivars need more researched and some of them, introduced either in the varietal assortments or in the improvement programs.

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ENHANCING *PHYSALIS IXOCARPA* L. CROP YIELD QUANTITY AND QUALITY USING BIOCHAR, WOOD VINEGAR AND CROPMAX: A SUSTAINABLE APPROACH

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Abstract

Physalis ixocarpa L., commonly known as tomatillo or Mexican husk tomato, is an economically valuable crop due to its nutritional value and culinary versatility. However, its growth and yield can be affected by various factors including soil quality, nutrient availability, and environmental stressors. In this study, we investigated the effects of employing biochar, wood vinegar, and CropMax—a proprietary organic fertilizer—on the growth, yield, and some quality parameters of Physalis ixocarpa L. Results demonstrated that the combined application of biochar, wood vinegar, and CropMax significantly improved the growth characteristics of Physalis ixocarpa L. The treatments applied positively influenced both quantitative and qualitative yield parameters compared to control variant. This study highlights the potential of employing biochar, wood vinegar, and CropMax as a sustainable approach to enhance the growth, yield, and soil health of Physalis ixocarpa. The findings underscore the importance of integrated management strategies for sustainable agriculture practices, emphasizing the potential for improving crop productivity while maintaining soil health and fertility

Key words*: tomatillo, pyrolized biomass, pyroligneous acid, eco-friendly produces, crop enhancer.*

INTRODUCTION

The imperative of addressing the exponential growth of the global population necessitates the establishment of sustainable and productive agroecosystems to meet current food demands while preserving crucial natural resources for future generations (Francis & Porter, 2011). An indispensable element in achieving sustainability lies in the conservation of biodiversity at the plant species level, with a specific emphasis on those less cultivated yet possessing significant potential (van Zonneveld et al., 2023).

Within this context, *Physalis ixocarpa* Brot., commonly known as "tomate verzi" (green tomato), "tomate de Cáscara" (husk tomato), or "tomatillo" (Svobodova & Kuban, 2018), emerges as an enigmatic gem in the horticultural domain. Belonging to the Solanaceae family, tomatillo is revered for its succulent and tangy green fruits enclosed in papery husks, primarily consumed fresh in Mexico, where the *Physalis* genus boasts over 70 species, mostly endemic (Mazova et al., 2020), the United States, and Central America (Mulato-Brito & Peña-Lomelí, 2007). The fruit develops within a protective calyx, offering defense against adverse climate conditions, diseases, insects, and environmental factors (Lim, 2013). Originating from Mesoamerica, this perennial plant has transcended its historical confines, evolving into a versatile and sought-after crop with diverse culinary applications. The distinctive flavor profile and ornamental allure of tomatillos have spurred increased interest in their cultivation, both in traditional open fields and sophisticated glasshouse environments.

Tomatillos hold global culinary significance, particularly in Mexican cuisine, where their zesty and citrusy notes enhance the flavors of salsas, sauces, and traditional dishes. Beyond their gustatory appeal, tomatillo plants, with their sprawling growth habit and verdant foliage, contribute aesthetic dynamism to agricultural landscapes and controlled environments.

From a medicinal perspective, tomatillos contain a substantial amount of phytochemicals, including polyphenols, recognized for their antioxidant properties (Silva et al., 2016), and have garnered attention for potential hypoglycemic effects (Gulati et al., 2012). Furthermore, tomatillos are acknowledged for their antibacterial, antitumor, immunomodulatory, and antipyretic actions (Guerrero-

Romero et al., 2021), traditionally employed in alternative treatments for various conditions, including liver disorders, asthma, malaria, and dermatitis (Zhang & Tong, 2016).
Moving to the challenges of commercial greenhouse cultivation of husk tomatoes, constraints include the necessity for appropriately designed greenhouses meeting plant specifications and a satisfactory availability of varieties suited to this cultivation system (Santiaguillo et al., 2009; Ponce et al., 2012).
Furthermore, achieving soil sustainability mandates the adoption of practices aimed at minimizing the inputs of synthetic chemical fertilizers. In recent years, biochar and wood vinegar have emerged as prominent alternative sources of inputs intended to enhance soil fertility.
Biochar, produced through the pyrolysis of waste biomass in controlled oxygen conditions (Lehmann, 2007), exhibits promise as a soil amendment. It improves soil physico-chemical properties, fosters plant growth, and mitigates soil pollution (Zheng et al., 2013). Although studies suggest that biochar can increase soil organic carbon and water holding capacity, benefiting plant growth (Jeffery et al., 2011), its impact on yield is not consistently positive; for instance, it may enhance nutrient availability without consistently improving crop yields (Vaccari et al., 2015). Wood vinegar, a by-product of biomass pyrolysis, has also been reported to enhance crop yields and nutrient uptake when applied to agricultural soils (Rui et al., 2014).

MATERIALS AND METHODS

The research has been carried out in 2023, into an unheated glasshouse within Vegetable Research and Development Station Bacău conventional agriculture plot, characterized by a well-developed alluvial soil with a loam-sandy composition, a pH level ranging from 6.2 to 6.8, and an organic matter content between 2% and 2.6%. The prior year's preceding crop was `Auria Bacaului` climbing bean. Tomatillo seedlings were grown in a controlled greenhouse environment using nutrient cubes and alveolar trays, with the initial sowing on March 14th and subsequent transplantation to the field on May 19th. The planting scheme included two distinct rows, with 70 cm between rows and 35 cm between plants/row, respectively. The tomatillo plants were not subjected to pruning throughout the vegetative season, instead being espaliered in accordance with the number of branches. Disease and pest management relied exclusively on copper-based products (two applications of Boille Bordellaise - 0.5% were conducted during the vegetation season). Irrigation was carried out using drip irrigation tape from May until the first ten days of September.

Experimental arrangement

The randomized experimental setup comprised four variants and three repetitions (10 plants per repetition), arranged in a pair of rows, as illustrated in Table 1.

Table 1. Randomized experimental setup

No.	Experimental variants	Applied doses	Main product description
V1	1 Biochar application * 2 Cropmax treatments	Biochar - consistent spreading at ground level, succeeded by shallow integration into the soil. Cropmax application - a quantity of 10 ml per 10 liters of water per 100 square meters.	Bio-GEKKA S (Expoclom GK SRL) - pyrolysis carbonization of biomass; compactness < 3 mm - 276 kg/m^3, specific surface area (BET) - 557.76 m^2/g, residue content (550 degrees) - 4.1% (w/w); carbon content (C) - 91.3% (w/w); overall nitrogen (N) - 0.66% (w/w); potassium (K) - 0.25% (w/w); sodium (Na) - 0.02% (w/w); calcium (Ca) - 1.1% (w/w); iron (Fe) - 0.09% (w/w); magnesium (Mg) - 0.05% (w/w); manganese (Mn) - 0.04% (w/w); sulfur (S) - 0.03% (w/w); water-holding capacity - 162.5%; dampness - 6%; acidity level - 8.76 CaCl2, EPA-PAH (devoid of LOQ) - 6 mg/kg.
V2	2 Wood vinegar * 2 Cropmax treatments	Wood vinegar application - an amount of 20 ml per 10 liters of water per 100 square meters. Cropmax application - a quantity of 10 ml per 10 liters of water per 100 square meters.	Bio-GEKKA L (Expoclom GK SRL); it is a byproduct of biomass carbonization through pyrolysis, containing acetic acid and pyroligneous acid; carbon content (C) - 14 g/l; Kjeldahl nitrogen - 3.37 mg/dm^3; potassium (K) - <20 mg/dm^3; boron (B) - <2 mg/dm^3; copper (Cu) - <0.4 mg/dm^3; iron (Fe) - 533 mg/dm^3; phosphorus (P) - <0.4 mg/dm^3; magnesium (Mg) - 0.809 mg/dm^3; manganese (Mn) - 3.42 mg/dm^3; acidity level - 4.24; nitrites (NO2-) <5 mg/dm^3; nitrates (NO3-) <5 mg/dm^3.
V3	2 Cropmax treatments	Cropmax treatment - an application rate of 10 ml per 10 liters of water per 100 square meters.	Cropmax is a highly concentrated foliar fertilizer; pH level - 7, nitrogen (N2) content at 0.2%, phosphorus pentoxide (P2O5) at 0.4%, potassium oxide (K2O) at 0.02%, iron (Fe) concentration of 220 mg/L, magnesium (Mg) content of 550 mg/L, and calcium (Ca) at 10 mg/L.
V4	Control (untreated)		

Quantitative Measurements

Various biometric characteristics, such as plant height, leaf count, stem diameter, no of branches, flower quantity and fruit count, fruit length, width and weight, were determined at plant physiological maturity (Figure 1).

Figure 1. Tomatillo plant during the growing season

Both chlorophyll pigments and anthocyanins were gauged using similar instruments, namely the CCM 200 plus and ACM 200 plus, manufactured by Optisciences. The results were quantified using indices that accurately reflect the overall concentration of chlorophyll pigments and anthocyanins, specifically the Chlorophyll Concentration Index and the Anthocyanin Content Index during a consecutive two-month period (June and July). The findings were displayed dynamically.

The yields obtained for the four different variations were recorded per plant, emphasizing the distinctions among them (Figure 2).

Figure 2. The yield of tomatillo fruits per plant

Quality Measurements

The quality assessment of tomatillo yield under the influence of the three examined treatments included the examination of several factors, including total dry matter (DM), total soluble solids (TSS), and titratable acidity (TA) (Figure 3).

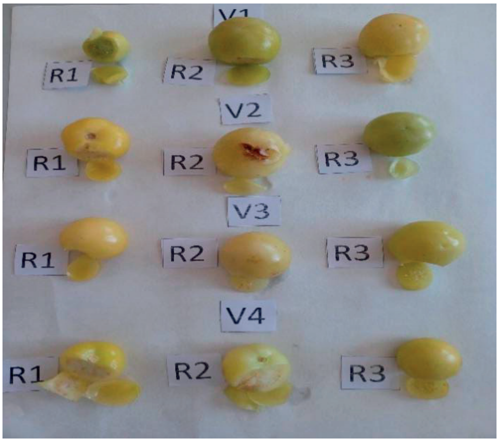


Figure 3. Preparing samples for qualitative determinations

Dry matter content (DM) - Fresh and uniform samples underwent drying in a forced air oven (Biobase) at a controlled temperature of 103 ± 2°C for 24 hours, adhering to AOAC (2000) guidelines. The results are expressed as a percentage.

Total soluble solids (TSS) were measured with a highly accurate handheld refractometer, and the outcomes are reported in degrees Brix, following the methodology described in AOAC (2005) section 932:12 (Figure 4).

Figure 4. Quantification of the total soluble solids content in tomatillo fruit

Titratable acidity (TA) - The percentage of malic acid was calculated using the formula: % malic acid = mL of NaOH x F x 25 x 2 x 0.0067. The results were reported as mean values, along with their respective standard errors. To assess the statistical significance of the total yield achieved per plant across the four different variants, an analysis was conducted using the ANOVA test.

RESULTS AND DISCUSSIONS

The results and analysis presented herein provide a comprehensive examination of key parameters influencing the growth, development, and quality of the tomatillo plants cultivated under various treatment conditions. The investigated parameters encompass a spectrum of key elements acting as indicators of plant growth, physiological status, and fruit quality, offering valuable insights into the efficacy of applied treatments. The multidimensional nature of the analysis allows for a holistic understanding of the effects of these treatments. The combination of Biochar application and Cropmax treatments in V1 leads to the tallest plants (83.22 cm) compared to other treatments (Figure 5). Biochar is known for improving soil fertility and water retention, which could contribute to enhanced plant growth (Jones et al., 2012; Schulz et al., 2013; Rawat et al., 2019; Dai et al., 2020). The positive correlation between Biochar application and increased plant height suggests a potential beneficial effect. V2, involving Wood vinegar and Cropmax treatments, results in plants with a moderate height (76.67 cm) and a relatively high flower count (16.67). Wood vinegar is often considered a natural plant growth promoter (Rogelio, 2018; Zhu et al., 2021; Yavaş et al., 2023). The correlation between Wood vinegar treatment and higher flower count hints at its potential role in influencing reproductive development. V3 involves Cropmax treatments without additional amendments, resulting in plants with a moderate height (72.89 cm) and a lower flower count (12.67). The control variant (V4) has the shortest plants (68.00 cm) but surprisingly exhibits the highest leaf count per branch (52.22) and a comparable flower count (16.11). This may indicate that untreated conditions lead to increased foliage development, possibly compensating for the shorter stature.

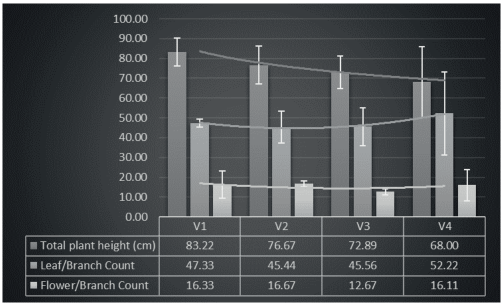


Figure 5. Some parameters of tomatillo plant growth and development influenced by the nutrient blend

Examining stem base diameter provides insights into the structural development of the plants. The relatively large stem base diameter in V1 suggests that the combination of Biochar application and Cropmax treatments contributes to robust structural development (Figure 6). This may be indicative of increased nutrient absorption and enhanced root growth. The high number of branches in V1 correlates with the large stem base diameter, indicating a positive relationship between structural development and branching patterns. This suggests that the treatments applied to V1 not only promote overall plant growth, but also influence branching architecture.

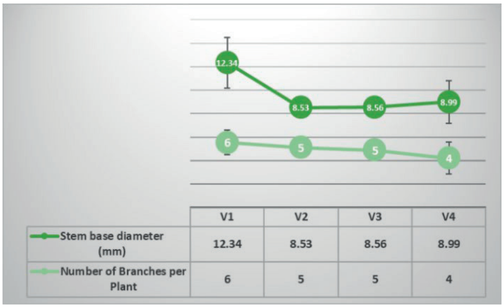
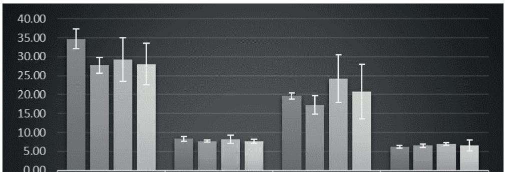


Figure 6. Tomatillo plant stem base diameter and number of branches per plant influenced by the nutrient blend

V2 exhibits a smaller stem base diameter compared to V1, suggesting that the inclusion of Wood vinegar in combination with Cropmax treatments may have a different impact on structural development. The moderate number of branches in V2 correlates with its stem base diameter, indicating a balanced structural development. V3 shows a similar stem base diameter to V2. This suggests that Cropmax treatments are influential in determining the structural development of the plant. Likewise, the consistent number of branches in V3, similar to V2, reinforces the notion that Cropmax treatments play a pivotal role in influencing branching patterns, irrespective of additional amendments.

The control variant (V4) elucidates that untreated tomatillo plants possess the capacity to attain a stem base diameter commensurate with treated counterparts. By contrast, the modest number of branches per plant underscores the consequential impact of fertilization interventions on the intricacies of branching architectures, accentuating the substantial influence treatments exert over the overarching structural development of plants.

The dynamic changes in Chlorophyll Content Index (CCI) and Anthocyanin Content Index (ACI) across the variants provide insights into the plant's physiological responses to different treatments (Figure 7).

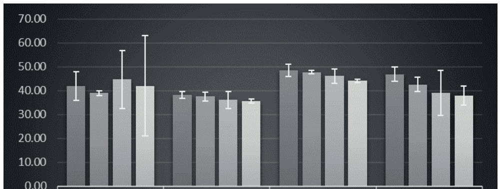


	Chlorophyll content index (CCI) -20.06	Anthocyanin content index (ACI) -20.06	Chlorophyll content index (CCI) -20.07	Anthocyanin content index (ACI) -20.07
V1	34.69	8.36	19.70	6.23
V2	27.74	7.77	17.29	6.51
V3	29.30	8.20	24.18	6.93
V4	28.07	7.69	20.81	6.61

Figure 7. Dynamics of chlorophyll and anthocyanin content of tomatillo leaves during the growing season

Across all variants, the observed dynamics suggest changes in chlorophyll and anthocyanin content over time, indicating potential responses to applied treatments or inherent plant developmental processes. Thus, the decreasing trend over the observed period could be attributed to alterations in plant pigmentation due to various factors, such as applied treatments influence, plant growth stages or environmental conditions.

The number of fruits per plant serves as a critical metric for overall productivity, and its correlation with other parameters provides insights into the holistic impact of applied treatments (Figure 8). Fruit length, width, and average fruit weight collectively contribute to the assessment of fruit quality, with each variant exhibiting unique characteristics influenced by the applied treatments.



	Number of fruits per plant	Fruit length (mm)	Fruit width	Average fruit weight (g)
V1	42.00	38.31	48.51	46.89
V2	39.00	37.51	47.74	42.56
V3	44.67	36.08	46.08	39.11
V4	42.00	35.65	44.09	37.87

Figure 8. The number of fruits per plant and other morphological characteristics of tomatillo fruits

Among the variants, V3 exhibits the highest number of fruits per plant at 44.67. This suggests that the application of Cropmax treatments alone has a pronounced positive impact on fruit yield. The robust yield in V3 may be attributed to enhanced nutrient availability, potentially promoting fruit set and development.

V1 showcases the longest fruit length at 38.31 mm, emphasizing the positive influence of Biochar application and Cropmax treatments on elongated fruit morphology. The extended fruit length in V1 may be indicative of improved cell elongation and enhanced overall plant growth. The same variant stands out with the widest fruit width at 48.51 mm. This suggests that the combined application of Biochar and Cropmax treatments contributes to broader fruit development, potentially influenced by increased nutrient availability and optimal environmental conditions. These results align with those obtained by Vdovenko et al. (2018).

V1 also records the highest average fruit weight at 46.89 g, highlighting the positive impact of Biochar application and Cropmax treatments on promoting heavier fruits. The substantial weight in V1 suggests enhanced fruit filling, potentially driven by improved nutrient uptake and utilization.

The highest recorded values in V3 for the number of fruits per plant showcase the positive influence of Cropmax treatments in promoting prolific fruiting. Meanwhile, V1's noteworthy values for fruit length, width, and average fruit weight underscore the combined effects of Biochar and Cropmax treatments in enhancing both fruit morphology and weight.

The overall importance of the applied treatments is evident in the consistent and nuanced patterns of total yield across variants (Table 2). While statistical tests may not reveal significant differences, the data underscores the need for a comprehensive understanding of treatment effects and the potential for further refinement to optimize agricultural practices.

Table 2. Tomatillo`s total yield/plant (g)

Variant	Total yield/plant (g)				Significance
	R1	R2	R3	Average	
V1	1620	2114	2176	1970	ns
V2	1560	1685	1729	1658	ns
V3	1587	2101	1387	1691	ns
V4	1111	2530	1123	1588	ns

Each variant exhibits a distinctive pattern in total yield across replicates, reflecting the impact of applied treatments. Even without statistically significant differences, the numerical variations in total yield values indicate that the treatments play a role in shaping overall productivity. In addition, the consistent yield levels across replicates within each variant highlight the reliability of the applied treatments in maintaining a stable and predictable crop output. Consistency is crucial for farmers seeking reliable and reproducible results in their agricultural endeavors. The outcomes concerning the total yield per plant exhibit a degree of similarity to those acquired by Cerri (2006).

Analyzing the recorded values for total soluble solids, titratable acidity, and total dry matter across variants offers valuable insights into the potential effects of applied treatments on fruit quality (Figure 9).

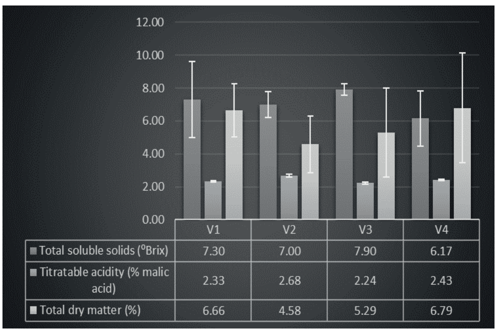


Figure 9. Some qualitative parameters of tomatillo fruits influenced by the nutrient blend

These insights can inform targeted strategies for crop management and contribute to the production of fruits with enhanced sensory attributes and nutritional value. The parameters measured collectively contribute to the overall quality and sensory characteristics of the fruit. Understanding the highest values for each parameter provides insights into the potential for enhancing flavor, acidity, and fruit composition

V3 exhibits the highest total soluble solids at 7.90 °Brix. This elevated level suggests a higher concentration of sugars and soluble compounds in the fruit, potentially indicating improved sweetness and flavor. The increased °Brix in V3 could be attributed to the influence of the applied Cropmax treatments, emphasizing their positive impact on fruit quality.

V2 records the highest titratable acidity at 2.68%. This suggests a higher concentration of malic acid in the fruit, contributing to overall acidity. The Wood vinegar and Cropmax treatments applied in V2 may have influenced the acidity levels, potentially impacting the fruit's taste profile and acidity balance.

Finally, V4 showcases the highest total dry matter content at 6.79%. This parameter reflects the percentage of solid components in the fruit, including sugars, acids, and other compounds. The higher total dry matter in V4, the untreated control group, raises intriguing questions about the natural development of fruit components in the absence of specific treatments. If the outcomes pertaining to the total dry matter content demonstrate commensurability, a discordance emerges in relation to the soluble dry substance, exhibiting an antagonistic disposition in contrast to findings reported by other researchers (Trejo-Téllez et al., 2004; González-Pérez & Guerrero-Beltrán, 2021)

CONCLUSIONS

The application of specific treatments, such as Biochar combined with Cropmax, has demonstrated a consistent positive impact on total plant height, leaf/branch count, and flower/branch count. These findings underscore the efficacy of these treatments in promoting tomatillo`s enhanced growth and morphological development.

Treatments involving Wood vinegar, Cropmax, and their combinations have showcased significant improvements in key tomatillo fruit quality parameters. Notably, elevated levels of total soluble solids (°Brix), favorable titratable acidity (% malic acid), and desirable total dry matter (%) have been observed, emphasizing the efficiency of the applied treatments in enhancing the overall quality of the fruits.

The nuanced variations observed among different treatment combinations highlight the specificity of tomatillo plant responses. Each treatment combination contributes uniquely to plant physiology and fruit characteristics, indicating the importance of tailoring treatments for specific outcomes.

Cropmax treatments have consistently influenced chlorophyll content index (CCI) and anthocyanin content index (ACI) positively. These alterations signify the treatments' efficiency in enhancing photosynthetic processes and secondary metabolite accumulation, contributing to improved plant health and stress response.

The treatments, particularly Biochar application combined with Cropmax, have resulted in robust stem base diameters and optimal branching architectures. These structural enhancements are indicative of the treatments' efficiency in promoting plant strength, stability, and overall architecture.

Distinctly, Cropmax applications have led to increased numbers of fruits per plant and improvements in fruit dimensions, suggesting the efficiency of these treatments in influencing both the quantity and size of the harvested fruits, addressing key aspects of crop productivity.

The analysis of total yield per plant across different variants has provided valuable insights into the efficiency of the applied products. The consistent yield levels observed underscore the robustness and reliability of the studied treatments. The lack of statistical significance should not overshadow the practical importance of the observed trends, as the treatments exhibit a tangible impact on the overall yield.

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PRELIMINARY STUDIES ON THE INFLUENCE OF DIFFERENT SUBSTRATES ON THE CULTIVATION OF PEPPERS (*CAPSICUM ANNUUM* L.)

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Abstract

The paper presents a summary of scientific and technical findings on the influence of different substrate types used in pepper crops in protected environments. Pepper cultivation has developed a lot over time, due to different factors, so that different possibilities of cultivation have emerged, in the field, in greenhouses, in seedlings, on natural or artificial substrate, in hydroponic systems. The substrate must meet certain requirements: it must have a suitable structure, ensure gas exchange with the atmosphere and be permeable to water and air, and be rich in nutrients. In particular, the paper refers to the use of organic substrate to improve the quality and quantity of pepper fruits. According to the literature cited, the use of organic substrate offers numerous advantages including better control of nutrition and irrigation, adequate aeration and drainage and better conditions for root system development. The aim is to obtain an earlier and higher yield by adapting the pepper cultivation technology in a limited space, by using certain doses and types of fertilizers leading to the optimization of nutrients.

***Key words**: organic substrat, cultivation tecnology, higher yield, protected environments.*

INTRODUCTION

In light of current global trends, which encompass concerns regarding burgeoning population growth, elevating standards of living, and the imperative to augment agricultural productivity, intensified attention is being directed towards the cultivation of agricultural crops, with a particular emphasis on vegetable crops. This necessitates a deliberative exploration of diverse growing media, encompassing both organic and inorganic substrates, to meet the escalating demand for food. Projections by Blok et al. (2021) forecast a substantial fourfold increase in the utilization of varied growing media by 2050, underpinning aspirations for enhanced sustainability, precision in water and nutrient management, and increased resistance against diseases.

A consensus prevails within the scientific community regarding the imperative to discover novel substrates conducive to horticultural and potted plant cultivation (Paradelo et al., 2012). Furthermore, organic materials are increasingly recognized as secure reservoirs of plant nutrients, posing minimal risks to crops or soil integrity (Hasanuzzaman et al., 2010).

Amidst the dynamic landscape of crop management, propositions for novel substrates in soilless production exhibit considerable variability (Papadopoulos, 1994). However, the efficacy of many innovative substrates is often compromised by suboptimal management practices. The selection of a specific substrate is intricately influenced by factors such as local availability, cost considerations, and growers' familiarity with substrate performance. Moreover, decisions regarding substrate choice necessitate a nuanced evaluation of various physical and chemical attributes (Cantliffe et al., 2001; Gruda et al., 2016).

Extant research underscores the profound impact of agricultural management practices on the physical attributes and chemical composition of fruits and vegetables. Variations in elemental concentrations are observed, owing to the interplay between intrinsic genetic factors and extrinsic environmental influences, notably interactions between soil and plants (Barcanu et al., 2020; Soare et al., 2017; Dinu et al., 2018).

Among vegetable crops, sweet peppers (*Capsicum annuum* L.) hold a distinct position, prized for their vibrant hues, delectable flavor profiles, and nutritional richness (Vega-Galvez et al., 2009). Analogous to numerous other vegetables, peppers are amenable to cultivation utilizing conventional substrates such as perlite, sand, rockwool, and other soilless systems, which have substantially supplanted traditional soil-based cultivation methods within greenhouse environments (Zhai et al., 2009).

Thus, a comparative analysis of various substrates utilized in sweet pepper cultivation, elucidating their distinct advantages and applications has been conducted. Through a comprehensive review of literature, substrates including peat, coconut fiber, sheep wool, rockwool, compost, rice hull, organic mulch, vermicompost, and plastic mulch are evaluated based on their unique properties and contributions to plant growth and soil management. Each substrate is examined in terms of its reusability, capacity for air maintenance, nutrient content, weed suppression, and impact on soil quality and microbial activity. The findings provide valuable insights into selecting suitable substrates for optimizing plant productivity while considering environmental sustainability.

MATERIALS AND METHODS

A comprehensive literature search was conducted using prominent academic databases including Google Scholar, Science Direct, and Springer, was conducted to identify relevant studies published within recent years. The search was conducted using a combination of key terms, including "organic substrate", "sweet pepper crop", "greenhouse crop", "hydroponics", and "crop management". Only peer-reviewed articles meeting specific criteria were considered for inclusion in this review. Selected studies were required to have relevance in the vegetable growing field and to present comparative data on the performance of different substrate types in sweet pepper (*Capsicum* sp.) cultivation within protected environments, such as greenhouses or solariums. Initial screening of articles was conducted based on title and abstract to identify potentially relevant studies. Full-text review was then performed to assess eligibility based on the inclusion criteria. A total of 154 papers were initially identified through the literature search, of which 67 studies met the selection criteria and were included in the final review. Data relevant to substrate types, cultivation methods, experimental design, crop performance metrics, and other pertinent variables were extracted from each selected study. Comparative analyses were conducted to evaluate the efficacy of different substrate types in terms of growth parameters, yield, nutrient uptake, disease incidence, and other relevant outcomes. Findings from the selected studies were synthesized to provide insights into the suitability and effectiveness of various substrate options for sweet pepper cultivation in protected environments.

RESULTS AND DISCUSSIONS

The substrate plays a pivotal role in regulating the water balance crucial for plant growth, primarily by its water retention capacity (Kirda et al., 2004). This capacity depends on the physical attributes of the substrate, including particle size, structure, and porosity (Ansorena, 1994; Burés et al., 1996). Substrates comprising particles ranging from 1 to 10 mm exhibit varying water retention capabilities (Sanchez-Molina et al., 2014), with smaller particles (<1mm) demonstrating increased water holding capacity (Ansorena, 1994).

The cultivation of peppers in organic substrates offers numerous advantages over traditional soil-based methods, particularly in terms of overall and early production yields. Soilless cultivation facilitates denser plant growth and balanced supply of air, water, and nutrients, thereby enhancing disease resistance. Moreover, according to Munoz et al. (2010), soilless cultivation minimizes weed growth and allows for the adoption of natural or biological pest control methods, while also facilitating easier elimination of soil-borne pests and diseases.

Hydroponics emerges as a prominent soilless cultivation technique renowned for its productivity, water and land conservation, and environmental sustainability (Savaas, 2003; Barcanu et al., 2018). This method provides precise control over plant nutrition, eliminates soil preparation requirements, and enhances cultivation duration and greenhouse yields. The transition to hydroponics aligns with environmental policies, facilitating reduced fertilizer usage and mitigating nutrient leaching into the environment (Avidan, 2000; Barcanu-Tudor & Draghici, 2018).

A suitable substrate should possess sufficient mechanical properties, high porosity, and the ability to evenly distribute oxygen and water for activity. It should also have a low soluble salt content and a pH between 5.0 and 6.5, as well as being sterile and chemically inert. Soilless substrates are derived from organic materials. Donnan (1998) suggests that materials with health benefits can improve product quality. Growing media serve several functions, including providing aeration and water, supporting maximum root growth, and physically supporting plants.

The physical and chemical properties of growing substrates are crucial factors when used as growing media in soilless culture.

In terms of physical properties, the most important characteristics of growing media are a high content of easily available water combined with adequate air supply. Bilderback et al. (2005) proposed ranges for physical substrate properties, including 0.19-0.70 g, 3 cm for bulk density, 10-30% for air porosity, and 50-85% for total porosity.

The degradation of soil quality due to environmental conditions and improper management practices necessitates a transition to soilless cultivation systems, which provide desirable conditions for crop growth and development and enhance resource use efficiency. One of the factors limiting growth in soilless cultivation systems is the type of growing substrate. The use of more sustainable resources and ecological growth substrates is a challenge that affects the soilless culture industry.

Peat

Peat, derived from the slow decomposition and partial carbonization of accumulated plant material in unaerated bogs, serves as a natural fertilizer renowned for its unique properties.

It emerges as a versatile option, offering reusable properties and economic advantages, especially in regions with abundant resources. Its unique formation under acidic, waterlogged conditions renders it highly productive for soilless cultivation (Kitir et al., 2018; Osvalde et al., 2021).

Following fertilization and liming, Sphagnum peat often stands as the primary constituent of numerous growing media due to its distinct advantages. Its inherently low pH and nutrient content allow for facile adjustment to cater to crop-specific requirements. Moreover, the mode of peat formation ensures its freedom from pests and pathogens, while controlled production processes further eliminate weed seeds (Schmilewski, 2008).

Peat emerges as a quality substrate, exhibiting superior characteristics compared to alternative growing media, thus rendering it the predominant choice in organic substrates. By comparing black and red peat, composted bark and sandy soil, through chemical analysis, biometric measurements and quantity of product, Popescu et al., (1995) highlighted the superiority of the two substrates, obtaining a double yield and a harvest two weeks earlier.

Moreover, the parameters measured on direct seeding on peat substrate, were higher than those obtained from transplanted seedlings such as fresh root mass, dry matter. Among the biometric parameters, plant height was higher in the transplanted plants, but other indices such as hypocotyl length, stem diameter, number of leaves, leaf area were higher in the transplanted plants (Jankauskienė & Laužikė, 2023).

Although peat is the most important constituent of growing media, other organic and mineral-organic materials are being promoted through research and development. It is important to note that the use of these materials should be clearly marked as subjective evaluations. For several years, more funding and effort have been invested in testing alternatives to peat than in peat itself. (Schmilewski, 2008)

The hot pepper plants grown in a big pot system have recorded the highest values for vegetative growth parameters, yield per square metre, and the percentage of nitrogen and phosphorus in leaves (Metwally, 2016).

Based on the study's findings, it may be advisable to use peat growing media to improve the quality and yield of pepper cultivars (Gungor &Yildirim, 2013)

Coconut fibre

Coconut fiber stands out as a promising and environmentally friendly substrate for soilless cultivation, characterized by its composition of lignin and cellulose particles with varying sizes. This substrate exhibits diverse capabilities, including cation exchange, absorption of gases or ammonia nitrogen, and adjustment of pH levels within the culture medium (Shinohara et al., 1997).

It presents notable attributes such as high air maintenance capacity and re-wettability, enhancing its suitability for hydroponic systems (Abad et al., 2005).

When employed in pepper cultivation within pots, the utilization of coconut substrate has demonstrated the potential to yield high-quality harvests while enhancing economic efficiency (Roşca & Novac, 2010).

Abad et al., (2005) provided regression equations aimed at formulating a coir dust medium with optimal physical characteristics tailored to specific soilless substrate culture systems. The preparation of this medium involves blending appropriate proportions of various particle size fractions. Notably, the particle size distribution of coir dust significantly influences its physical properties, impacting aspects such as water retention, aeration, and relative hydraulic conductivity. These properties, heavily influenced by particle size distribution, play a pivotal role in enhancing irrigation management within containers utilizing coir dust as a substrate.

Sheep wool

Sheep wool, often regarded as a byproduct of sheep farming, has garnered attention as a potential resource for agricultural applications, particularly as a fertilizer or substrate. Although less conventional, it serves as a nitrogen-rich substrate, contributing to plant nutrition and potentially mitigating waste streams (Bradshaw and Hagen, 2022).

Concerns surrounding its safe utilization prompted investigations into its efficacy in enhancing plant growth and yield. In a study conducted by Górecki & Górecki (2010), the impact of washed sheep wool as an amendment to a peat-based growing substrate was evaluated concerning the growth and yield of sweet pepper, tomato, and eggplant. The experimental setup involved spreading a layer of wool on a 5 cm thick stratum of substrate, followed by covering with the same substrate at a rate of 10 g of wool per 1 dm^3 of substrate. The plants were individually cultivated in containers.

Results from the study revealed that the incorporation of wool led to notable enhancements in yield, with increases of up to 33%, particularly evident for tomato and pepper crops. Furthermore, the addition of wool induced alterations in the nutrient content of both the substrate and leaves, signifying its potential as a valuable and environmentally friendly fertilizer.

Bradshaw & Hagen (2022) noted that mulching increases soil temperature, which results in a higher release of nitrogen from sheep's wool. The performances of wool pellets were very close to those of organic fertilizers, which indicates that wool can be an alternative as a growing medium in ecological agriculture.

Rockwool

Mineral wool products are engineered to possess notable water and air retention capacities, facilitating root growth and nutrient uptake in hydroponic systems. Thus, rockwool stands out for its inertness, sterility, and efficient drainage properties, making it indispensable in hydroponic setups while ensuring disease and pest control. Furthermore, rockwool exhibits a rapid response to fluctuations in liquid feeding electrical conductivity (EC) or pH requirements, enabling precise control over nutrient delivery and maintaining optimal growing conditions (Bussel & Mckennie, 2004). The fibrous structure of mineral wool provides robust mechanical support for plant development. However, the naturally high pH of mineral wool renders it unsuitable for immediate plant growth, necessitating conditioning processes to attain a stable and appropriate pH level. Conditioning typically involves pre-soaking mineral wool in a nutrient solution adjusted to pH 5.5 until bubbling ceases (Alexander et al., 1994).

Water absorbent rockwool is extensively utilized in horticulture as a substrate for propagation and growth. Typically formed into plugs, blocks, or slabs, rockwool serves as an intermediary medium for cultivating crops until they reach transplanting maturity. Various types of blocks and slabs are manufactured, differing in density, fibre orientation, size, and shape. While some are single-use with lower fibre density and shorter lifespan, others are more durable with higher fibre density. The orientation of fibres in rockwool slabs, whether horizontal or vertical, impacts water holding capacity and aeration, with horizontal fibres and dual density slabs exhibiting superior performance (Bussel & Mckennie, 2004).

Water-repellent rockwool is commonly used in horticulture as flock or granulate in coarse, medium, and fine grades. Coarse grade water-repellent rockwool may occasionally be used as a soil conditioner in the greenhouse. The main use of all grades of water-repellent rockwool is in container mixes, which also include water-absorbent rockwool flock or other substrates such as peat, bark, and soil. Nursery, houseplants, hydroponic herbs, and commercial cut flower crops are grown in containers using these mixes. (Bussell & Mckennie, 2004).

Despite its prevalent use in Europe, rockwool as a growing medium presents several drawbacks. It is non-biodegradable, costly to produce due to high energy consumption, non-recyclable, and entails significant storage expenses (Osvalde et al., 2021).

Compost

The utilization of compost in agricultural practices offers a multifaceted array of benefits that contribute to soil health and productivity. As elucidated by Cortellini et al., (1996), the incorporation of compost enriches soil organic matter content, thereby fostering a conducive environment for nutrient cycling and microbial activity. Furthermore, Maynard (1995) highlights the enhancement of soil cation exchange capacity, facilitating improved nutrient retention and availability for plant uptake. Compost's capacity to augment soil water holding capacity, as evidenced by Paino et al. (1996), ensures sustained moisture levels crucial for plant growth and resilience to drought stress.

Moreover, Serria-Wittling et al. (1996) underscore its role in ameliorating soil acidity, thereby promoting optimal pH levels conducive to plant nutrient uptake and microbial activity. Additionally, Rothwell and Hortenstine (1969) demonstrate the stimulatory effect of compost on soil microbial and enzymatic activity, vital for nutrient cycling and soil health maintenance. Finally, Turner et al. (1994) high light its ability to mitigate soil compaction, thereby enhancing soil structure and aeration.

When applied as a mulch to the soil surface, organic compost exhibits a slower decomposition rate compared to when it is incorporated into the soil, allowing for its utilization throughout an entire growing season. This extended presence improves the water holding capacity of the soil and reduces the apparent density in the upper root zone (Wang et al., 2010).

Post-composting, the volume of organic compost decreases while its density increases, as observed in studies such as that by Paun et al. (2021). Despite this, mineralized nitrogen content in compost tends to be low due to inherent processes and nitrogen loss through leaching. To augment mineralized nitrogen levels, effective management of the composting process and utilization of diverse nitrogen sources are recommended (Younis et al., 2022).

Although compost can be integrated into seedling growth mixtures, it is typically not employed as the sole planting substrate due to various factors. These include rapid drying, potential presence of phytotoxins, and nitrogen immobilization by lignin. Consequently, compost is often utilized in mixtures comprising 20-30% for transplanting seedlings, as noted by Morel & Guillemain (2004).

Furthermore, compost application has been linked to enhancing plant immunity against diseases and pests, contributing to overall plant health and vigor (Ahmad et al., 2019).

Vermicompost

Vermicompost, enriched with humic substances and beneficial microorganisms, plays a pivotal role in soil health maintenance, nutrient cycling, and root protection, warranting its consideration in integrated soil management strategies (Lara & Quintero, 2006)

Its physical, chemical, and biological properties, emerges as a substrate conducive to promoting the growth of various vegetable plants within greenhouse environments. This comprehensive attributes enable it to meet the nutritional requirements of these plants effectively.

In the realm of organic chili pepper production, organic fertilizers represent a cost-effective, environmentally friendly, and sustainable alternative to chemical fertilizers (López-Espinosa et al., 2013). By reducing reliance on synthetic inputs, organic fertilizers contribute significantly to the ecological integrity of agricultural systems.

Humus derived from earthworm activity garners widespread acclaim, not only within conventional agriculture but also within the realm of organic farming (Bravo-Lozano et al., 2011).

Rice hull and straw

Rice hull is an agricultural by-product that is often underutilised. According to Okafor & Okonkwo (2009), over 100 million tons of rice hull are generated annually worldwide. Due to the increasing difficulty and expense of collecting and disposing of rice hull, it is often left unused as waste.

It demonstrates multifaceted benefits including weed suppression, improved soil nutrient availability, and control of soil-borne pathogens, making it an environmentally friendly alternative (Dobermann & Fairhurst, 2002)

The management of rice straw disposal presents a notable environmental concern if not handled appropriately, with options including incorporation into soil or field burning, as outlined by Mansaray & Ghaly (1997). Despite its disposal challenges, rice straw possesses favorable properties as a growing medium, characterized by its lightweight nature, inertness regarding nutrient absorption and desorption, good drainage, aeration, and slow decomposition rate (Saparamadu, 2008).

The utilization of rice husk is recommended only after it has been fully immersed in water for a week.

Transplanting pepper plants onto rice straw culture media led to notably higher root fresh weight, number of leaves per plant, number of branches per plant, and plant height when compared to sandy soil (El-Sayed et al.; 2015). Furthermore, sweet pepper plants grown on rice straw exhibited significantly higher early, marketable, and total yields compared to those grown on sand or other media. Analysis of leaf nutrient concentrations revealed that nitrogen and potassium levels were highest in sandy soil, while they were lowest in straw culture. Conversely, phosphorus and calcium concentrations were highest in straw culture, suggesting a significant impact on leaf chlorophyll content and phosphorus levels.

Rice straw could be recommended as a growing substrate to replace naturally infested soil. It can improve the production of pepper plants under greenhouse conditions while saving 35-38% of irrigation water and fertilizers. Additionally, it presents a solution for soil salinity and alkalinity, and avoids the serious pollution resulting from burning rice straw for disposal.

Moreover, the adoption of rice straw substrates holds promise for enhancing the exportation of organic sweet peppers by eliminating the need for soil chemical disinfectants and nematicides typically employed to combat soil-borne pathogens and nematodes.

Wood fibres

Wood fibers are derived from wood and wood waste through mechanical or thermal processes. However, only mechanically treated wood is deemed suitable as raw material for this purpose. Wood subjected to gluing, coating, lacquering, painting, or treatment with organic or inorganic substances is prohibited due to potential adverse effects.

To mitigate the risk of nitrogen immobilization by wood fibers, which can pose challenges in commercial horticulture, the addition of a nitrogen fertilizer to the wood chips prior to extrusion is recommended. This precautionary measure aims to prevent potential cultivation difficulties associated with nitrogen immobilization.

Perlite

Perlite stands as a prevalent growing substrate within vegetable cultivation, notably in the cultivation practices of sweet peppers (*Capsicum annuum*). This lightweight, inert material is derived from volcanic glass and is characterized by its high porosity and excellent drainage properties. Several investigations have indicated that in the initial phases of development (up to 90 days post-transplantation), sweet pepper plants cultivated in a perlite-based medium exhibited a notable reduction in all parameters related to vegetative growth compared to those grown in sandy soil. It should be emphasized that this research specifically targets the early developmental stages. The physical attributes of the perlite utilized in this examination were delineated as follows: a bulk density of 0.13 g/cm, moisture content, water holding capacity of 69.7%, pH level of 7.8, and porosity of 68% (Raviv et al. (2008).

The escalation in bulk density is intricately associated with a diminution in the overall pore volume, primarily impacting growth by constraining free pore space. This discovery corroborates the findings of Abd-El-Baky et al. (2010), whose investigation revealed that plant height, stem diameter, and root fresh weight exhibited noteworthy augmentation in sand-based substrates compared to those in perlite.

Organic mulch

The integration of organic mulch into agricultural and horticultural practices embodies a holistic approach to soil management, offering advantages that encompass both agronomic and environmental considerations. The utilization of organic mulch confers multifaceted benefits, each contributing to the overall enhancement of soil health and productivity (Lalande et al., 1998; Feldman et al., 2000).

Firstly, organic mulch serves as a formidable tool in moisture conservation, effectively mitigating water loss through evaporation and enhancing soil water retention capacity. This attribute is paramount in arid and semi-arid regions where water scarcity poses a significant challenge to agricultural sustainability. Moreover, the ability of organic mulch to reduce soil erosion, as highlighted by Feldman et al. (2000), underscores its role in safeguarding soil integrity and mitigating the detrimental effects of water and wind erosion, thereby preserving soil structure and fertility.

Furthermore, organic mulch serves as a natural barrier against weed proliferation, effectively suppressing weed growth and competition for resources such as water, nutrients, and sunlight. This not only reduces the need for labor-intensive weed management practices but also mitigates the use of synthetic herbicides, aligning with principles of sustainable agriculture and environmental stewardship.

Importantly, the cost-effectiveness of organic mulch, as underscored by Lalande et al. (1998), presents a compelling advantage for resource-limited agricultural systems. Its affordability and accessibility make it a viable option for smallholder farmers and subsistence agriculturalists, facilitating widespread adoption and implementation.

Additionally, the incorporation of organic mulch into soil systems augments soil quality through the gradual decomposition of organic matter, enriching soil with essential nutrients and organic carbon. This process stimulates soil microbial communities, promoting biodiversity and enhancing soil fertility and structure over time.

Plastic mulch

Plastic mulches play a crucial role in vegetable crop cultivation, serving various functions such as modifying soil temperature and moisture, controlling weeds, deterring insects, and impacting plant photobiology.

The prevalent material for plastic film mulching is low-density polyethylene, synthesized through ethylene polymerization under high pressure (Lamont, 2005). Besides these benefits, plastic mulch acts as a protective barrier against soil-borne pathogens and microorganisms, ensuring food safety and quality in agricultural production (Lamont, 2005).

Foremost, the utilization of plastic mulch markedly reduces evaporation of water from the soil, thereby conserving precious moisture resources crucial for plant growth and development. This attribute is particularly advantageous in regions prone to water scarcity, where efficient water management is imperative for ensuring crop viability and resilience to drought stress.

Furthermore, plastic mulch serves as a formidable barrier against weed encroachment, effectively suppressing weed growth and competition for essential resources such as water, nutrients, and sunlight. This not only alleviates the need for labor-intensive weed control measures but also mitigates the reliance on chemical herbicides, thereby fostering environmentally sustainable crop production systems.

Moreover, the implementation of plastic mulch has been consistently associated with increased crop yields, as demonstrated by Narayan et al. (2017). This elevation in yield potential is attributed to several factors, including enhanced soil temperature regulation, optimized moisture retention, and minimized competition from weeds, collectively facilitating optimal conditions for plant growth and productivity.

Additionally, plastic mulch contributes to improved fruit quality, characterized by attributes such as size, color, flavor, and nutritional content. The insulation provided by the mulch maintains consistent soil temperatures, promoting uniform fruit ripening and minimizing physiological disorders, thereby enhancing marketability and consumer appeal.

The expedited maturation afforded by plastic mulch translates into earlier harvests, providing growers with a competitive edge in seasonal markets and optimizing resource utilization. Furthermore, the inhibition of weed growth and reduction in soil compaction associated with plastic mulch contribute to improved soil health and structure, facilitating root development and nutrient uptake efficiency (Bosland & Votava, 1999; Tindall, 2001).

Importantly, plastic mulch serves as a barrier against soil-borne pathogens and microorganisms, safeguarding fruits from contamination and spoilage, thus ensuring food safety and quality assurance. This multifaceted array of benefits underscores the pivotal role of plastic mulch in modern agricultural practices, exemplifying its potential to revolutionize crop management strategies and foster sustainable food production systems for future generations.

The microclimate of a field can be altered by adjusting the surface's radiation budget and reducing soil water evaporation.

This has been demonstrated in studies by Liakatas et al. (1986), Romero-Sierra & Tanner (1974) and Ham et al. (1993).

The study found that the black polythene mulch (double coated 30 micron) had a higher soil moisture content (16.74%) compared to the double coated white polythene (15.22%) and the control group (10.10%). The increase in moisture retention capacity resulting from polythene mulching can be attributed to the reduction in moisture evaporation from the soil. Additionally, the water that evaporated from the soil was trapped beneath the mulches, resulting in vapours that dropped back into the upper soil layer. Wang et al. (1998) reported that all types of polythene mulch increased soil moisture content in a chilli field compared to the control. At the time of crop harvesting, weeds were removed from different plots and dried to obtain their dry weight (g/plot). The results showed that polythene mulches were more effective in reducing weed density compared to organic mulches. In plots with polythene mulch, weeds only emerged through the punch holes and no weeds were found under the plastic. This may be due to the lack of light penetration through the black plastic. The density of weeds was lowest in plots covered with black polythene mulch (74.81 g/plot), followed by those covered with white polythene mulch (32.42 g/plot), and highest in the control group (418 g/plot). Mulch affects the microclimate surrounding the plant through its interaction with water and soil. The use of mulch resulted in a higher number of fruits per plant, a decrease in the number of aborted fruits, and an increase in fruit weight, ultimately leading to higher production per plot (Narayan et al., 2017). When using various types of plastic mulches (black, aluminum, silver, and white), it was observed that the dry weight of the shoots was higher in the mulched soil compared to the control soil. Additionally, a greater quantity of nutrients (N, K, and S) were found in the leaves (Canul-Tun et al., 2017)

The analysis of the optical properties of plastic mulches in shortwave and longwave spectrums demonstrated a wide range of characteristics in modern agricultural plastics. Field experiments revealed that the optical characteristics of the mulch affected the subsoil and surface temperatures of mulched beds.

Ham et al. (1993) conducted their experiments without a crop to maximise solar irradiance on the surface and intensify the effect of mulch optical properties. The shading of the bed by the developing crop canopy would likely moderate differences between mulches, as well as the effect of mulching in general.

The combination of soil plastic mulching and the application of organic amendments shows promise for controlling soilborne pathogens in temperate regions through various mechanisms. These include the accumulation of toxic volatile compounds generated during organic matter decomposition, the creation of anaerobic conditions in the soil, and an increase in soil suppressiveness due to high levels of microbial activity (Gamliel et al., 2000). The use of organic amendments followed by soil plastic mulching may be a viable solution for controlling *Phytophthora* stem and root rot in pepper crops under temperate climates. The decrease in disease incidence observed was attributed, at least in part, to a reduction in oospore viability, an increase in soil microbial activity and functional diversity, and the production of NH_3, which may have resulted in pathogen suppressiveness.

After being warmed up, the root zone temperature in the covered raised bed was approximately 2 degrees Celsius higher than in the uncovered raised bed. The temperature of the environment and soil was influenced by the colour of the mulch and the year, while the air temperature was affected by the covering of the pepper rows. The covered area experienced a higher temperature by 2-5°C (Ham et al., 1993).

The selection of substrates for sweet pepper cultivation should be guided by a thorough understanding of their respective advantages and applications. Integrating diverse substrates into cultivation systems enables growers to address specific requirements while promoting sustainable practices and enhancing overall productivity. Future research endeavors should focus on optimizing substrate combinations and exploring novel materials to further advance horticultural production systems in a resource-efficient and environmentally sustainable manner.

CONCLUSIONS

The selection of suitable substrates for sweet pepper cultivation is crucial for optimizing growth and yield potential. Organic substrates such as peat, coconut fiber, and vermicompost offer distinct advantages in terms of water retention, nutrient availability, and disease suppression, while mineral substrates like rockwool provide precise control over plant nutrition in hydroponic systems. Understanding the attributes and applications of different substrates is essential for tailoring cultivation practices to specific crop requirements and environmental conditions.

Soilless cultivation systems, including hydroponics and organic substrate-based methods, offer viable alternatives to traditional soil-based approaches, particularly in regions with limited arable land or soilborne disease pressures. The adoption of soilless cultivation facilitates resource-efficient crop production, enhances disease resistance, and promotes sustainable agriculture practices by minimizing water and nutrient wastage while maximizing yields.

The integration of organic mulches into agricultural systems presents numerous agronomic and environmental benefits, including moisture conservation, weed suppression, soil erosion control, and enhancement of soil health. Organic mulches contribute to sustainable soil management practices by improving soil structure, fertility, and microbial activity, thereby supporting long-term agricultural productivity and ecosystem resilience.

Future research efforts should focus on optimizing substrate formulations, exploring alternative materials, and assessing the long-term impacts of soilless cultivation systems on crop productivity, soil health, and environmental sustainability. By advancing our understanding of substrate-plant interactions and cultivation practices, we can develop innovative solutions to address emerging challenges in horticultural production and promote the adoption of sustainable agricultural practices on a global scale.

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EFFECT OF BIOSTIMULANT TREATMENT ON THE ANTIOXIDANT ACTIVITY IN TOMATO FRUIT

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Abstract

One of the most widely grown and consumed vegetable, tomato (Lycopersicon esculentum Mill.) is valuable both for their rich taste and for their nutritional value and antioxidant properties. Vitamin C, carotenoids (lycopene, β-carotene) and polyphenols are the main bioactive compounds present in tomato, their regular consumption being correlated with a low risk of various cancers and some cardiovascular diseases. Biostimulants products represent nowadays a sustainable alternative to the chemical fertilizers used for improving crop yields and quality. A product obtained from fish gelatin and collagen hydrolysate was physicochemically characterized and used for foliar application on tomato crop in the fruiting stage. The objective of this study was to assess the effect of the biostimulant treatment on antioxidant activity of tomatoes. The tomatoes were analyzed regarding carotenoids, ascorbic acid, phenolic compounds content and antioxidant activity. The treated tomatoes presented higher content by 26.64% for lycopene, by 12.8% for ascorbic acid and by 17.25% for total phenolics. These positive effects can be attributed to the supplementary amino acids amount provided by the biostimulator product.

Key words*: antioxidant activity, ascorbic acid, fish gelatin, lycopene, phenolic compounds.*

INTRODUCTION

Recent research has reported protective effects on human health of a diet rich in vegetables and fruits, among which tomatoes occupy an important place. One of the most widely grown and consumed vegetable, tomato (*Lycopersicon esculentum* Mill.) is valuable both for their rich taste and for their nutritional value and antioxidant properties. The main bioactive compounds present in tomato are antioxidants such as carotenoids (lycopene, zeaxanthin, β-carotene), vitamin C and polyphenols which are involved in inhibition of the harmful effects of free radicals, preventing oxidative damage in DNA and proteins. The health-beneficial properties of tomato have been proven by epidemiological evidence that suggests their role in reducing serum levels of oxidative stress biomarkers. Research performed by Abete et al. (2013) demonstrated that daily consumption of 160 g of tomato sauce rich in lycopene produces a decrease in oxidized LDL cholesterol levels. Consequently, a diet rich in tomato was correlated with a low risk of various cancers and some cardiovascular diseases (Collins et al., 2022).

The nutritional content of the tomato fruit depends on the cultivation conditions and on the growth environment that must ensure the maximum potential accumulation of the bioactive compounds under organic farming conditions. Since the EU Council Directive 91/676/EEC called for a significant reduction in the amount of nitrogen-containing fertilizers used in agriculture and horticulture, environmentally friendly agricultural techniques to improve crop yields and quality were adopted. As an alternative to chemical fertilizers, biostimulants began to be used on a large scale to promote plant growth, so that the market size for biostimulants is expected to reach USD 4.14 billion by 2025 (Madende & Hayes, 2020). Although they do not supply nutrients directly to plants, the biostimulants can facilitate the nutrients achievement by supporting metabolic processes in plants improving the general health and vitality of plants.

Hydrolysates of food by-products such as animal tissue (Cristiano & De Lucia, 2021; Luta et al., 2024) or fish waste (Chalamaiah et al., 2012; Madende & Hayes, 2020) have been used as biostimulants provided by animal sources. Fish processing produce large quantities of waste (head, skin, bones, tail, viscera) representing a source of environmental pollution if improperly disposed. Only about 30% of the 91 million tons of fish harvested every year is transformed into fishmeal (Madende & Hayes, 2020). But fish by-products have potential applications as plant biostimulants. The residues rich in collagen and gelatin are processed by chemical or enzymatic hydrolysis and converted into protein hydrolysates containing free amino acids, short-chain peptides and proteins as active agents that positively influence some physiological processes, including photosynthesis, assimilation and translocation of nutrient in plants, and also accumulation of valuable compounds (Malécange et al., 2023). Various studies have shown that fish hydrolysates can improve nutrient utilization by plants, achieving beneficial effects on root and leaf growth, inducing flowering, improving fruit set, and also reducing fruit drop (Chalamaiah et al., 2012; Yakhin et al., 2017; Xu & Mou, 2017).

The present study was focused on testing a product obtained from fish gelatin and collagen hydrolysate with inorganic potassium added. This protein gel was physico-chemically characterized and used for foliar application on tomato in the fruiting stage. The aim was to assess the effect of the tomato plants treatment with the biostimulant product on the antioxidant compounds content and on the total antioxidant capacity of tomato fruit compared to the plants grown in conventional cultivation.

MATERIALS AND METHODS

Obtaining of biostimulant gel (protein hydrolysate)

A new product representing a protein gel made from fish gelatin and collagen hydrolyzate enriched with macroelements (K) with a ratio of NPK 1:1:3 was obtained and tested for biostimulant properties by application on tomato plants during the fruiting stage.

Fish gelatine was obtained from fish scales treated for 1 h with 0.1 M NaOH to remove non-collagen proteins, then demineralization using 1 M HCl (1.5 hours) have been made. The demineralized fish scales were subjected to a hot water extraction to produce gelatin, which was then filtered in several stages to obtain a clear gelatin solution. The biostimulant product was obtained by mixing equal parts of fish gelatine with collagen hydrolysate and inorganic potassium. The collagen hydrolysate was obtained from the residues of bovine hide after extraction of gelatine, which were dispersed in water in a ratio solid to liquid 1:2 at pH 8 and 50°C. Then a hydrolysis with Protamex (edopeptidases) added in a ratio of 1.6 to residue weight was made for two hours. The potassium was added in a ratio of 3:1 to total nitrogen. The obtained gel was analyzed regarding certain chemical characteristics (Table 1). Determination of the dry substance, total ash, total nitrogen and protein content, pH, bloom test, and viscosity were made using standards in force.

Table 1. Physico-chemical characteristics of protein gel

Characteristics	Analytical methods	Parameters value
Dry substance (%)	SR EN ISO 4684:2006	15.18
Total ash (%)	SR EN ISO 4047:2002	2.7
Total nitrogen (%)	SR EN ISO 5397:1996	1.20
Protein content (%)	SR EN ISO 5397:1996	6.74
pH	STAS 8619/3:1990	7.02
Bloom test (g)	European Pharmacopoeia. Gel strength	54
Viscozity (mPa*s)	European Pharmacopoeia. Viscosity.	1.75

Biological material

The experiment was conducted in the experimental greenhouse of the University of Agronomic Sciences and Veterinary Medicine from Bucharest in 2023 on pink tomato crop from BPK 16021 hybrid (*Lycopersicon esculentum* Mill.), provided by Marcoser SRL (Matca, Galati, Romania). The experimental variants were: V1 - untreated plants (control) and V2 - plants which received treatment with fish gelatin based gel. The substrate used for cultivation was Traysubstrate, a well structured mixture of blond peat and black peat, with addition of macro and microelements and a pH of 5.5-6.5. Three successive foliar treatments with dilluted solution of protein gel (10% v/v) were applied, once every seven days, between May 22 and June 5, 2023. No treatment with other fertilizers was administered except for biostimulant applications with tested protein gel. The tomato harvests started on June 26, 2023. Random tomato samples were collected from each variant and were analyzed in laboratory regarding bioactive compounds.

Biochemical analysis of the tomato fruit

Tomato fruit harvested at the red-ripening stage were subjected to biochemical analyses. The extractions were made according to the protocol provided by the analysis method used.

Carotenoids (lycopene and *β-carotene)* were determined according to the protocol described by Anthon & Barrett (2007). An amount of blended tomatoes was homogenized with a triple mixture of hexane: ethanol: acetone (2:1:1), then sonication and shaking were applied so that two phases were separated. A sample was collected from the upper phase and the absorbance was measured at two wavelength (503 and 444 nm). Using the extinction coefficient lycopene and β-carotene contents has been calculated. Results were expressed as mg/100 g FW.

Vitamin C content was determined using titration with potassium iodate solution by volumetric method described by Elgailani et al. (2017). A 2% solutrion of oxalic acid was used for the extraction of vitamin C with the purpose to prevent the oxidation. The extracts were treated with KI solution, hydrochloric acid and starch solution, then were subjected to titration with iodate KIO_3 until the a blue color appears indicating the end of the reaction. Results were expressed as mg/100 g FW.

Total polyphenols content was performed using the modified Folin-Ciocalteu method as Singleton et al. (1999) described. The determination is based on the reduction of the Folin-Ciocalteu reagent, obtaining a blue colored compound, followed by measuring the absorbance at 750 nm. The results were expressed as gallic acid equivalents (mg GAE/g FW).

Total antioxidant activity was measured according to the method adapted by Brand-Williams et al. (1995) after Blois (1958), using the stable free radical DPPH. 100 μM solution of DPPH in methanol were mixed with different concentrations of a tomato extract in 80% aqueous methanol, then were maintained 30 min in a dark place at room temperature. After that the absorbance (A) was measured at 515 nm. The percentage of the radical scavenging activity (RSA) was calculated as follows:

$$\% \text{ RSA} = (1-[A_{sample}/A_{control\ t=0}])/100$$

A DPPH solution in 80% methanol was used as control. The linear regression curve of the sample extracts (mg/mL) against the percentage of the radical scavenging activity was made and used for calculating EC_{50} for each sample. The EC_{50} parameter is defined as the concentration of sample which is required to scavenge 50% of DPPH free radicals.

Statistical analysis was performed with the one-way Analysis of Variance (ANOVA) using Microsoft Excel Office 2019 for Windows. Comparisons of means were calculated using the Duncan's test at the 5% significance level ($p < 0.05$). All measurements were carried out in five replicates, and results are presented as means ± standard deviation (S.D.). In charts, values marked with different letters show significant differences ($p < 0.05$).

RESULTS AND DISCUSSIONS

Effect of the biostimulant treatment on the antioxidant compounds of the tomato

Biostimulants act by inducing a physiological response in the treated plants, influencing metabolic processes of plants and consequently, their growth and development. The current research aimed to assess the

response of the tomato crop to the application of tested protein gel regarding the antioxidants content of the fruit.
Vitamin C is the most commonly found vitamin in tomato fruit. Considered as a major natural antioxidant, vitamin C is involved in limiting the damaging effects of free radicals therefore it is supposed to help prevent or delay the development of certain diseases caused by oxidative stress. Besides that, vitamin C stimulates the immune system and improves the absorption of iron and calcium (Bianchi et al., 2023).

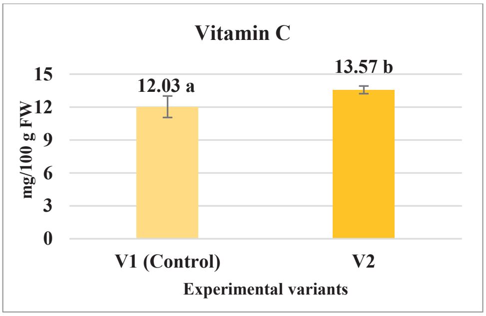

Figure 1. Vitamin C content in the analyzed tomato samples

The application of the biostimulant protein gel resulted in increased vitamin C content in tomato fruit. Significant differences were noted between the control and the treated variant (Figure 1), which recorded a 12.8% higher vitamin C content than the untreated plants.
Also previous research performed by Luta et al. (2024) found that the biostimulant treatment of tomato plant with a bovine gelatine based gel stimulated the accumulation of vitamin C, which was 1.29 times higher compared to the untreated plants. These results are in according to Tallarita et al. (2021), which reported an increasing of vitamin C in tomato fruit when applied 9 times the biostimulant treatment with an enzymatic protein hydrolysate. On the contrary, evaluating the effects of three biostimulants (protein hydrolysate, plant and seaweed extract) on the nutritional quality of greenhouse tomato, Colla et al. (2017) noted no influence of the treatment on the total ascorbic acid.
Lycopene and ***β-carotene*** are the main carotenoids detected in tomato fruit, representing almost 75% of tomato carotenoids (Hernández-Herrera et al., 2022). Lycopene and other carotenoids are suggested to protect against carcinogenesis through antioxidant mechanisms. Thorough studies demonstrated by in vitro experiments that both lycopene and β-carotene manifest anticancer activities, being able to induce apoptosis in cancer cells and to inhibit their proliferation (Jayappriyan et al., 2013; Arathi et al., 2016).
The analysis of the obtained results highlighted a 26.64% increase in the lycopene content from 7.17±0.2 mg/100 g FW in the untreated tomato fruit to 9.09±0.11 mg/100 g FW in the variant treated with fish gelatin based gel (Figure 2).

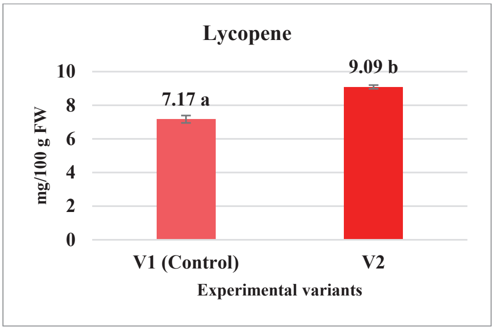

Figure 2. Lycopene content in the analyzed tomato samples

And the β-carotene synthesis in the tomato increased by 20.37% under the same biostimulant treatment (Figure 3).

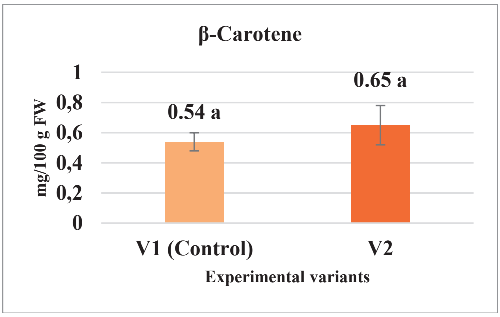

Figure 3. β-carotene content in the analyzed tomato samples

Tallarita et al. (2021) noted also that lycopene content recorded a quantitative improvement (from 11.05 to 14.13 mg/g FW) after repeated application of the tested protein hydrolysate on the tomato crop.

An increased by 125% accumulation of lycopene has been reported by Colla et al. (2017) under the foliar application of a commercial legume-derived protein hydrolysate. Consequently, a positive correlation between lycopene and potassium concentration was suggested, reporting that biostimulant application appeared to stimulated lycopene accumulation through the increase in mineral absorption.

Polyphenols are bioactive compounds with major role in free radical scavenging activity and in plant protection against stress.

Moreover, polyphenols are involved in plant development and growth (Sun et al., 2024) and also a correlation between total phenols of plants and antifungal activity has been reported (Mohamed et al., 2017).

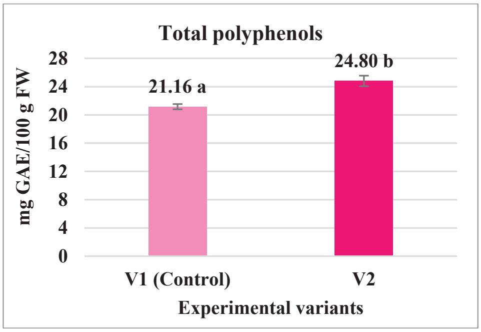

Figure 4. Total polyphenols content in the analyzed tomato samples

Figure 4 shows a stimulating effect for the total polyphenols biosynthesis under the treatment with the tested fish gelatin based gel. The obtained values were between 21.16±0.37 and 24.80±0.75 mg GAE/100 g FW, indicating a 17.25% increase in the average content of total polyphenols in the treated tomato compared to the untreated control.

Also previous studies reported that using of biostimulants enhanced the polyphenol content in tomato (Ali et al., 2021), broccoli (Kałuzewicz et al., 2017), and zucchini (Abd-Elkader et al., 2022).

Effect of the biostimulant treatment on the antioxidant activity of the tomato

Various studies have reported that biostimulants facilitate plant resilience, mainly by increasing antioxidant activity in the plants under unfavorable environmental conditions (Colla et al., 2015; Gonzalez-Morales et al., 2021; Malécange et al., 2023). The antioxidant capacity in plants is correlated with the content of compounds with antioxidant potential, such as carotenoids, flavonoids, vitamin C, polyphenols. Furthermore, a linear correlation of the increase in polyphenol content with antioxidant capacity is reported by previous research (Asadi et al., 2022; Balan et al., 2023).

Samples of tomato collected from the experimental variants has been screened for a potential radical scavenging activity, and the EC50 values were calculated. Significant differences between the analyzed variants were noted (Figure 5).

The result indicated that variant V2 (tomato treated with protein gel) recorded the highest antioxidant activity (91.21±0.81 mg/mL expressed as the EC50 value), as expected due to the higher content in total polyphenols compared to the untreated variant, which required a higher concentration (121.39±0.80 mg/mL) to scavenge 50% of DPPH free radicals.

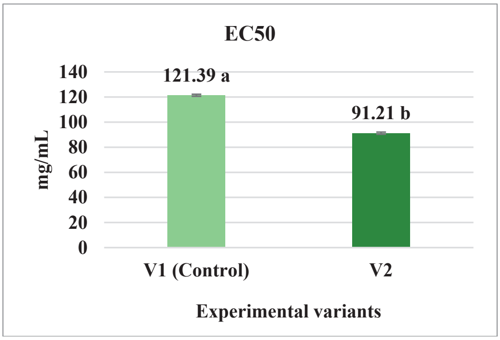

Figure 5. EC_{50} values of DPPH scavenging activities in the analyzed tomato samples

Overall, the treatment with biostimulant protein gel positively impacted the tomato plants growth and an improved fruit quality has been obtained.

These beneficial effects can be attributed to the supplementary amino acids amount provided by the new biostimulant product. Positive effects of the foliar application of amino acids and biostimulants based on amino acids on quality parameters have been reported also as a result of research performed on *Foeniculum vulgare* Mill. (Elsayed et al., 2022), *Achillea* *millefolium* L. (Shafie et al., 2021), *Nigella sativa* L. (Ayyat et al., 2021), *Ocimum basilicum* L. (Aghaye et al., 2019). Amino acids are molecules essential in metabolite synthesis promoting cell growth and plant development (Sun et al., 2024). The free amino acids present in the tested fish gelatin based gel represent a support for the development of tomato plants, helping the rapid biosynthesis of different types of proteins. Important for plant is not only the presence of these free amino acids, but also their accessibility for use in biosynthesis processes (Rentsch et al., 2007) therefore providing ready-for-uptake amino acids represents an advantage for plants, especially during critical periods of plant development such as the flowering or the fruiting stage (Bulgari et al., 2015).

CONCLUSIONS

Biochemical determinations performed in this study revealed beneficial effects of the treatments with biostimulant fish gelatin based gel on the tomato fruit quality.

Applications of the tested protein gel resulted in a good accumulation of the antioxidant compounds and a high radical scavenging activity of the tomato fruit, increasing their potential in preventing oxidative stress.

The tested new biostimulant could be considered better alternative for chemical fertilizers in obtaining vegetable with valuable nutritional and health-related properties.

This research also encourages the reuse of waste products supporting the circular economy in favor of a healthier environment.

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IMPACT OF URBAN CONDITION ON BRASSICACEAE DEVELOPMENT

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Abstract

Urban agriculture refers to the practice of cultivating plants within city environments. It faces challenges such as accessing resources, soil, water, and air quality in urban environment. Field experiments were carried out in 2023 on alluvial-meadow soil located in Sofia city, focusing on growing cabbage and kohlrabi. Biometric analysis, absolute dry matter, and crop yields were conducted for both crops. Soil analyses were performed at the beginning of the experiment. The soil showed weak humus content, ranging 1.5% to 1.82%. The data obtained for kohlrabi yields and total available water, along with the correlation coefficient R2-0.77, indicate that an increase in irrigation rate results in decreased yield. Yields obtained in urban conditions ranged between 14-15 t/ha for kohlrabi and 34-47 t/ha for cabbage.

Key words: *urban agriculture, drip irrigation, yield, soil condition.*

INTRODUCTION

In an era marked by rapid urbanization and environmental concerns, the concept of urban gardening has emerged as a promising avenue for sustainable living and resilient communities (Eigenbrod, C. & Gruda, N., 2015; Saha, M. & Eckelman, M. J., 2017). Urban gardening leads to sustainability and community resilience in the face of rapid urbanization and environmental degradation. However, its realization is not without hurdles, particularly in the realm of resource access and environmental quality. Access to resources forms the bedrock of successful urban gardening endeavours. Furthermore, the quality of soil in urban environments poses a significant challenge to successful gardening practices (Dobson, M. C. et al., 2021). Soil contamination, resulting from industrial activities, vehicular emissions, and improper waste disposal, jeopardizes plant health and compromises food safety. Water scarcity and quality issues also loom large in urban gardening pursuits (Bediakoh, A. W. et al., 2024). Competition for water resources, coupled with erratic precipitation patterns exacerbated by climate change, underscores the importance of efficient water management strategies. Rainwater harvesting, drip irrigation systems, and grey water recycling techniques offer promising avenues for conserving water and reducing reliance on municipal water supplies. The ability of drip irrigation to save water is unparalleled, making it a crucial tool in addressing water scarcity while ensuring food security and agricultural sustainability. The irrigation of late cabbage and kohlrabi holds significant importance in determining both the quantity and quality of the yield. Cultivating Brassicaceae can be effectively managed even under conditions of deficit irrigation. Moreover, employing a deficit irrigation approach enables crops to sustain a certain level of water deficit with minimal impact on yield, as noted by Abdelkhalik (2019). This strategy holds promise for enhancing water use efficiency and conserving water resources. Moreover, alert monitoring of water quality parameters is imperative to safeguarding plants from contaminants and ensuring the safety of harvested produce (Orsini, F. et al., 2013). In conclusion, navigating the complex challenges of resource access and environmental quality is paramount to the success and sustainability of urban gardening initiatives. Through collaborative efforts and innovative solutions, urban gardening holds immense potential to transform our cities into greener, healthier, and more equitable spaces for generations to come.

The aim of study is to establish the influence of urban condition and deficit irrigation regimes on cabbage and kohlrabi quality of yield for the conditions of Sofia city region.

MATERIALS AND METHODS

The experiment was conducted in 2023 in the suburban area of Sofia city. The soil type is alluvial-meadow. The tested crops are late cabbage and purple kohlrabi. The study was carried out on long plot method in four replicates, featuring harvest plots measuring 12 square meters each. Sowing followed a two-row strip pattern with an inter-row spacing of 70 by 50 centimetres.

To assess the impact of the irrigation regimen on the growth, development, and yield of cabbage and kohlrabi, the vegetation period is segmented into two distinct sub-periods: "vegetative" and "reproductive".

Different variants of the irrigation regime (IR) were tested as follows:

IR 1. Irrigation with 40% of the irrigation rate determined in the optimal variant.

IR 2. Irrigation with 70% of the irrigation rate determined in the optimal variant.

IR 3. Irrigation with full irrigation rate (100% m) - optimal irrigation (control).

IR 4. Irrigation with an increased irrigation rate (130% m) - determined in the optimal variant.

IR 5. Without irrigation during the "planting-vegetative growth" period.

IR 6. Without irrigation during the "head formatting" period.

In the optimal variant (IR3), irrigation will be conduct when soil moisture reaches 80% of the field capacity (FC) within the 0-40 cm layer. The irrigation rate will be adjusted to ensure adequate moisture throughout the active soil layer (0-60 cm). To achieve this, soil moisture dynamics will be monitored over a period of 7 days using the Gravimetric Soil Moisture Detection method (S.G. Reynolds, 1970).

$$GWC\ (\%) = [(\text{mass of moist soil (g)} - \text{mass of dry soil (g)})/\text{mass of dry soil (g)}] \times 100$$

The irrigation rate in the control variant will be calculated based on the water balance equation. (Z. Stoyanov et al., 1981).

Drip hoses will be used to irrigate the experimental plots, allowing for precise control over the dosage of irrigation water. The water supplied to each irrigated plot will be measured based on an hourly flow rate.

The water source is a borehole near a livestock farm. Fertilizer is not applied during the crop's vegetation period to stimulate growth.

Precipitation levels are recorded each morning using a rain gauge. The average daily temperature during the crop's vegetation was recorded.

Soil and water analyses were conducted in a certified laboratory according to approved methodology.

The quantity and quality of the yield was evaluated from 10 plants of each replication.

RESULTS AND DISCUSSIONS

The average annual air temperature in Sofia city for the period 1956-1995 was 9.8°C. The coldest is January (-1.3°C), and the warmest is July (20.0°C). The average annual temperature in the centre of the capital is 10.2°C. Differences in air temperatures between the centre and peripheral urban areas indicate the presence of a heat "island" in the ground part of the atmosphere, which is a specific feature of the urban climate. This "heat island" has been particularly pronounced in the last three decades.

The average annual rainfall in the city and its adjacent territory is about 550-600 mm.

A curve of precipitation availability has been drawn for a 37-year period. According to the obtained results, the year 2023 is characterized by a precipitation availability of 45.9%, which defines it as a wet year. The annual precipitation amount is 638.3 mm, which is about 30 mm more than the previous year.

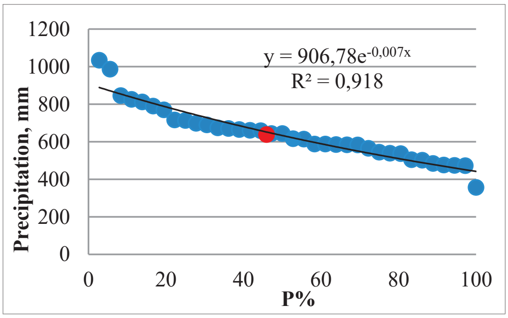

Figure 1. Curve of precipitation availability for the period 1987-2023

Brassicaceae crops typically prefer cooler temperatures for optimal growth. They thrive in temperatures ranging from around 15°C to 25°C during the growing season. Extreme heat can cause bolting (premature flowering), which negatively affects the quality of the crop. Temperatures exceeding 25-30°C can induce heat stress, negatively impacting cabbage head formation and yield (Červenski, J. et al., 2022). In terms of water requirement of the crops moderate amount of rainfall or irrigation is sufficient, but it's important to avoid both drought stress and waterlogging.

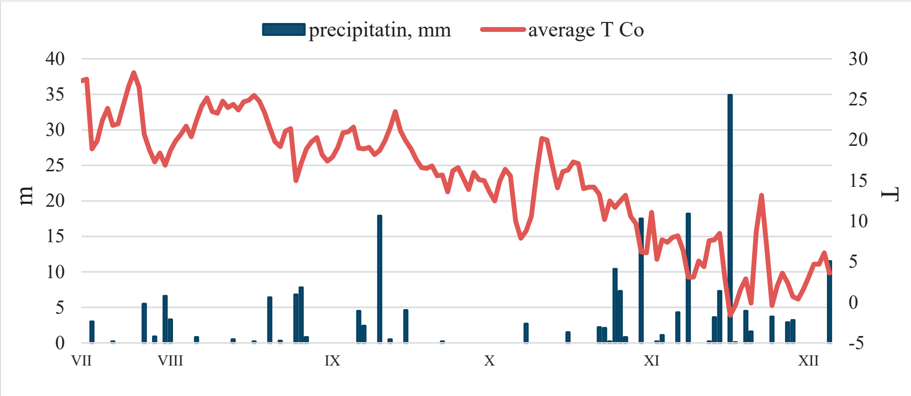

Figure 2. Precipitation and average daily temperatures during the growing season of cabbage and kohlrabi in 2023

There is an even distribution of precipitation during the growing season of both crops. However, the recorded precipitation until the beginning of November rarely exceeds 10 mm. At the beginning of the vegetable crop development period, the average daily temperatures reach 28.3°C, while at the end of the period before harvesting, negative values are recorded.

After the recorded temperature peak, a significant decrease in temperatures was noted during the second ten days of August. Towards the completion of head formation, the average daily temperatures fall within the optimal range for crop development.

Soil condition is the other important factor for successful cabbage and kohlrabi growth in urban condition.

Table 1. Agrochemical characteristics of the soil

Depth	Humus	pH	NH_4^+-N	NO_3^--N	P_2O_5	K_2O
cm	%	H2O	mg/kg	mg/kg	mg/100 g	mg/100 g
0-40	1.68	7.25	46.75	9.07	48.09	23.1

The NH_4 content of 67.75 mg/kg soil, as obtained from soil probe data, suggests a moderate level of ammonium in the soil. Ammonium is an essential source of nitrogen for plant growth, but excessive levels can potentially lead to nutrient imbalances or toxicity issues, particularly for sensitive plants. The NH_3 content of 9.07 mg/kg soil, as obtained from soil probe data, indicates the presence of ammonia in the soil. Ammonia can serve as a source of nitrogen for plant growth, although it's typically found in lower concentrations compared to other forms of nitrogen such as nitrate (NO_3^-) and ammonium (NH_4^+). Soil data obtained before vegetables planting, show a good degree of storage of K_2O, a very high content of available P_2O_5. The soil reaction is slightly alkaline. The soil is poorly humus with humus content for the arable soil layer.

This high content of NH_4 and P_2O_5 is probably due to unprepared storage of manure from keeping a few cows nearby. Thera is some evidence for spreading the manure in the area.

The samples of water used for irrigation on the experimental plots have been taken and analyzed. The boreholes are located near the experimental site, and the well-used to supply the drip irrigation system has a flow rate of 2 l/sec. The data from the water analyses conducted in an independent certified laboratory are presented in Table 2. The analysis conducted is fully compliant with

Regulation No. 18 of May 27, 2009, regarding the quality of water for irrigation of agricultural crops.

Table 2. Water quality analysis

Name of index	Unit	Results	MAC
Solids	mg/dm^3	216	-
Total hardness	$mg\ eqv/dm^3$	3.75	12
pH		7.18	6.5-9.5
Electro conductivity	μS/cm	311	2000
Voidability, permanganate	$mg\ O_2/dm^2$	0.82	5
Florida/F	mg/dm^3	0.25	1.5
Chlorides/Cl	mg/dm^3	10.59	250
NO2	mg/dm^3	<0.05	0.5
NO3	mg/dm^3	69.77	50
PO4	mg/dm^3	<0.1	0.5
SO4	mg/dm^3	50.75	250
HCO3	mg/dm^3	102	-
CO3	mg/dm^3	<5	-
NH4	mg/dm^3	<0.005	0.5
Ca	mg/dm^3	52.72	150
K	mg/dm^3	2.08	-
Mg	mg/dm^3	13.55	80
Na	mg/dm^3	11.3	200
Al	mg/dm^3	<0.005	0.2
As	mg/dm^3	<0.001	0.01
Cd	mg/dm^3	<0.001	0.005
Cr	mg/dm^3	<0.001	0.05
Cu	mg/dm^3	<0.001	2
Fe	mg/dm^3	0.077	0.2
Mn	mg/dm^3	<0.001	0.05
Ni	mg/dm^3	0.0031	0.2
Pb	mg/dm^3	<0.001	0.01
Zn	mg/dm^3	0.0112	5

The electrical conductivity of water for drinking and household needs should not exceed 2000 μS/cm, which is approximately equal to a total mineralization of 1000 mg/l. In the borehole used for drip irrigation, the conductivity is only 311 μS/cm, indicating a low content of soluble salts.
The water has a weakly alkaline pH. Elevated levels of nitrate forms are observed, which are 1.4 times higher than the norm. Nitrates in underground water mainly occur due to excessive use of fertilizers, animal waste, and the proximity of underground water sources to irrigation water.
High nitrate content in underground water can often be attributed to improper manure storage practices. When manure is not stored properly, such as in uncovered or inadequately sealed facilities, it can lead to the leaching of nitrates into the soil and eventually into groundwater. This is because manure contains nitrogen compounds that can break down into nitrate through microbial processes.

Excessive nitrates in the soil are most commonly found in agricultural areas. Boreholes constructed in shallow sandy soils are more vulnerable to nitrate contamination due to their increased mobility. Improper disposal of manure has led to groundwater pollution.
Cabbage and kohlrabi plants are relatively sensitive to water stress, particularly during the head formation stage. Severe water stress during this critical period can lead to reduced head size, uneven head formation, and decreased yield. However, moderate water stress during early growth stages may stimulate root development and enhance water use efficiency without significantly compromising yield.
The largest cabbage heads in terms of diameter were observed in the variants with 70% of the irrigation rate and in the variant with skip irrigation during the vegetative phase. The smallest diameter was reported for the variant with 130% of the irrigation rate, resembling the size of a rosette. This is likely due to restricts oxygen availability to plant roots and leads to root suffocation, nutrient leaching, and increased susceptibility to diseases.
Similar results were observed in the tuber of the kohlrabi, with approximately the same widths, lengths, and heights across variants. There is a directly proportional tendency of average weight increase relative to the irrigation rate for the variants at 40%, 70%, 100%, and 130%.
While deficit irrigation may reduce cabbage yield under severe water stress conditions, it can also improve yield quality in some cases. Moderate water stress may result in firmer heads with higher dry matter content and increased resistance to diseases such as tip burn.
Obtained yield of cabbage and kohlrabi is reported by variants and is equated in tons per hectare.
It is clear that skipping the irrigation during the vegetative phase does not lead to a significant decrease in yield, with only about a 10% reduction observed.
Reduced irrigation at 70% of the norm and canceling irrigation during the vegetative phase of cabbage development provide grounds for the successful implementation of deficit irrigation regimes in late-season cabbage production.

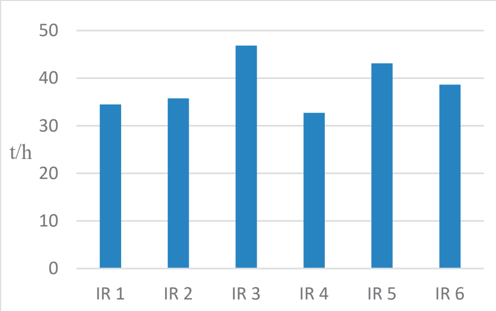

Figure 3. Cabbage yield 2023

From the data, we can observe some variation in kohlrabi yield among the different irrigation condition conditions. The average yield appears to be around 15.5 tons per hectare, with individual conditions ranging from approximately 14.7 to 16.6 tons per hectare.

In variants whit high irrigation rate 130% the lowest yield is observed, which defines kohlrabi as sensitive to waterlogging. Kadiri, L. et al. (2017) obtain a similar result according to authors Waterlogging can have significant effects on the yield of kohlrabi, as it can adversely affect plant growth, nutrient uptake, and overall productivity.

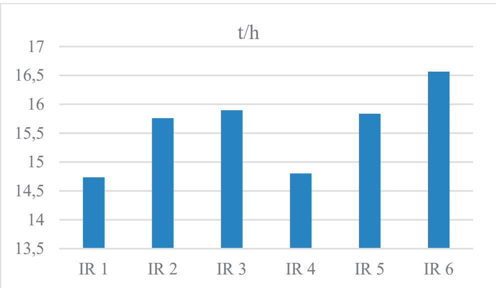

Figure 4. Kohlrabi yield 2023

The statistical processing of the data for the yield of the two crops with ANOVA. Shows statistically proven differences between irrigation variants with $P < 0.01$.

Total Available Water (TAW) refers to the amount of water present in the soil that is available for plant use.

The obtained data for kohlrabi yield, as well as the derived dependency, indicate that increasing the irrigation rate results in a decrease in kohlrabi yield. This is due to the low amount of air in the soil pores, resulting from their filling with water. The good results obtained with reduced irrigation rates show that kohlrabi is a crop that does not tolerate overwatering and can be successfully grown under conditions of disrupted irrigation regimes.

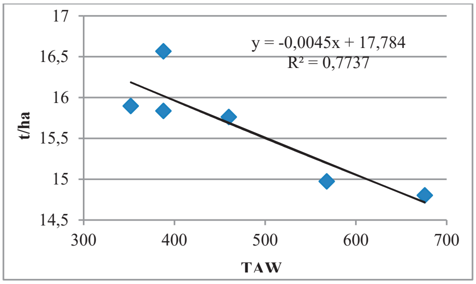

Figure 5. Dependency between yield and irrigation rate for kohlrabi 2023

Monitoring the quality of vegetables, particularly regarding nitrate content, is crucial for ensuring consumer safety and health. Vegetables such as cabbage and kohlrabi are known to have the propensity to accumulate nitrates, which can have implications for human health if consumed in excess.

Nitrate accumulation in vegetables can occur from various natural sources, as highlighted by Putnik-Delić, M. et al. (2023). These sources may include nitrogen-containing fertilizers, organic matter decomposition in soil, and environmental factors such as temperature and moisture levels. Understanding and managing these factors are essential for mitigating nitrate accumulation and ensuring the production of high-quality vegetables.

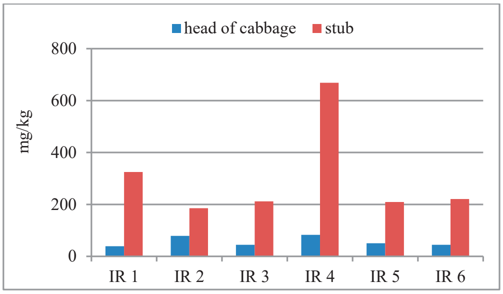

Figure 6. Nitrate content in heads and cobs of cabbage 2023

There is variation in nitrate content across different irrigation conditions for both the head and stub of cabbage. Variant IR 4 has significantly higher nitrate content compared to the other conditions in stub. Interestingly, there are differences in nitrate content between the head and stub of cabbage within each condition. This suggests that nitrate accumulation may vary between different parts of the cabbage plant. The data suggests that different irrigation regimes may influence nitrate accumulation in cabbage. For example, IR 4 shows notably high nitrate levels compared to other variants, indicating a potential correlation between irrigation practices and nitrate content.

The measured levels of nitrates in the edible part of the cabbage do not exceed the maximum permissible concentration of 500 mg/kg.

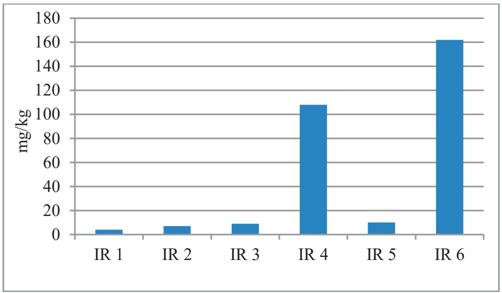

Figure 7. Nitrate content in the productive part of kohlrabi 2023

The data shows variations in nitrate content across different experimental conditions. Particularly, IR 4 and IR 6 exhibit significantly higher nitrate levels compared to other conditions.

Water stress conditions caused by over watering can alter plant metabolism, including the assimilation of nitrates. Under water stress, plants may prioritize the assimilation of nitrates over other metabolic processes, leading to increased nitrate accumulation in plant tissues.

Morard, P. et al. (2000) obtain similar result for tomato plants. The authors report that 12 hours after the onset of oxygen deprivation could be attributed to the reduction of nitrates by the root system of tomato plants. This reduction process might utilize oxygen, derived from the reaction, to facilitate essential processes like water and nitrate uptake, which are pivotal for plant nutrition. Consequently, it appears that under conditions of root asphyxia, the plant may acclimate by resorting to a metabolism akin to "nitrate respiration".

CONCLUSIONS

Based on the research findings, it can be concluded that the climatic conditions within the Sofia city region are conducive to the cultivation of cabbage and kohlrabi crops. However, the presence of nearby livestock may have adverse effects on soil and water quality, posing a risk of potential contamination. Nevertheless, current assessments indicate that the quality of both soil and water remains satisfactory and does not pose any immediate threat to human health.

The irregular distribution of rainfall throughout the growing season necessitates regular irrigation practices. Cabbage crops exhibit positive responses to controlled irrigation deficits, with the most promising outcomes observed when watering is withheld during the vegetative phase. However, it's crucial to note that excessive soil moisture resulting from over-irrigation can induce stress in plants, adversely affecting both yield and nutrient absorption. The data obtained regarding kohlrabi yield, along with the derived dependencies, suggest that an increase in irrigation rates correlates with a decrease in kohlrabi yield.

Under conditions of water stress, plants may prioritize the assimilation of nitrates over other metabolic processes, leading to heightened nitrate accumulation in plant tissues. However, it's worth noting that measured levels of nitrates in the edible portion of cabbage and kohlrabi do not surpass the maximum permissible concentration of 500 mg/kg, indicating compliance with safety standards.

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QUALITATIVE AND AGROPRODUCTIVE RESULTS REGARDING SUCCESSIVE CROP OF SWEET PEPPER IN PROTECTED SPACES

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Abstract

Sweet peppers are among the most appreciated vegetable species, renowned for their important nutrients and organoleptic properties. Although their cultivation is mainly carried out in the main crop, for the present study, the sweet peppers were established in a tunnel, in a successive system of culture, after the early cabbage. The aim of this study was to evaluate the influence of the fertilization regime and the cultivar on biochemical and agroproductive characteristics of three sweet pepper hybrids grown in tunnel in a successive system. The experimental protocol consisted in the organization of a bifactorial experience, placed in subdivided plots, with three repetitions: Factor A – Sweet pepper hybrid (a1 - Reno F1; a2 - Traian F1; a3 - Bihar F1) and Factor B – Fertilization regime (b1 - unfertilized; b2 - organic fertilization; b3 - chemical fertilization). Results showed significant influence of the fertilization regime on yields, the best quantitative values being obtained following chemical fertilization (1.52 kg/plant). Conversely, the highest vitamin C content (127.40 mg/100 g product) was observed with organic fertilization.

Key words: *sweet pepper, hybrid, fertilization, yield, protected spaces.*

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.), an annual herbaceous plant also known as pepper or capsicum, belongs to the *Solanaceae* family. It is part of the genus *Capsicum*, which encompasses approximately thirty species. Among these, it is one of the five domesticated species, alongside *C. chinense* Jacq., *C. baccatum* L., *C. frutescens* L., and *C. pubescens* (Akhtar et al., 2021). The most farmed spice in the world, *Capsicum annuum* L., is highly appreciated for its intense flavor, aroma, and vibrant color features. Vegetables serve as the primary dietary sources of bioactive compounds essential for human health and well-being (Florescu et al., 2022). Sweet peppers are utilized for various home dishes as fresh, preserved, and dried. However, the freshness of red peppers gradually diminishes during storage due to natural processes occurring under different conditions (Bita et al., 2009). Red fruits peppers are highly nutritious (Brezeanu et al., 2022), high in antioxidant-producing polyphenolic compounds, as well as a good source of vitamins A, C and minerals (Vlahova et al., 2011; Narayan et al., 2009; El-Ghorab et al., 2013; Kantar et al., 2016). However, several variables, including species, seasonality, environmental condition, and the plant's life cycle, affect the chemical profile of peppers (Antonio et al., 2018). High yields can be obtained through the judicious selection of hybrids, using an appropriate technology, with a particular focus on fertilization. Several factors also influence the vitamin C content in horticultural products, including cultivar, eco-pedoclimatic conditions, cultivation methods, harvest time and storage conditions. According to Szafirowska and Elkner (2008), pepper fruits grown in an organic system had increased levels of beta-carotene, vitamin C, and total flavonoids. Caruso et al. (2019) assessed the effects of conventional and organic farming, with microorganism-enriched fertilization, on two pepper cultivars 'Brillant' and 'Yolo Wonder'. Conventionally grown plants had higher assimilatory pigment levels, while microorganism-enriched fertilization increased yields. 'Yolo Wonder' red fruits had higher carotenoids and antioxidants, particularly under organic farming with microorganism-enriched fertilization. Barcanu et al. (2021) explored the impact of organic and chemical fertilization on 'Regal' and 'Cantemir' sweet peppers. Their findings revealed that the 'Cantemir' responds differently to fertilization compared to 'Regal' which remained unaffected. More recent, Stoleru et al. (2023) investigated the cultivation of two sweet pepper cultivars, 'Blancina' and 'Brillant', using different fertilization approaches: chemical, organic, and biological. Biological and organic fertilizations significantly improved most parameters analyzed, such as yield, acidity, and phytonutrient content. However, chemical treatment outperformed them only in mineral content.

The objective of this study was to assess the influence of fertilization regime and the hybrid on the biochemical and agroproductive characteristics of three sweet pepper hybrids grown in a wood tunnel using a successive crop system.

MATERIALS AND METHODS

The biological material and working methodology

The research was carried out in an experimental farm in the village of Corod, Galați County, in 2023 (Figure 1). The experimental protocol was organized as bifactorial in the form of subdivided plots with three repetitions. The two experimental factors studied were represented by Factor A - Sweet pepper hybrid (a1 - Reno F1; a2 - Traian F1; a3 - Bihar F1) and Factor B - Fertilization regime (b1 - unfertilized; b2 - organic fertilization; b3 - chemical fertilization).

The biological material used consisted of three sweet pepper hybrids suitable for cultivation both in the field and in protected spaces.

The Reno F1 cultivar is an early sweet pepper variety with good stress tolerance. Its excellent fruits guarantee a high production yield, featuring bright red color, thick flesh, and a weight ranging between 160-180 grams.

The Traian F1 cultivar is a semi-late sweet pepper hybrid with indeterminate growth and high production capacity. The fruits are large, intensely red, with a weight of 150-190 grams and a high dry matter content.

The Bihar F1 cultivar is characterized by indeterminate growth and good resistance to dropping. The fruits are large, dark red, with thick flesh and a fruit weight ranging between 150 and 220 grams.

Figure 1. Aspects from the experimental crop of sweet pepper

The experimental fertilization regime involved a control variant - unfertilized, and two differrent types of fertilizers - organic and chemical.

Organic fertilization was carried out with Orgevit, while chemical fertilization was done with Nutri Top NPK 5.10.15, applied once, at the preparation of the seedbed, before planting the seedlings.

Orgevit is a granular fertilizer with a 100% content of organic substances of natural origin (poultry), containing all the necessary micro and macroelements for plants (65% organic matter, 90% dry matter, 4% N, 3% P_2O_5, 2.5% K_2O, 1% MgO, 0.02% Fe, 0.01% Mn, 0.01% B, 0.01% Zn, 0.001% Cu, 0.001% Mo).

Nutri Top NPK 5.10.15 is a solid chemical fertilizer containing humic extracts, macro and microelements (5% N, 10% P_2O_5, 15% K_2O, 37% SO_3, 10% CaO, 1.3% MgO, 0.5% Fe, 0.01% Mn, 0.02% B, 0.01% Zn, 0.007% Cu), with a complex effect on plants and soil, maximizing nutrient availability and yields.

The crop was established by planting the seedlings on May 31, as a successive crop after early cabbage, in a tunnel covered with polyethylene film. Cultivation practices were carried out according to recommendations from specialized literature (Ciofu et al., 2003; Munteanu, 2003). Pepper fruits harvesting was done in four stages, starting with 801 BBCH (Meier, 2001), followed by productivity indicators and biochemical determinations.

Productivity indicators

The yield determinations aimed at analyzing the main productivity indicators such as: the number of fruits per plant, the total yield per plant, the average weight of a fruit, and the height and diameter of the fruits.

Color parameters

To determine color parameters, the MiniScan XE Plus device manufactured by HunterLab, Reston, VA, USA, was employed. The parameters examined included L, a, and b. L denotes brightness (ranging from 0 to 100), a indicates redness to greenness (0 to 100 = red and −80 to 0 = green), and b represents yellowness to blueness (0 to 70 = yellow; −100 to 0 = blue).

Biochemical analyses

Total Soluble Solids content (TSS) was evaluate using a Refractometer. The results were expressed in °Brix according to OECD standards, 2018 and Irimia, 2013.

Titratable acidity (TA) was determined by titrimetric method. Sweet pepper samples were homogenized with distilled water and titrated with NaOH until reaching of 8.1 pH. Results were expressed as % citric acid.

Ascorbic acid (Vitamin C) content was determined with a Reflectoquant, a dispositive that measures light reflected from the test strip. The determination range is between 25 and 450 mg/L ascorbic acid and the results are expressed in mg/100 g fresh product (Irimia, 2021).

Moisture (M) was carried out by oven drying method at 105 degrees for 4 hours and Total Dry Matter was calculated based on the formula, TDM % =100 - M%.

Ash (A). Sweet pepper samples were set on fire at 550 ± 20ºC in ash oven (AOAC, Method Number 930.05, http://www.eoma.aoac.org/methods/info.asp?ID=31326).

Biochemical parameters were determined in triplicate according to the standards, averages being statistically analyzed.

Statistical analyses

The experimental data obtained were analyzed using appropriate statistical methods recommended by the specialized literature (Jitareanu, 1999; Leonte and Simioniuc, 2018; Chiruță, 2019).

The results were reported as means ± standard error after data processing through ANOVA, and differences between variants were highlighted using the Tukey test, employing SPSS software v21.

RESULTS AND DISCUSSIONS

Sweet pepper productivity

The influence of the hybrid and fertilization regime on sweet pepper productivity indicators is presented in Table 1.

Table 1. Results regarding the influence of the hybrid and fertilization regime on the main productivity indicators

Experimental variant	No. of fruits/plant	Fruit weight (g)	Total yield (kg/plant)	Plant height (cm)	Fruit diameter (cm)
Hybrid					
Reno F1	9.08 ± 0.21 ns	140.56 ± 3.92 ns	1.28 ± 0.04 ns	5.02 ± 0.14 ns	8.50 ± 0.08 b
Traian F1	8.78 ± 0.39 ns	128.51 ± 0.36 ns	1.12 ± 0.05 ns	5.07 ± 0.15 ns	8.76 ± 0.03 a
Bihar F1	9.55 ± 0.55 ns	123.15 ± 5.76 ns	1.19 ± 0.10 ns	5.06 ± 0.04 ns	8.55 ± 0.02 ab
Significance	ns	ns	ns	ns	*
Fertilization regime					
Unfertilized	7.50 ± 0.39 b	126.30 ± 2.57 ns	0.94 ± 0.06 b	4.20 ± 0.12 b	7.87 ± 0.03 c
Organic	8.69 ± 0.10 b	130.09 ± 1.34 ns	1.13 ± 0.00 b	4.90 ± 0.25 b	8.60 ± 0.06 b
Chemical	11.22 ± 0.63 a	135.84 ± 2.48 ns	1.52 ± 0.08 a	6.03 ± 0.07 a	9.30 ± 0.00 a
Significance	*	ns	*	*	*

Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at p<0.05.

Regarding the hybrid factor, there were no significant differences observed in the total number of fruits per plant, total yield per plant, fruit weight, and plant height, between the Reno, Traian, and Bihar hybrids.
The significant differences between the hybrids were only noticed in fruit diameter, with the highest value belonging to the hybrid Traian. However, concerning the fertilization regime, significant variations were observed.
The variants to which chemical fertilization was applied recorded the highest values for all the indicators measured, with significant differences except for the average fruit weight, which showed statistically insignificant differences.
Conversely, plants in the unfertilized variants displayed the lowest values across these productivity indicators.
The interaction effect of the studied factors on sweet peppers productivity indicators is presented in Table 2. Chemical fertilization in different combinations with the hybrid factor resulted in the highest values for each of the productivity indicators considered. Thus, the chemically fertilized Bihar hybrid variant recorded the highest values for total fruit/plant and total yield/plant, with statistically significant differences being achieved. The Reno hybrid variant, which also received a chemical fertilization, registered the highest values for plant height and fruit diameter, with significant differences, but also for fruit weight, in which case the differences were insignificant.
These findings underscore the importance of considering both cultivar characteristics and fertilization methods to optimize the sweet pepper productivity in agricultural practices.

Table 2. Results on the influence of hybrid x fertilization regime interaction on the main productivity indicators

Experimental variant	No. of fruits/plant	Fruit weight (g)	Total yield (kg/plant)	Plant height (cm)	Fruit diameter (cm)
Reno F1 x Unfertilized	7.63 ± 0.93 b	134.26 ± 3.38 ns	1.01 ± 0.10 bc	3.82 ± 0.26 e	7.29 ± 0.08 d
Reno F1 x Organic fertilization	8.77 ± 0.54 ab	140.89 ± 10.19 ns	1.22 ± 0.02 abc	4.82 ± 0.39 cde	8.80 ± 0.20 abc
Reno F1 x Chemical fertilization	10.93 ± 0.70 ab	146.53 ± 7.93 ns	1.59 ± 0.01 ab	6.41 ± 0.36 a	9.40 ± 0.21 a
Traian F1 x Unfertilized	7.43 ± 0.64 b	125.20 ± 5.88 ns	0.92 ± 0.07 c	4.23 ± 0.03 de	8.32 ± 0.07 c
Traian F1 x Organic fertilization	8.77 ± 0.43 ab	127.34 ± 3.46 ns	1.11 ± 0.04 abc	4.75 ± 0.27 cde	8.58 ± 0.14 bc
Traian F1 x Chemical fertilization	10.20 ± 1.93 ab	133.00 ± 7.85 ns	1.34 ± 0.23 abc	6.23 ± 0.21 ab	9.37 ± 0.15 a
Bihar F1 x Unfertilized	7.53 ± 0.72 b	119.44 ± 8.66 ns	0.89 ± 0.10 c	4.57 ± 0.08 cde	8.07 ± 0.15 c
Bihar F1 x Organic fertilization	8.60 ± 0.49 ab	122.04 ± 7.11 ns	1.04 ± 0.01 abc	5.09 ± 0.21 bcd	8.48 ± 0.17 bc
Bihar F1 x Chemical fertilization	12.60 ± 1.32 a	127.98 ± 4.14 ns	1.62 ± 0.21 a	5.51 ± 0.24 abc	9.09 ± 0.13 ab
Significance	*	ns	*	*	*

Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at p<0.05.

Sweet pepper biochemical composition

The results regarding the influence of hybrid and fertilization regime on various chemical parameters of sweet peppers are summarized in Table 3.
Bihar hybrid recorded the highest TSS (8.38°Bx) and dry matter (10.32%) values, correlated with the lowest titratable acidity (0.19% citric acid), moisture (89.68%) and ash content (0.28%) values. The highest titratable acidity (0.21% citric acid) and moisture (90.55%) values were found in the Reno hybrid and the highest ash content in the Traian hybrid (0.55%). The differences between the experimental variants were significant in this case.
Regarding the influence of fertilization regime, the differences obtained between the different experimental variants were statistically confirmed. The chemically fertilized variant determined the highest values of total soluble

solids (8.08°Bx) and ash content (0.50%), with significant differences compared to the other two variants. The non-fertilized variant registered the highest values for titratable acidity (0.20% citric acid) and total dry matter (9.98%), while the organically fertilized variant had the highest moisture content (90.66%), the differences found were also significant.

Table 3. Results regarding the influence of hybrid and fertilization regime on biochemical parameters of sweet pepper

Experimental variant	TSS (°Bx)	TA (% citric acid)	Moisture %	TDM %	Ash %
Hybrid					
Reno F1	7.40 ± 0.03 b	0.21 ± 0.00 a	90.55 ± 0.10 a	9.45 ± 0.10 b	0.44 ± 0.01 b
Traian F1	7.52 ± 0.05 b	0.19 ± 0.00 b	90.52 ± 0.09 a	9.48 ± 0.09 b	0.55 ± 0.01 a
Bihar F1	8.38 ± 0.07 a	0.19 ± 0.00 b	89.68 ± 0.01 b	10.32 ± 0.01 a	0.28 ± 0.00 c
Significance	*	*	*	*	*
Fertilization regime					
Unfertilized	7.73 ± 0.02 b	0.20 ± 0.00 a	90.02 ± 0.02 b	9.98 ± 0.02 a	0.35 ± 0.00 c
Organic fertilization	7.49 ± 0.03 c	0.19 ± 0.00 b	90.66 ± 0.04 a	9.34 ± 0.04 b	0.42 ± 0.01 b
Chemical fertilization	8.08 ± 0.03 a	0.19 ± 0.00 ab	90.08 ± 0.19 b	9.92 ± 0.19 a	0.50 ± 0.01 a
Significance	*	*	*	*	*

Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. TSS - Total soluble solids; TA - Titratable acidity; TDM - Total dry matter.

Total Soluble Solids (TSS) is a quality index for vegetables and fruits. The Bihar hybrid has a high potential for accumulating soluble solids matter, with the unfertilized variant showing the highest value for this parameter (8.93 °Bx), followed by the chemically fertilized variant of the same hybrid (8.67 °Bx). In the case of each experimented cultivar, chemical fertilization positively influenced the accumulation of water-soluble biochemical compounds to a greater extent than organic fertilization (Table 4).

Table 4. Results regarding the influence of the hybrid x fertilization regime interaction on biochemical parameters of sweet pepper

Experimental variant	TSS (°Bx)	TA (% citric acid)	Moisture %	TDM %	Ash %
Reno F1 x Unfertilized	6.60 ± 0.12 d	0.24 ± 0.00 a	90.27 ± 0.03 bc	9.73 ± 0.03 bc	0.32 ± 0.00 d
Reno F1 x Organic fertilization	7.77 ± 0.03 b	0.20 ± 0.00 b	90.60 ± 0.05 b	9.40 ± 0.05 c	0.45 ± 0.01 c
Reno F1 x Chemical fertilization	7.83 ± 0.07 b	0.20 ± 0.00 b	90.78 ± 0.30 b	9.22 ± 0.30 c	0.54 ± 0.01 b
Traian F1 x Unfertilized	7.67 ± 0.09 b	0.20 ± 0.00 b	90.35 ± 0.04 bc	9.65 ± 0.04 bc	0.45 ± 0.02 c
Traian F1 x Organic fertilization	7.17 ± 0.03 c	0.18 ± 0.00 d	91.62 ± 0.02 a	8.38 ± 0.02 d	0.47 ± 0.01 c
Traian F1 x Chemical fertilization	7.73 ± 0.03 b	0.18 ± 0.00 d	89.59 ± 0.24 d	10.41 ± 0.24 a	0.72 ± 0.01 a
Bihar F1 x Unfertilized	8.93 ± 0.09 a	0.16 ± 0.00 e	89.43 ± 0.03 d	10.57 ± 0.03 a	0.28 ± 0.01 e
Bihar F1 x Organic fertilization	7.53 ± 0.03 b	0.19 ± 0.00 c	89.77 ± 0.05 cd	10.23 ± 0.05 ab	0.33 ± 0.00 d
Bihar F1 x Chemical fertilization	8.67 ± 0.09 a	0.20 ± 0.00 bc	89.86 ± 0.04 cd	10.14 ± 0.04 ab	0.22 ± 0.01 f
Significance	*	*	*	*	*

Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. TSS - Total soluble solids; TA - Titratable acidity; TDM - Total dry matter.

Every horticulture product's distinctive flavor must be correlated with the levels of organic acids (malate and citrate) and sugars (sucrose, glucose, galactose, and fructose).

Regarding the accumulation of organic acids (TA), the unfertilized Reno hybrid

demonstrated the highest potential, with a titratable acidity of 0.24% citric acid, the difference being significant compared to the rest of the variants.
Other researchers show higher values of acidity of peppers compared to those obtained in this study, the values registered by Pérez-Grajales et al. (2019) varying between 0.31 and 0.45% citric acid.
The highest moisture value of 91.60% was obtained by the Traian F1 organically fertilized, followed by Reno F1 chemically fertilized (90.78%), the differences being statistically ensured.
Total dry matter (TDM) registered the highest value at the unfertilized variant of Bihar F1 (10.57%), followed closely by the Traian F1 under chemical fertilization (10.41%), with differences ensured at a significant statistically level.
The significant influence of the tested hybrid can be noticed in the following two values of TDM content, which also belong to the Bihar hybrid, in organic (10.23%) and chemically (10.14%) fertilized variants.
Ash content serves as an index for the mineral content of food. This parameter was influenced by both factors, the hybrid selected and the fertilization regime applied. The highest value was determined for the chemically fertilized hybrid Traian (0.72%), followed by the chemically fertilized hybrid Reno (0.54%). The subsequent values belong to the organically fertilized variants of the same hybrids, Traian (0.47%) and Reno (0.45%), as well as the non-fertilized hybrid Traian (0.45%), with the differences found to be significant.
Bihar F1 exhibited the highest potential for ascorbic acid accumulation, with a value of 122.7 mg/100 g product, followed by Traian hybrid with a value of 110.8 mg/100 g product (Figure 2). Organic fertilization favored ascorbic acid accumulation (127.4 mg/100 g product) to a greater extent than Chemical (118.4 mg/100 g product) and Unfertilized one (78.7 mg/100 g product).

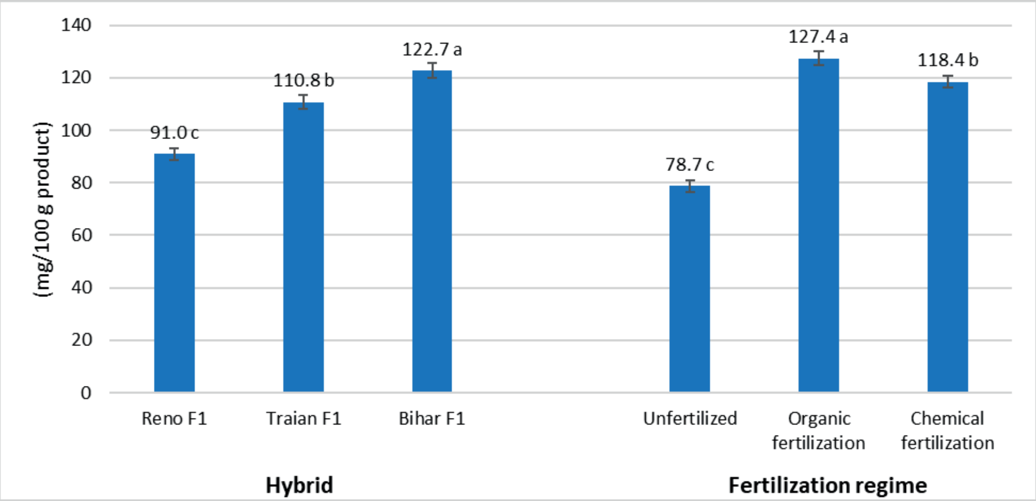

Figure 2. The influence of hybrid and fertilization regime on Vitamin C content of sweet pepper
(values associated to different letters are significantly different according to Tukey's test at $p<0.05$)

The influence of organic fertilization and the hybrid on the vitamin C content is shown through the interaction of these factors, represented in Figure 3. The mixture of the Bihar hybrid and organic fertilization resulted in the highest ascorbic acid content (148.6 mg/100 g product), followed by the Traian hybrid which also received organic fertilization (136.1 mg/100 g product). Similar results with high vitamin C content were obtained by other researches (Perez-Lopez, 2007; Brezeanu, 2022), the organic fertilization being in dependence with the cultivar, maturity, and fertilization regime. According to Hamed et al., 2019, ascorbic acid precursors like exposure to light and glucose level are responsible for the greater ascorbic acid levels in the developed stage.

The same two hybrids, Bihar and Traian, to which chemical fertilization was applied, produced the next highest values for ascorbic acid content (134.9 mg/100 g product; 120.6 mg/100 g product). In the opposite, the lowest vitamin C content was determined in the non-fertilized hybrid Traian (75.6 mg/100 g product).

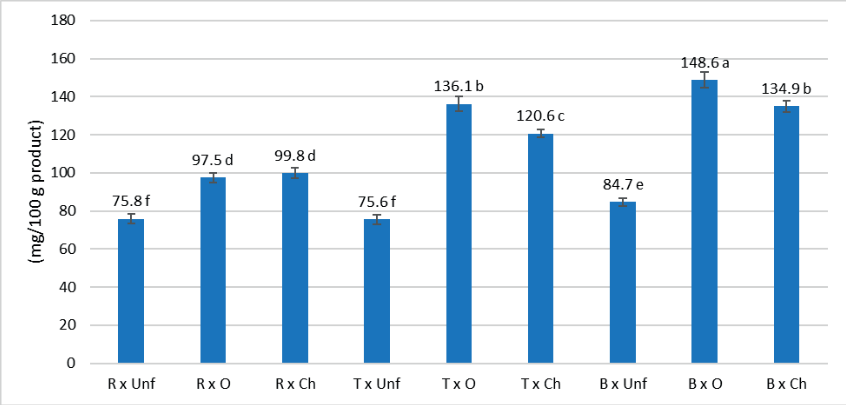

Figure 3. The influence of the interaction between hybrid and fertilization regime on Vitamin C content of sweet pepper (T-Traian F1, R-Reno F1, B-Bihar F1, Unf-Unfertilized, O-Organic fertilization, Ch-Chemical fertilization; values associated to different letters are significantly different according to Tukey's test at $p<0.05$)

CONCLUSIONS

Sweet pepper hybrids grown in tunnel and nutritional regimes had significant effects on yield and biochemical characteristics, the study revealing the importance of considering both hybrid selection and fertilization strategies to optimize this cultivation system.

The influence of the experimented hybrid determined values of the productivity indicators with insignificant differences between the experimental variants, except for fruit diameter.

Chemical fertilization had the strongest impact on productivity indicators, recording the highest values among these, with statistically significant differences, except for fruit weight which showed insignificant differences.

Chemical fertilization in various combinations with the hybrid factor resulted in the highest values for each of the productivity indicators considered. Thus, the chemically fertilized Bihar hybrid recorded the highest values for number of fruits per plant and sweet pepper yield per plant, while the Reno hybrid, also chemically fertilized, stood out for the other three productivity indicators (fruit weight, plant height and fruit diameter).

The fertilization application at Traian hybrid led to the highest values of moisture, dry matter and ash content, with statistically significant differences. The unfertilized variants of Bihar and Reno hybrids resulted in the highest values of total soluble solids and titratable acidity respectively with also significant differences.

The judicious choice of hybrid and fertilization regime significantly influences the content of vitamin C. The Bihar F1 hybrid stands out as having the highest potential for accumulating ascorbic acid, while the application of organic fertilization favors the synthesis of vitamin C to a greater extent compared to the other two fertilization regimes.

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ASSESSING THE ALLELOPATHIC POTENTIAL OF VARIOUS SPECIES FOR WEED CONTROL IN ORGANIC FARMING ON A CLIMBING BEAN CROP

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Abstract

The aim of the research was to assess the allelopathic potential of certain species for weed control in climbing bean crop. Field trials were carried out to examine the allelopathic impacts of the following allelopathic species: white clover (Trifolium repens), red clover (Trifolium pratense), sainfoin (Onobrychis viciifolia), oil radish (Raphanus sativus var. oleiformis), yellow mustard (Sinapis alba), oats (Avena sativa), barley (Hordeum vulgare), two-rowed barley (Hordeum distichon) and Japanese grass (Lolium perene, Festuca rubra and Poa pratensis). These species were sown with "Auria Bacăului" climbing bean (Phaseolus vulgaris) on intercropping system. It has been observed that main weed species identified in climbing bean crop were: red-root amaranth (Amaranthus retroflexus), cockspur (Echinochloa crus-galli), Canada thistle (Cirsium arvense), perennial sow thistle (Sonchus arvensis), pale knotweed (Persicaria lapathifolia), groundsel (Senecio vulgaris), bindweed (Convolvulus arvensis), guasca (Galinsoga parviflora), flower-of-an-hour (Hibiscus trionum) and petty spurge (Euphorbia peplus). In conclusion, intercropping beans with allelopathic species such as red clover, yellow mustard, and oil radish, along with red clover, oats, sainfoin, two-rowed barley and barley, resulted in a substantial diminishment in weed infestation.

***Key words**: ecological agriculture, biological phenomenon, weed infestation, intercropping system.*

INTRODUCTION

The impacts of regional and global climate shifts are becoming evident across different aspects of life on our planet. However, the repercussions on agriculture and food supply stand out as potentially among the most significant threats to sustaining life (Malhi et al., 2021). Assessment of the effects of global climate change factors on agriculture and farming practices is important to anticipate and adapt practices that maximize agricultural production in future climate scenarios. To attain sustainable crop production in unpredictable environments, a comprehensive strategy is essential. This strategy should not only aim to boost crop productivity but also to prioritize the effective management of agricultural pests like weeds (Varanasi et al., 2016). Weeds, given their intricate nature, exert significant adverse effects on agriculture, forestry, rangelands, public health, and various human activities. In contrast to sporadic and irregular outbreaks of pests and diseases, weeds present a constant challenge, causing severe issues in crop production (Kostov and Pacanoski, 2007). Despite widespread herbicide usage, crop production continues to suffer losses due to weed-related issues.

Abiotic factors such as atmospheric CO_2 levels, temperature variations and the availability of water or nutrients play a crucial role in influencing the physiology and growth of weeds. Weeds exhibit rapid responses to changes in resources and possess a higher capacity for adaptation and proliferation in diverse habitats, primarily due to their extensive genetic diversity and physiological adaptability compared to crops. As climate change introduces new constraints on resources vital for plant growth, the dynamics of interactions between crops and weeds, as well as crop losses attributed to weeds, are likely to be influenced accordingly (Valerio et al., 2013).

Effectively tackling both cost and ecological considerations involves implementing diverse weed management strategies. One notable innovative approach to weed control involves utilizing the allelopathic phenomenon, as highlighted by studies such as Jabran and Farooq (2012) and Zeng (2014). In specific cropping systems, such as organic farming, allelopathic weed control may serve as a standalone strategy. Additionally, it can be integrated with other methods to establish a comprehensive approach known as integrated weed management, as discussed by Jabran et al. (2015).

Therefore, the combination of allelopathy and competition emerges as a promising environmentally friendly tool, particularly for weed management. However, a comprehensive understanding of this phenomenon is essential for its successful application, given the currently limited available knowledge. The judicious utilization of allelopathic crops in agriculture holds the potential to diminish pesticide usage, consequently reducing environmental and food pollution. This approach also has the potential to lower costs in agriculture, enhance food security in impoverished regions, improve soil productivity and contribute to increased biodiversity and sustainability within the agro-ecosystem (Farooq et al., 2020).

Controlling weeds in organic agriculture stands out as one of the most challenging aspects of this farming approach (Archer et al., 2007; Cavigelli et al., 2008; Munteanu and Stoleru, 2012). It primarily relies on preventive methods, encompassing practices such as utilizing cover crops, employing mulches, incorporating green manure, and implementing intercropping, with allelopathy potentially playing a significant role in these strategies (Kalinova, 2010; Tesio and Ferrero, 2010; Liebman and Davis, 2015).

The objective of this study was to assess the impact of some allelopathic species on weed control, including white clover (*Trifolium repens*), red clover (*Trifolium pratense*), oats (*Avena sativa*), sainfoin (*Onobrychis viciifolia*), oil radish (*Raphanus sativus* var. *oleiformis)*, yellow mustard (*Sinapis alba*), barley (*Hordeum vulgare*), two-rowed barley (*Hordeum distichon*) and Japanese grass (42% *Festuca rubra* 'Trac Maxima 1', 10% *Festuca rubra* 'Gz Greenmile', 20% *Lolium perene* 'Fabian', 25% *Lolium perene* 'Greenway', 3% *Poa pratensis)*.

MATERIALS AND METHODS

Site description

The research was carried out at the Vegetable Research and Development Station, located in the North-Eastern region of Bacau, during 2023 vegetation season, as part of a series of experiences on climbing bean crop, under ecological farming conditions.

Plant material

The biological material utilized in the study consisted of the "Auria Bacăului" climbing bean cultivar, a variety patented by the Vegetable Research and Development Station Bacau. The bean seeds were sown at a spacing of 40 cm. The species with allelopathic potential utilized in the experiment were: white clover (12 kg/ha), red clover (20 kg/ha), yellow mustard (25 kg/ha), sainfoin (90 kg/ha), oil radish (30 kg/ha), two-rowed barley (200 kg/ha), barley (180 kg/ha), oats (120 kg/ha) and Japanese grass (200 kg/ha).

Experimental design

The study was conducted in randomized block design, with three replicates. The experimental factor was represented by climbing bean in an intercropping system with allelopathic species. A number of six experimental variants were investigated (including Control) as is shown in Table 1.

Table 1. Variants used in the study

Variants	Species with allelopathic properties
V1	- red clover;
V2	- white clover, red clover, sainfoin;
V3	- red clover, Japanese grass;
V4	- red clover, oil radish, yellow mustard;
V5	- red clover, oats, sainfoin, two-rowed barley, barley;
VM	- soil tillage (the control).

The allelopathic species were sown on 20th March, while the climbing bean on 3rd of May. Mowing was performed twice, with a garden strimmer. The control was tillaged two times manually and once mechanically.

The research methods used

Weed presence was evaluated utilizing a metric frame. The fresh matter and number of weeds per m^2 were assessed. The identification of weed species was conducted using a weed descriptor (Gurău, 2007). The assessment of weed infestation occurred at two intervals: A- before the first tillage/mowing, B- before the second tillage/mowing. The calculations for the proportions of competitive and allelopathic effects in the experiments were determined using the formula established by Sturm et al. (2018):

Overall weed biomass reduction (%) = 1 - (Weed biomass with allelophatic species)/(Weed biomass without allelopathic species)*100.

The level of chlorophyll and anthocyanins was measured using OPTI-SCIENCES Chlorophyll Content Meter and OPTI-SCIENCES Anthocyanin Content Meter. Leaves for all measurements were randomly sampled from three plants in each replicate. The concentration of total soluble solids was measured using a highly precise handheld portable refractometer. The analysis utilized homogenized juice obtained by pressing fresh pods, and the findings are reported in °Brix, following the 932.12 methodology (AOAC, 2005; Brezeanu et al., 2022). Fresh pods were randomly sampled from three plants for each replicate.

During harvest, the total seed yield (kg/ha) and seed yield per plant (g) were recorded. Towards the end of the harvest, stem diameter (mm), root length (mm) and root weight (g) were analyzed.

Statistical analysis

The statistical ANOVA was used to process the experimental data. In IMB SPSS Statistics 20, means were separated using a Tukey's HSD test at $P < 0.05$.

RESULTS AND DISCUSSIONS

A number of 10 weed species were identified in the climbing bean crop, belonging to both dicotyledonous and monocotyledonous classes (Table 1). The weeds observed in the experiment belong to the following botanical families: *Asteraceae*, *Amaranthaceae*, *Convolvulaceae*, *Poaceae*, *Malvaceae*, *Euphorbiaceae* and *Polygonaceae*.

Table 2. The weed species identified within the bean crop

Classification	Species
Annual dicotyledons	red-root amaranth (*Amaranthus retroflexus*)
	petty spurge (*Euphorbia peplus*)
	gallant soldier (*Galinsoga parviflora*)
	lower-of-an-hour (*Hibiscus trionum*)
	curlytop knotweed (*Persicaria lapatifolia*)
	groundsel (*Senecio vulgaris*)
Perennial dicotyledons	creeping thistle (*Cirsium arvense*)
	field bindweed (*Convolvulus arvensis*)
	perennial sow thistle (*Sonchus arvensis*)
Annual monocotyledons	cockspur (*Echinochloa crus-galli*)

The results of the ANOVA highlighted the existence of a significant difference between the variants ($p < 0.001$) regarding the number of weeds/m^2 in both observations (Figure 1). Tukey's post-hoc analysis revealed that the control variant exhibited a significantly higher number of weeds/m^2 compared to the other variants, prior to the first mowing/tillage. The control variant exhibited the highest number of weeds/m^2, whereas variants V2, V3, V4 and V5 were positioned at the opposite end, displaying the lowest level of weed infestation, in the observation conducted before the second mowing/tillage.

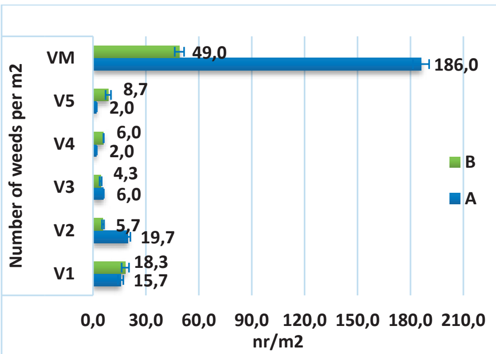

Figure 1. Number of weeds per m^2: A - before the first tillage/mowing; B - before the second tillage/mowing

The ANOVA results highlighted a significant difference between the variants ($p < 0.001$) in terms of weed biomass g/m^2 in both observations (Figure 2). According to Tukey's post-hoc analysis, the weed biomass g/m^2, in the control variant and V1 significantly differed from all other variants before the first mowing/tillage. Conversely, variants V3, V4 and V5 exhibited reduced weed biomass. The control variant showed the highest weed infestation level, while variants V2, V3, V4 and V5 displayed the lowest levels of weed infestation in the observation conducted before the second mowing/tillage.

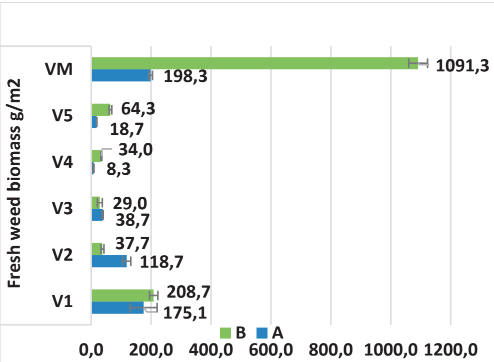

Figure 2. Fresh weed biomass g/m^2: A - before the first tillage/mowing; B - before the second tillage/mowing

It was observed that the weed biomass was reduced for all variants. The average value for the percentage reduction of weed biomass ranged from 11.20% to 97.34%, with the highest value recorded for variant V3, before the second mowing/tillage (Figure 3).

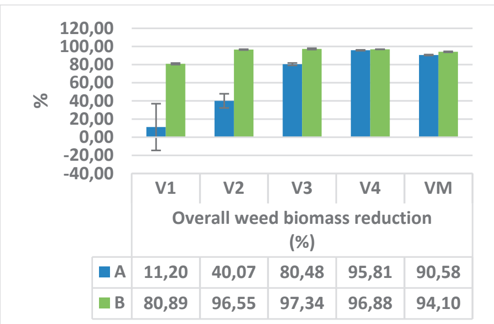

Figure 3. Overall weed biomass reduction (%): A - before the first tillage/mowing; B - before the second tillage/mowing

The results of the ANOVA indicated a significant difference between the variants ($p < 0.003$) regarding the total seed production. According to Tukey's post-hoc analysis, variant V5 exhibited a significant higher total seed production, compared to the control variant and V3 (Figure 4).

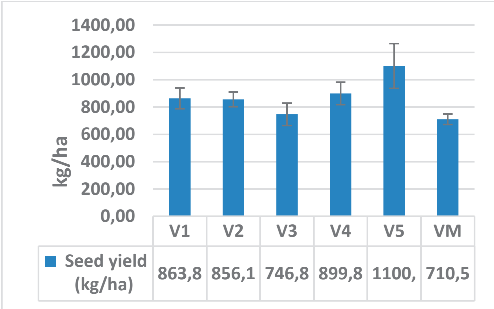

Figure 4. Seed yield (kg/ha) at climbing bean "Auria Bacăului" cultivar

The ANOVA results highlighted a significant difference between the variants ($p < 0.046$), in terms of seed yield per plant. Variant V5 exhibited a significant higher total seed production per plant, compared to the control variant (Figure 5).

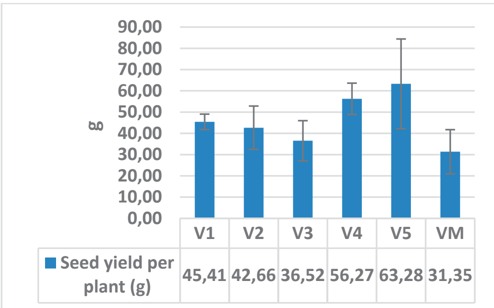

Figure 5. Seed yield per plant (g) at climbing bean "Auria Bacăului" cultivar

It was observed that there were no statistical significant differences between the variants, in terms of leaf chlorophyll content ($p < 0.068$) and soluble solid content in green pods ($p < 0.463$), according to the ANOVA test. However, a significant difference was noted in the anthocyanin content ($p < 0.346$) of the leaves (Figure 6). According to Tukey's post-hoc analysis, variant V1 had a significant lower anthocyanin content, compared to the control.

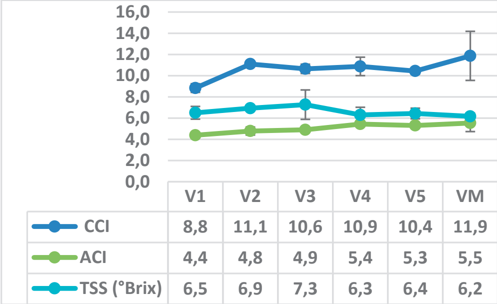

Figure 6. Chlorophyll content index (CCI), Anthocyanin content index (ACI) and Total soluble solids (TSS) at climbing bean "Auria Bacăului" cultivar

Roots play a crucial role in plants' absorption of water and nutrients (Polania *et al.*, 2017). Phenotypic assessments of root traits in common beans under drought stress have highlighted the significance of various rooting patterns, such as deep rooting, enabling access to water from deeper soil layers (Burridge et al., 2016). Fine roots and root hairs possess the capacity to traverse a substantial soil volume, requiring minimal carbon and energy for their functioning (Rao et al., 2016). It was observed that there were no statistically significant differences between the variants in terms of stem diameter (mm) and root weight (g). Concerning root length, subtle differences were observed among the variants (Table 3).

Table 3. Results of the biometric measurements within the bean crop

Variant	Stem diameter (mm)	Root length (mm)	Root weight (g)
V1	9.08±0.4	86.77±1.5bc	3.53±0.5
V2	9.44±0.6	80.99±6.9c	4.6±0.6
V3	9.28±0.3	89.71±4.6ab	4.15±0.1
V4	8.84±1.0	98.22±1.5a	3.50±0.8
V5	9.4±0.6	94.86±1.3ab	4.75±0.7
VM	9.3±0.3	83.52±5.0bc	3.63±0.4
	ns	*	ns

The results are presented as means ± SD. Distinct letters indicate significant differences between the groups, as determined by the Tukey post-hoc test ($P < 0.05$): a–the highest value for the test performed, *–significant differences; ns- non-significant.

The observed differences in weed biomass, seed production, and anthocyanin content underscore the potential of integrating allelopathic species into organic cultivation strategies. It is important to note that allelopathic species did not have a negative impact on the bean crop. However, long-term studies are necessary to fully understand their effects on production, soil, and weed species. From observations in the previous years, it has been noted that the timing of sowing allelopathic species plays a crucial role. Optimal results are achieved when these species are sown simultaneously with the bean seeds.

CONCLUSIONS

The degree of weed infestation was significantly reduced by the intercropping of bean, red clover, oil radish, yellow mustard variant and bean, red clover, barley, two-rowed barley, oats and sainfoin.

This research additionally revealed that plant species possessing allelopathic properties did not adversely affect the seed yield of the climbing bean crop.

It was found that there was no significant difference in the level of chlorophyll content, soluble solid content, stem diameter and root weight for the bean crop.

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THE INFLUENCE OF LIGHT INTENSITY ON YIELD AND MINERAL CONTENTS OF FOUR LETTUCE SPECIES CULTIVATED IN NUTRIENT FILM TECHNIQUE (NFT)

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Abstract

Lettuce cultivation in hydroponic systems using the Nutrient Film Technique (NFT) is widely practiced globally, and it can be done under either natural light or LED light conditions. LED lights are commonly employed in plant factories or as supplemental lighting during periods of insufficient natural light, such as the winter season. To achieve optimal growth and quality of vegetables grown in NFT system, various factors need to be considered, including temperature, light intensity, humidity, and others. Our study aimed to investigate the yield and mineral content of lettuce cultivars grown under natural light and LED light conditions. The experiment was conducted at a greenhouse in USAMV. We observed that lettuce cultivated under natural light conditions exhibited higher fresh weight, dry matter, phosphorus, iron, and copper content. Conversely, lettuce grown under LED light conditions showed higher nitrate, potassium, calcium, magnesium, and zinc levels.

Key words: *Lettuce, natural light, LED light, macro and micronutrients.*

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is indeed one of the most extensively studied vegetables in terms of cultivation in either open field or hydroponic system (Chan et al., 2022). It is a leafy green vegetable known for its nutritional value, providing essential vitamins, minerals, and protein, Lettuce has been reported to offer various health benefits, including reducing the risk of heart disease and cancer, as well as other health-related functions (Nicolle et al., 2004; Gunes & Dogu-Baykut, 2018).

Most crops grown in modern structures with hydroponic systems are indeed influenced by a range of environmental factors. These factors include light, temperature, CO_2 concentration, humidity, and others. Each of these environmental variables plays a critical role in shaping the growth and development of crops in hydroponic systems. Among these factors, light intensity and temperature play crucial roles in regulating the growth and development of lettuce. Their interaction is known to co-regulate various physiological processes in plants. When light intensity and temperature increase simultaneously, it often leads to enhanced growth and improved nutritional values of lettuce (Fu et al., 2012; Chen et al., 2021). In regions where sunlight is limited, especially during the winter months, supplemental lighting provided by LEDs can compensate for the lack of natural sunlight. By delivering the appropriate light spectrum and intensity, LEDs ensure that plants receive the necessary light for photosynthesis, growth, and development throughout the year (Li et al., 2016). Some research suggests that red and blue light spectra can effectively promote the accumulation of biochemical compounds and yield in lettuce and other vegetable species (Mohamed et al., 2021; Rahman et al., 2021; Lin et al., 2018). Furthermore, replacing a portion of red light, blue light, or both with green light has been found to be more effective in promoting both plant growth and quality. While low light intensities have been observed to affect plant growth in several ways. For instance, light intensity led to increased plant height and specific leaf area. However, low light intensities may also result in reductions in leaf number, leaf thickness, and ultimately yield. These observations are supported by studies conducted by Dong et al. (2014), Hou et al. (2010), and Steinger et al. (2003). Moreover, harvesting at low light intensity could enhance the Ca, K, Mg (Colonna et al., 2016). All of these research studies highlight the significant impact of light intensity on plant growth parameters and mineral content, which are useful for enhancing both the quantity and quality of produce for vegetable growers.

Our study aimed to evaluate how natural light and LED red light influence the yield and mineral contents of lettuce species. By comparing the effects of these two lighting conditions on lettuce growth and nutrient composition, we sought to better understand how different light sources influence the productivity and nutritional quality of lettuce crops.

MATERIALS AND METHODS

The The research was conducted in January 2023 in the greenhouse of the USAMV of Bucharest, Faculty of Horticulture, Research Center for Quality Control of Horticultural Produce.

The lettuce varieties used in the experiment included Lollo Bionda and Lollo Rossa from Amia Seeds Company, as well as Lugano and Carmesi from Rijk Zwaan Seed Company.

At the beginning of the experiment, the seeds were sown in a plastic tray filled with a mixture of peat and perlite (75% and 25%, respectively). After approximately one week, when the seedlings had emerged and developed cotyledon leaves, they were then transferred into jiffy pots for further growth. Once the seedlings reached the age of 22-25 days and had developed four true leaves, they were transplanted into the Nutrient Film Technique (NFT) system for the duration of the experiment.

The experiment followed a Randomized Complete Block Design (RCBD) with 3 replications. Each lettuce variety had a total of 15 plants, with 5 plants per replication. EC was monitored daily and maintained at 1.2-1.4 mS/cm for the first week after seedling transfer. It was then increased to 1.8-2.1 mS/cm until harvest. pH levels were kept within the range of 5.8 to 6.2. The average temperature in natural light was 16°C, humidity, and CO_2 levels were automatically controlled at the same level for both conditions throughout the experiment. In the Nutrient Film Technique (NFT) system under natural light, light intensity varied depending on weather conditions, with a range of 300-500 µmol $m^{-2}s^{-1}$. In the vertical farm, LED red lights were installed in a closed chamber. The temperature was maintained at a constant 20°C, and the lighting schedule was set to 16 hours of light and 8 hours of darkness. The average light intensity was maintained at 220 µmol $m^{-2}s^{-1}$.

At the harvesting stage (30 days in NFT), data were recorded for five individual plants for each lettuce variety. Parameters recorded included fresh weight and dry mass. Dry mass was determined by cutting leaves into fine pieces and drying at a constant temperature of 105°C for 24 hours. For mineral determination, samples weighing 0.250 g were weighed using an analytical balance and placed in Teflon recipients. Then, 8 ml of concentrated ultrapure HNO_3 (65%) and 2 ml of H_2O_2 (30%) were added to the samples for mineralization. Mineralization was carried out using microwave digestion for 30 minutes with the ETHOS UP microwave digestion system. After mineralization, clear solutions were transferred quantitatively into volumetric flasks (50 mL) and made up with Milli-Q ultrapure water. Analysis of mineral content was conducted using the Agilent Series ICP-MS spectrometer with quadrupole analyzer 7700x and MassHunter Workstation software (Agilent Technologies). Calibration curves were performed using the ICP-MS multi-element calibration standard containing various elements in specified concentrations dissolved in 5% HNO_3 (Dobrin et al., 2018). Statistical analysis was used STATISTICA, StatSoft software (version 10) to perform the analysis.

Figure 1. Lettuces cultivated in NFT under natural light and LED red light

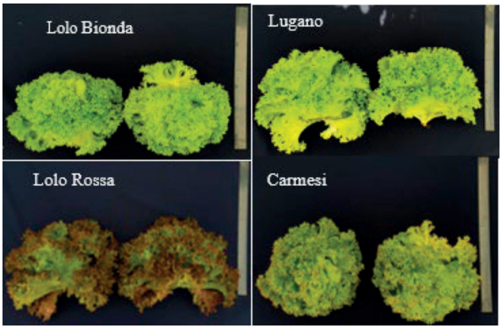

Figure 2. Lettuce varieties used in the experiment

RESULTS AND DISCUSSIONS

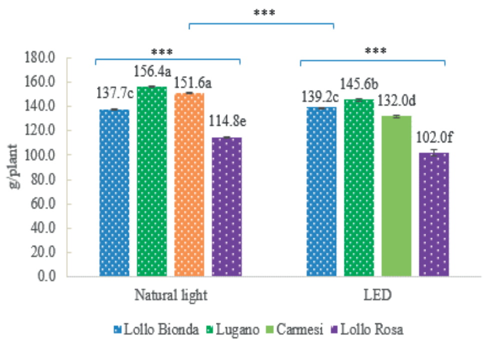

Figure 3. Fresh mass of lettuce cultivated under both light conditions. The value represents the means, standard error and significant level. The letter indicates the significant at p<0.05

Fresh mass. A strong interaction was found between lighting conditions and variety regarding fresh mass at $p \leq 0.001$ (Figure 3). Both natural light condition and LED light condition were significant at $p < 0.001$. Under natural light conditions, Lugano and Carmesi achieved the highest yields (156.4 and 151.6 g/plant, respectively), followed by Lollo Bionda and Lollo Rosa (137.7 and 114.8 g/plant, respectively). Under LED light, Lugano had the highest fresh mass (145.6 g/plant), followed by Lollo Bionda, Carmesi, and Lollo Rosa (139.2, 132.0, and 102.0 g/plant, respectively). Overall, the average fresh mass under natural light was greater than under LED light (140.1 and 129.7 g/plant, respectively). Our results are consistent with Fu et al. (2012), and Zhou et al. (2019) who reported that lettuce mass reached its highest yield when exposed to light intensity between 350-600 µmol $m^{-2}s^{-1}$. In our experiment, the light intensity under natural conditions (300-500 µmol $m^{-2}s^{-1}$) was higher than under LED conditions (220 µmol $m^{-2}s^{-1}$).

Dry matter: There was an interaction between lighting conditions and varieties regarding dry matter at $p \leq 0.05$ (Figure 4). Under natural light, the dry matter of the varieties was highly significant at $p \leq 0.001$, while under LED it was significant at $p \leq 0.01$. Lollo Rosa and Lollo Bionda exhibited the highest dry matter values under natural light (7.0% and 6.9%, respectively), followed by Carmesi and Lugano (6.6% and 5.6%, respectively). Under LED light, Lollo Rosa had the highest dry matter content (5.8%), followed by Lollo Bionda, Carmesi, and Lugano (5.7%, 5.7%, and 5.5%, respectively). On average, the dry matter content under natural light was higher than under LED light (6.5% and 5.7%, respectively). Our results are consistent with Jin et al. (2023), who conducted an experiment on gradually increasing light intensity up to 300 µmol $m^{-2}s^{-1}$ and found that dry matter increased with increasing light intensity compared to constant light intensity (220 µmol $m^{-2}s^{-1}$).

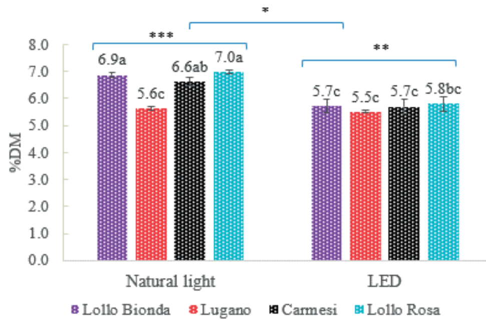

Figure 4. Dry matter of lettuce cultivated under both light conditions. The value represents the means, standard error and significant level. The letter indicates the significant at p<0.05

Nitrate. A strong interaction was found between lighting conditions and variety over nitrate content in fresh lettuce leaves at $p \leq 0.001$ (Figure 5). Both natural light condition and LED light condition were significant at $p \leq 0.001$. Under natural light, Lugano had the highest nitrate content (1567 mg/kg), followed by Lollo Rosa, Lollo Bionda, and Carmesi (1500, 1489, and 1222 mg/kg, respectively). Under LED red light, Lollo Rosa had the highest nitrate content (2078 mg/kg), followed by Carmesi, Lollo Bionda, and Lugano (1600, 1589, and 1519 mg/kg, respectively). However, this content is below the maximum level of nitrate content set by the European Union. The average nitrate content under LED light was higher than under natural light conditions (1697 and 1444 mg/kg, respectively). Lettuce grown under low light intensity usually uptakes nitrate beyond the reduction rate, leading to nitrate accumulation in the leaves, as evidenced by our experiment.

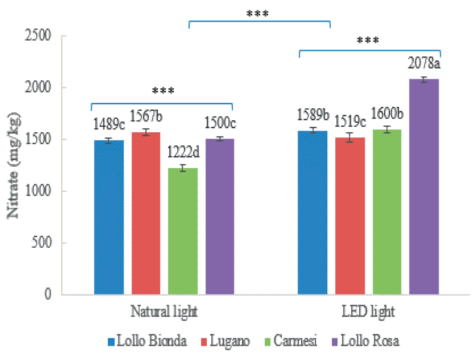

Figure 5. Nitrate of lettuce cultivated under both light conditions. The value represents the means, standard error and significant level. The letter indicates the significant at p<0.05

Potassium (K). Based on our results, under natural light conditions, Lollo Rosa had the highest potassium (K) content (3479 mg/kg), followed by Lollo Bionda, Carmesi, and Lugano (3346, 3255, and 3133 mg/kg, respectively) (Figure 6).

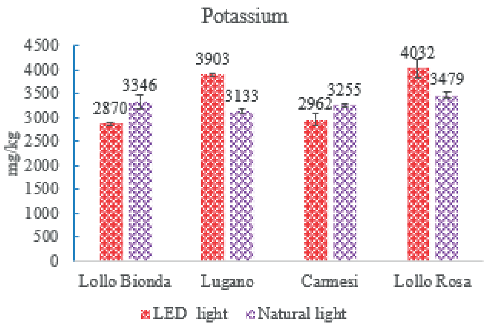

Figure 6. Potassium in lettuce's leaves cultivated under both light conditions. The value represents the means, standard deviation

Lollo Rosa maintained a high K content under LED light as well (4032 mg/kg), followed by Lugano, Carmesi, and Lollo Bionda (3903, 2962, and 2870 mg/kg). The average K content under LED light was higher than under natural conditions (3441.8 and 3303.1 mg/kg, respectively). This result consistent with Colonna et al. (2016) found that harvested at low light intensity enhanced K.

Calcium (Ca). Under natural light conditions, Lugano exhibited the highest calcium (Ca) content (1136 mg/kg), followed by Lollo Bionda, Lollo Rosa, and Carmesi (819, 756, and 566 mg/kg, respectively) (Figure 7). Under LED light, Lugano retained the highest Ca content (1474 mg/kg), followed by Lollo Rosa, Carmesi, and Lollo Bionda (1006, 824, and 739 mg/kg, respectively). The average Ca under LED light was greater than natural light condition (1010.7 and 819.1 mg/kg). Our results contrast with those of Sango (2016), who found that calcium levels in the outer leaves of headed lettuce increased with higher light intensity, ranging from 150 to 300 µmol $m^{-2}s^{-1}$.

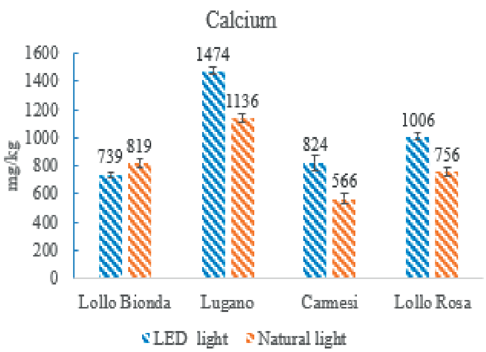

Figure 7. Calcium in lettuce's leaves cultivated under both light conditions. The value represents the means, standard deviation

Phosphorus (P). Regarding lettuce cultivated under natural light conditions, Lollo Bionda showed the highest phosphorus (P) content (469 mg/kg), followed by Carmesi, Lollo Rosa, and Lugano (422, 409, and 363 mg/kg, respectively) (Figure 8). Under LED light, Lollo Bionda maintained a high P content (430 mg/kg), followed by Lollo Rosa, Lugano, and Carmesi (374, 324, and 248 mg/kg). A significant disparity in P content between the two conditions was observed in Carmesi. Overall, all the varieties under natural light conditions demonstrated better P content compared to LED light, with average values of

415.7 and 343.8 mg/kg. Our result was similar to Chen et al. (2014), who found that phosphorus content in the shoot of white lupin plants was higher under high light intensity when the nutrient solution provided to the plants was sufficient.

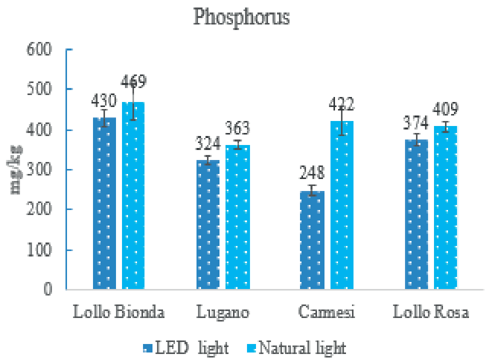

Figure 8. Phosphorus in lettuce's leaves cultivated under both light conditions. The value represents the means, standard deviation

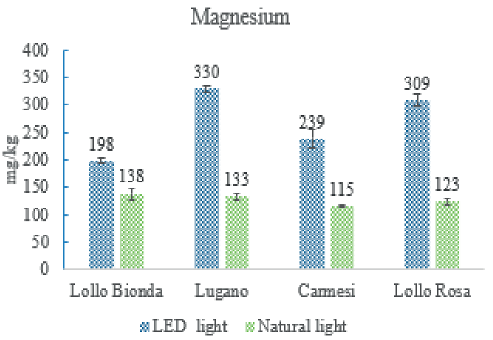

Figure 9. Magnesium in lettuce's leaves cultivated under both light conditions. The value represents the means, standard deviation

Magnesium (Mg). Under natural light conditions, Lollo Bionda exhibited a higher content of Mg at 138 mg/kg, followed by Lugano, Lollo Rosa, and Carmesi (133, 123, and 115 mg/kg) (Figure 9). However, under LED light, Lugano had the highest Mg content at 330 mg/kg, followed by Lollo Rosa, Carmesi, and Lollo Bionda (309, 239, and 198 mg/kg). The Mg content under LED light was 1.4 to 2.5 times greater than under natural light, with averages of 269 and 127.4 mg/kg. Our results are similar to those of Colonna et al. (2016), who found that magnesium levels increased under low light intensity.

Zinc (Zn). According to our results, Lollo Bionda under natural light conditions had the highest value of Zn at 5.2 mg/kg, followed by Carmesi, Lugano, and Lollo Rosa (3.9, 3.6, and 3.5 mg/kg) (Figure 10). Furthermore, Lollo Bionda maintained its lead in Zn content under LED light conditions (8.1 mg/kg), followed by Lugano, Lollo Rosa, and Carmesi (7.0, 4.3, and 2.4 mg/kg). All varieties under LED light demonstrated higher Zn content compared to those under natural light, except for Carmesi, with average values of 5.5 and 4.0 mg/kg.

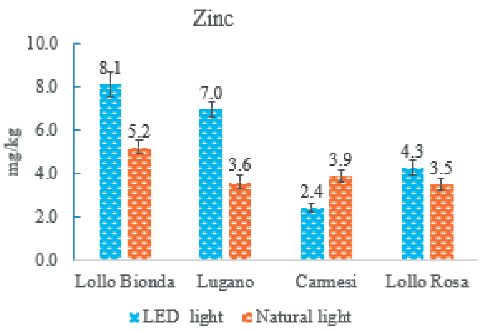

Figure 10. Zinc in lettuce's leaves cultivated under both light conditions. The value represents the means, standard deviation

Copper (Cu). Regarding the copper content in lettuce, under natural light conditions, Lollo Bionda exhibited the highest Cu content at 1.023 mg/kg, followed by Carmesi, Lugano, and Lollo Rosa (1.008, 0.965, and 0.799 mg/kg, respectively) (Figure 11).

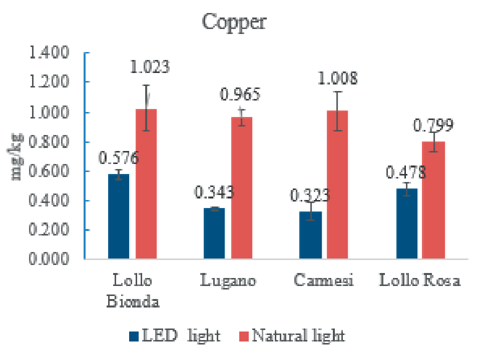

Figure 11. Copper in lettuce's leaves cultivated under both light conditions. The value represents the means, standard deviation

Furthermore, Lollo Bionda maintained a high level of Cu under LED conditions (0.576

mg/kg), followed by Lollo Rosa, Lugano, and Carmesi (0.478, 0.343, and 0.323 mg/kg). Overall, the average Cu content was higher under natural light compared to LED (0.949 and 0.430 mg/kg).

Iron (Fe). Based on the results obtained from the analysis, under natural light conditions, Lugano had the highest value of Fe (35.3 mg/kg), followed by Lollo Bionda, Lollo Rosa, and Carmesi (25.3, 22.4, and 13.6 mg/kg.fw, respectively) (Figure 12). However, under LED light conditions, Lollo Rosa had the highest value of Fe (41.9 mg/kg), followed by Carmesi, Lugano, and Lollo Bionda (20.8, 5.3, and 4.5 mg/kg). It is noteworthy that Lollo Bionda and Lugano had higher Fe values under natural light, while Carmesi and Lollo Rosa had higher Fe values under LED light. The average Fe content was higher under natural light compared to LED (24.2 and 18.1 mg/kg).

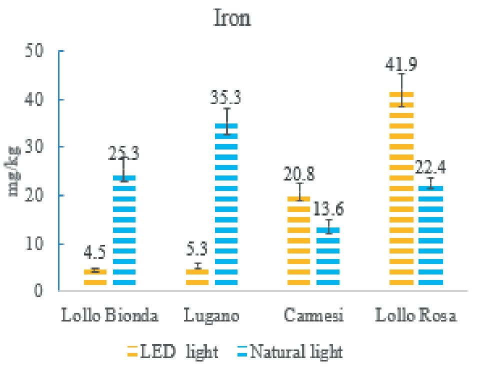

Figure 12. Iron in lettuce's leaves cultivated under both light conditions. The value represents the means, standard deviation

CONCLUSIONS

Based on our experiment's results, we conclude that cultivating lettuces species in NFT under both natural light and LED light conditions influenced various parameters, including fresh mass, dry matter, nitrate content in leaves, and macro and micronutrient levels.

Under natural light conditions, lettuce exhibited higher fresh weight, dry matter, phosphorus, iron, and copper content.

On the other hand, lettuce cultivated under LED light conditions showed higher nitrate content, potassium, calcium, magnesium, and zinc levels.

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STUDY ON THE EFFECTIVENESS OF SOME INSECTO-FUNGICIDE COMBINATIONS IN THE CONTROL OF PATHOGENS AND PESTS IN SWEET POTATO CROP IN THE FIELD

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Abstract

In the conditions of the sandy soils of Dăbuleni, in the period 2020-2022, the aim was to identify some combinations of active substances, to test their effect in preventing and combating pathogens and pests reported during the vegetation period in the sweet potato crop, but also to establish the effectiveness of the application of these products. The best control over the attack of the harmful organisms taken in the study was ensured by the variants in which the Cabrio top and Ortiva 250 SC fungicides were applied in complex with the Mospilan 20 SG insecticides. The combinations of products used in the experimental variants had different effectiveness in terms of the control of harmful organisms that appeared during the vegetation period in the sweet potato crop, depending on the year of the study. Thus, in the variants in which the Ortiva 250 SC product was used in combination, the effectiveness in combating the pathogen Alternaria spp. had values between 82.0% and 89.5%, and in combating the pathogen Botrytis cinerea the effectiveness was between 74.7% and 88.0%.

Key words*: sweet potato, treatment, fungal diseases, effectiveness, pest control.*

INTRODUCTION

Climatic changes in the last decade, manifested by increased air temperature, drought or excessive precipitation, have led to changes in the frequency and virulence of the attack of harmful organisms on different plant species, including sweet potato. In this sense, different combinations of insect-fungicide products have been tested in order to combat diseases and pests. Recent research has highlighted the results obtained in the sweet potato in combating *Alternaria* spp. pathogens by using the mixture of azoxystrobin-difenoconazole which was the most effective in reducing the intensity of the disease. Fungicides based on pyraclostrobin-boscalid, unizeb, azoxystrobin-chlorothalonil and cymoxanil-mancozeb were also effective (KANDALO et al., 2016). The active substances pyrimethanil, cyprodinil, fludioxonil, azoxystrobin, difenoconazole and potassium bicarbonate have been used successfully in the USA as control measures (OLSON et al., 2012). In our country, the sweet potato plant is considered to be relatively less susceptible to diseases and pests, compared to countries that have a tradition of growing it. However, in case of non-compliance with the technology, it can be affected by viral, bacterial or fungal diseases, but also by nematodes or foliar pests and tuber pests (DIACONU et al., 2018). The use of virus-free tested material can increase yields up to 7 times (Loebenstein et al., 2009). In vegetative beds, the most destructive diseases are rhizoctoniosis and rots, and *Fusarium* wilt, root rot and stem rot can be responsible for severe yield losses (Clark and Moyer, 1988). In the field, the most important diseases are anthracnose, *Fusarium* wilt and *Alternaria*, and storage or post-harvest diseases include *Rhizopus* soft rot, Java black rot and charcoal rot, among others. These diseases usually develop after harvest or after packaging for long-distance transport (Clark and colab., 2009; Ames and colab., 1997). After Ames et al., 1997, Clark et al., 2015, Ekman and Lovatt, 2015, cited by Boiu-Sicuia et al., 2015, and 2018, mycotic diseases commonly encountered in culture are represented by soil pathogens, foliar diseases and storage diseases.

More than 40 species of arthropods harmful to the sweet potato crop are listed globally, grouped according to the importance of the attack (Iamandei et al., 2014; Davis, 2014). Soil insects can be controlled by applying insecticides before planting or at planting (Brandenberger et al., 2014).

MATERIALS AND METHODS

The experience was located at RDSPCS Dăbuleni, Romania, located on the 4th terrace of the Danube at an altitude of 44 m. The geographic coordinates are: North latitude: 43° 48′, and 24° 5′ East longitude. During the experience, the effectiveness of combinations of phytosanitary products in the control of Alternaria spp. and gray rot (*Botrytis cinerea*) was monitored, and among the pests reported in the culture on the aphids and larvae of defoliating insects, especially *Spodoptera* sp. and *Helicoverpa armigera*. The period analyzed in the experience was 2020-2022, with excessive temperatures accompanied by drought and with precipitation during the sweet potato vegetation period with values below the sum of multiannual precipitation. The aim was to identify combinations of active substances for the complex combating of pathogens and pests in the sweet potato crop in the field. The monofactorial experiment was arranged in randomized blocks with 10 variants in three replications, using the genotype "KSP 1". The surface of the experimental plot was 7.65 m2 and contained 3 rows with 10 plants per row, spaced 30 cm apart. between plants in a row and at 90-100 cm. between the lines. The experimental variants were:

V 1 - Cabrio top - 0.2 % + Mospilan 20 SG - 0.02%;
V 2 - Cabrio top - 0.2 % + Laser 240 SC - 0.05%;
V 3 - Cabrio top - 0.2 % + Karate Zeon - 0.02%;
V 4 - Score 250 EC - 0.05 % + Mospilan 20 SG - 0.02%;
V 5 - Score 250 EC - 0.05 % + Laser 240 SC - 0.05%;
V 6 - Score 250 EC - 0.05 % + Karate Zeon - 0.02%;
V 7 - Ortiva 250 SC - 0.1 % + Mospilan 20 SG - 0.02%;
V 8 - Ortiva 250 SC - 0.1 % + Laser 240 SC - 0.05%;
V 9 - Ortiva 250 SC - 0.1% + Karate Zeon – 0.02%;
V 10 - Untreated control variant.

Attack frequency, attack intensity, attack grade and efficacy were calculated for each treatment option. The interpretation of the obtained results was done by the statistical method, both for each year of experimentation and for their average (2020-2022). The effectiveness of treatments (%) was calculated according to the formula (ABBOT, 1925):

$E\% = [(DA_{Mt} – DA_{v}) / DA_{Mt}] \times 100$, where:

DA_{v} = degree of attack (%) in the treated variant.

DA_{Mt} = degree of attack (%) in the control version.

Score 250 EC is a systemic fungicide with rapid uptake into the plant. The active substance difenoconazole 25% acts against fungi at the moment of penetration into the plant (germination of fungal spores) and during the formation of haustoria and stops the development of pathogenic fungi (https://www.syngenta.ro/product/crop-protection/fungicid/score-250ec).

Cabrio top, contact fungicide, effective thanks to the active substance pyraclostrobin. It has preventive action and is approved for the control of *Alternaria solani* in tomatoes.

Ortiva 250 SC, contact fungicide, but also with local systemic action, contains azoxystrobin and difenoconazole, and ensures the fight against alternariosis in several crops, both in the greenhouse and in field conditions.

Mospilan 20 SG (acetamiprid 20%), systemic insecticide, with fast and effective action in combating different pests, regardless of their development stage. In the southeastern United States, the insecticide is used successfully to control aphids and other insects harmful to the sweet potato plant (Vegetable crop handbook for Southeastern United States, 2020).

Karate Zeon (lambda-cyhalothrin 50 g/l), insecticide that acts by contact and ingestion, pyrethroid, with action on the insect's nervous system.

Laser 240 SC (spinosad 240 g/l) is an ecological insecticide with high activity in the plant, acting on harmful insects by contact and ingestion. It belongs to a new family of biological insecticides: Naturalyte, which includes insecticides derived from metabolites of living organisms.

Three treatments were made during the sweet potato vegetation period, applied preventively, based on the forecast and warning. Observations were made on the frequency and intensity of the attack of the pathogens *Alternaria* spp. and *Botrytis cinerea*, and among the pests appearing in the crop, on aphids (*Aphis* sp.) and *Spodoptera* sp. larvae, after which the degree of attack was calculated (DA %) and the effectiveness of treatment options (E %).

RESULTS AND DISCUSSIONS

The factors that determine the appearance and evolution of pathogens and a large number of pests are: unfavorable temperatures, humidity in the soil and air outside the required limits, abundant fertilization with nitrogen, water drops on the leaves (from sprinkler irrigation, heavy rains) and heavy rainfall or excessive drought. The three years studied were very different from a climatic point of view. The average monthly air temperature exceeded the multiannual average temperature in all months of the plant's vegetation period. In the year 2020 (Figure 1), the precipitation during the sweet potato vegetation period (335.4 mm.) had values below the sum of the multiannual precipitation, but the precipitation was distributed much more evenly in all months.

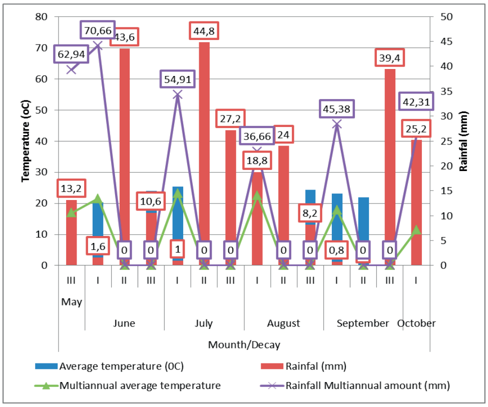

Figure 1. Meteorological characterization of the year 2020 during the sweet potato vegetation period

In the year 2021, the precipitation during the sweet potato vegetation period had values below the sum of the multiannual precipitation, they were missing in the first decade of July, in the first and second decades of August, as well as in the first decade of September (Figure 2).

During the vegetation period of the sweet potato plant in 2022, the average air temperature showed values between 13.3°C in October and 25.2°C in July, with maximum temperatures above 40 °C (respectively 41.6°C in July), as early as May registering 31.8°C. Precipitation was reduced, their value being lower than the multi-annual amount.

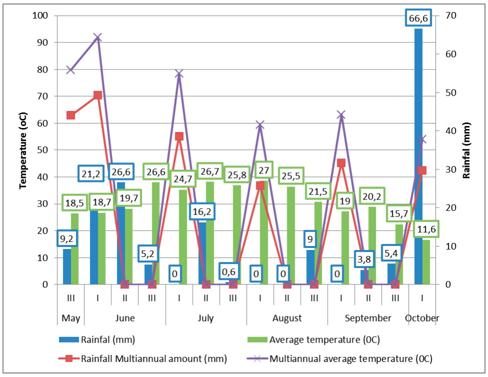

Figure 2. Meteorological characterization of the year 2021 during the sweet potato vegetation period

The product combinations used in the experimental variants performed differently in terms of prevention and control of harmful organisms appearing during the vegetation period in the sweet potato crop. Thus, in 2020 (Table 1), the best combinations of insecticides and fungicides to combat *Alternaria* spp. were those used in the variant V 7 (Ortiva 250 SC 0.1% + Mospilan 20 SG 0.02%) and V 1 (Cabrio top 0.2% + Mospilan 20 SG 0.02%), the effectiveness of the treatment being 88.9%. In the variant V 9 (Ortiva 250 SC 0.1% + Karate Zeon 0.02%), the effectiveness in combating *Botrytis cinerea* was 83.3%, and for combating aphids and *Spodoptera* sp. the best variant was V 4 (Score 250 EC 0.05% + Mospilan 20 SG 0.02%), having efficacy of 87.1% and 85.0% respectively.

Table 1. The effectiveness of treatment variants on pathogens and pests in the sweet potato crop in the field in 2020

Variant	Pathogens and pests							
	Alternaria spp.		*Botrytis cinerea*		*Aphis* sp.		*Spodoptera* sp.	
	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)
V 1	1.7	88.9	3.3	76.5	2.5	82.4	3.3	74.9
V 2	4.5	70	4	71.8	8.7	39	8.2	38.6
V 3	2.8	81.1	2.8	80	6.2	56.6	5.3	59.9
V 4	2	86.7	3.7	74.2	1.8	87.1	2	85
V 5	3.3	77.8	4.2	70.7	4	71.8	5	62.4
V 6	6.7	55.6	3.7	74.2	8.7	39	5.7	57.4
V 7	1.7	88.9	2.7	81.2	3.4	75.8	4	69.9
V 8	2.7	82	2.5	82.4	7.2	49.3	6.7	49.9
V 9	2	86.4	1.8	87.6	5.4	61.7	5.8	56.1
V 10	15	Mt	14.2	Mt	14.2	Mt	13.3	Mt

In the year 2021 (Table 2). the best combination of insecticides and fungicides was the one used in the V 7 variant (Ortiva 250 SC 0.1% + Mospilan 20 SG 0.02%). variant where the effectiveness in combating *Alternaria* spp. it was 89.5%. and in combating aphids by 79.9%. For the control of *Botrytis cinerea* and *Spodoptera* sp. larvae the most effective combination was in the V 9 variant (Ortiva 250 SC 0.1% + Karate Zeon 0.02%) with an effectiveness of 88.0 % for both.

Table 2. The effectiveness of treatment variants on pathogens and pests in the sweet potato crop in the field in 2021

Variant	Pathogens and pests							
	Alternaria spp.		*Botrytis cinerea*		*Aphis* sp.		*Spodoptera* sp.	
	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)
V 1	2.5	83.7	3.2	74.7	3.2	79.4	2.5	80.0
V 2	4.5	70.6	4.0	68.0	4.5	70.8	4.0	68.0
V 3	4.5	70.6	2.8	77.3	6.5	57.8	2.8	77.3
V 4	2.5	83.7	3.2	74.7	4.2	72.9	3.2	74.7
V 5	2.3	84.7	2.8	77.3	4.2	72.7	2.8	77.3
V 6	4.2	72.8	3.8	69.9	7.8	49.1	3.8	69.9
V 7	1.6	89.5	2.7	78.7	3.1	79.9	2.7	78.7
V 8	2.3	84.7	3.2	74.7	7.0	54.5	3.2	74.7
V 9	2.5	83.4	1.5	88.0	5.0	67.3	1.5	88.0
V 10	15.3	Mt	12.5	Mt	15.4	Mt	12.5	Mt

In the year 2022 (Table 3). the best combination of insecticides and fungicides was the one used in the V 7 variant (Ortiva 250 SC 0.1% + Mospilan 20 SG 0.02%). variant where the effectiveness in combating *Alternaria* spp. was 87.2%. *Botrytis cinerea* - 83.3%. *Aphis* sp. - 87.6% and *Spodoptera* sp. - 75.8%.

Also. the combination of Ortiva 250 SC 0.1% and Laser 240 SC 0.05% applied in variant V 8. had an efficacy of 84.2% in the control of Alternaria and 76.7 % in the control of gray rot (*Botrytis cinerea*). and the combination of Ortiva 250 SC 0.1% and Karate Zeon 0.02%. applied to variant V 9 had 84.2% effectiveness in controlling *Alternaria* spp. and 80.0% in controlling gray rot (*Botrytis cinerea*).

Table 3. The effectiveness of treatment variants on pathogens and pests in the sweet potato crop in the field in 2022

Variant	Pathogens and pests							
	Alternaria spp.		*Botrytis cinerea*		*Aphis* sp.		*Spodoptera* sp.	
	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)	DA (%)	E (%)
V 1	2.5	81.2	3.3	66.7	4.2	72.2	3.3	71.5
V 2	3.7	72.4	4.8	51.7	5.7	62.2	6.8	41.6
V 3	3.2	76.2	3.2	68.3	3.5	76.7	5.7	51.6
V 4	3.0	77.4	4.3	57.0	3.8	74.4	4.5	61.5
V 5	4.2	68.7	3.3	66.7	8.3	44.4	5.3	54.4
V 6	4.2	68.7	4.5	55.0	5.7	62.2	6.5	44.4
V 7	1.7	87.2	1.7	83.3	1.9	87.6	2.8	75.8
V 8	2.1	84.2	2.3	76.7	9.6	36.0	6.8	41.6
V 9	2.1	84.2	2.0	80.0	6.4	57.6	7.5	35.9
V 10	13.3	Mt	10.0	Mt	15.0	Mt	11.7	Mt

Regarding the attack of the pathogen *Alternaria* spp., the values of the degree of attack in the three years of the study oscillated between 1.7 % in the variants V 1 (Cabrio top - 0.2% + Mospilan 20 SG - 0.02%) and V 7 (Ortiva 250 SC - 0.1% + Mospilan 20 SG - 0.02%) and 15.0% in the control version (in 2020), in 2021 between 1.6% in V 7 (Ortiva 250 SC - 0.1% + Mospilan 20 SG - 0.02%) and 15.3% in the control variant, and between 1.7% in the V 7 variant (Ortiva 250 SC - 0.1 % + Mospilan 20 SG - 0.02 %) and 13.3 % in the control variant (in 2022). The attack of the pathogen *Botrytis cinerea* recorded values between 1.8% in the V 9 variant (Ortiva 250 SC - 0.1% + Karate Zeon

- 0.02%) and 14.2% in the control variant (in 2020), in 2021 - between 1.5% in the variant V 9 (Ortiva 250 SC - 0.1% + Karate Zeon - 0.02%) and 12.5% in the control variant, and between 1.7% in the V 7 variant (Ortiva 250 SC - 0.1% + Mospilan 20 SG - 0.02%) and 10.0% in the witness version (in 2022). As can be seen from these data. the values of the degree of attack in the control variant were higher in the case of the pathogen *Alternaria* spp. oscillating between 13.3% and 15.0% and in the case of *Botrytis cinerea* between 10.0% and 14.2%. The degree of attack in the case of aphids had values between 1.8% in the V 4 variant (Score 250 EC - 0.05% + Mospilan 20 SG - 0.02%) and 14.2% in the control variant (in 2020), between 3.1% in the V variant 7 (Ortiva 250 SC - 0.1% + Mospilan 20 SG - 0.02%) and 15.4% in the control variant in 2021 and between 1.9% in the V 7 variant and 15.0% in the control variant (in 2022). In the case of defoliating caterpillars belonging to the species *Spodoptera* sp. the degree of attack had values that oscillated between 2.8% in the V 4 variant (Score 250 EC - 0.05% + Mospilan 20 SG - 0.02%) and 13.3% in the control variant (in 2020), between 4.5% in the V 9 variant (Ortiva 250 SC - 0.1% + Karate Zeon - 0.02%) and 12.5% in the untreated control variant (in 2021) and between 2.8% in the V 7 variant (Ortiva 250 SC - 0.1% + Mospilan 20 SG - 0.02%) and 11.7% in the control version (in 2022). The results showed that among the analyzed pests, the highest values of the degree of attack in the control variant were recorded in the case of aphids, values that oscillated between 14.2% and 15.4%, while, in the case of the defoliator *Spodoptera* sp., the values were between 11.4% and 13.3%.

Analyzing as a whole the data obtained in the three years of the study regarding the effectiveness of the treatment variants, it was found that the best control over the attack of the harmful organisms taken in the study. It was ensured by the variants in which Cabrio top and Ortiva 250 SC fungicides were applied in complex with Mospilan 20 SG insecticides.

On the other hand, it is observed that the combinations of products used in the experimental variants had different effectiveness in terms of the control of harmful organisms that appeared during the vegetation period in the sweet potato crop, depending on the year of the study.

Thus, in the variants in which the product Ortiva 250 SC was used in combination, the effectiveness in combating the pathogen *Alternaria* spp. had values between 82.0% and 89.5%, and in combating the *Botrytis cinerea* pathogen, the effectiveness was between 74.7% and 88.0%.

In the combinations where Cabrio top fungicide was used the biological efficacy was between 70.0% and 88.9% in the case of the pathogen *Alternaria* spp. and between 51.7% and 80.0% in the case of the pathogen *Botrytis cinerea* (Figure 3).

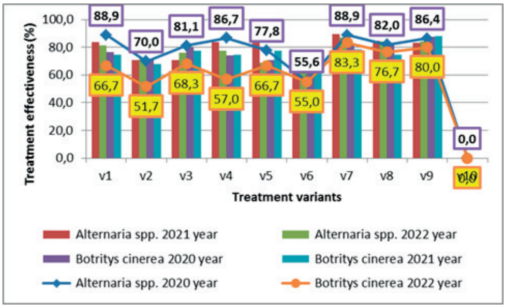

Figure 3. Effectiveness of treatments in combating *Alternaria* spp. and *Botrytis cinerea*

In the variants where the product Mospilan 20 SG was applied alongside the fungicide. efficacies ranging between 72.2% and 87.6% in combating aphids and between 61.5% and 80% in combating defoliating larvae were recorded (Figure 4).

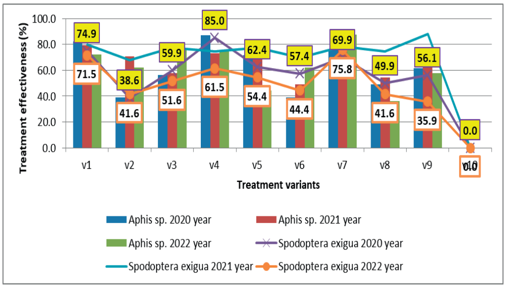

Figure 4. Effectiveness of treatments in combating aphids and defoliating larvae

CONCLUSIONS

- By applying the treatments during the vegetation period to the sweet potato grown on sandy soils with different combinations of insecto-fungicides, a good protection of the plants was ensured against the attack of the studied diseases and pests: *Alternaria* spp. *Botrytis cinerea, Spodoptera* sp. and *Aphis* sp.
- The treatment with the product Ortiva 250 SC - 0.1% was effective in combating alternariosis (efficacy 87.2-89.5%) and the pathogen *Botrytis cinerea* (efficacy 80.0-88.0%). and in the variants in which the product Mospilan 20 SG was applied alongside the fungicide. efficacies ranging between 72.2% and 87.6% in combating aphids and between 61.5% and 80% in combating defoliating larvae were recorded.
- The treatment variant with Ortiva 250 SC 0.1% + Karate Zeon 0.02% gave good results in combating the pathogen *Botrytis cinerea* (efficacy 80.0-88.0%). The best insect-fungicide combination was the one between Ortiva 250 SC 0.1% + Mospilan 20 SG 0.02%. combination where the effectiveness in combating *Alternaria* spp. was between 87.2-89.5%, for *Botrytis cinerea* - between 78.7 and 83.3%, for *Aphis* sp. - 75.8-87.6% and for *Spodoptera* sp. - between 69.9% and 78.7%.

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THE INFLUENCE OF BENEFICIAL BACTERIA ON SOIL AND TOMATO ROOTS

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Abstract

The soil is one of the most important resources of mankind, being the physical support as well as providing the supply of nutrients that plants need to grow and bear fruit. It is well known from specialized research that the application of products based on microorganisms can improve the physical and chemical qualities of the soil and also the productivity of culture. The present study examined the efficacy of two products Rizobac and Bactilis on 3 tomato hybrids Kingset (red fruit), Bucanero (black fruit), Buffalosun (yellow fruit) grown in protected spaces (plastic tunnels), in the Scărişoara district of county Olt. The experiment took part in 2 years, 2022 and 2023. The culture was established by seedling (seedling maturity = 60-65 days) and after planting in the plastic tunnels, the technology specific to tomatoes was applied. The results showed that were considerable differences regarding length (cm) and root volume (cm^3) from 16% in terms of root length to 98% higher volume for plants treated with products used. Furthermore, the application of Rizobac and Bactilis improved soil apparent density, total porosity, and compaction degree. In summary, based on statistical correlation tests, it can be concluded that as the total nitrogen content decreases, the number of bacterial colonies increases proportionally.

***Key words**: biological, fertilizers, microorganisms, PGPR, soil.*

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is a highly nutritious and widely consumed product globally, known for its rich flavor and versatility in culinary applications, are consumed fresh in salads, cooked in various vegetable dishes, and processed into a wide array of products including ketchup, sauces, purees, syrups, juices, and more. The fruits are abundant sources of vitamins, minerals, amino acids, and pigments while being low in calories (Dinu et al., 2017; Soare et al., 2015).

These products are enjoyed on a broad scale worldwide, showcasing the versatility and popularity of tomatoes in culinary applications (Javaria et al., 2012).

Due to its high nutrient demands, tomato plants typically require substantial amounts of chemical fertilizers to meet their nutritional needs. Despite this, tomatoes rank third in terms of global vegetable production (Helal et al., 2022; Shi & Maguer, 2000).

Tomatoes are cultivated in a wide range of soil types, from small-scale home gardens to large commercial farms, where they are grown as a profitable cash crop by vegetable growers (Pandey & Chandra, 2013). As a heavy-yielding crop, tomatoes demand substantial nutrient inputs to support their growth and maximize yield potential. Tomatoes exhibit positive responses to a variety of technological interventions, including both physical methods such as increasing the number of stems to enhance yield, as well as the application of biofertilizers containing substances like arginine and cysteine (Apahidean et al., 2019; Becherescu et al., 2021; Hoza et al., 2019).

In recent years, the concentrations of heavy metals in soils have risen significantly to

hazardous levels, primarily due to unsustainable practices such as sewage irrigation, excessive use of chemical fertilizers, and widespread pesticide application in agriculture. These practices pose serious threats to soil biological systems and overall ecosystem health (Jaishankar et al., 2014).
The demand for organic tomatoes is surging worldwide, driven by their superior nutritional quality, extended shelf-life, and sustainable yield. Similarly, there is growing momentum in restoring metal-contaminated soils and preserving soil health through the application of organic inputs and microbial inoculations. In recent years, farmers are increasingly adopting good agricultural practices, transitioning to organic farming, and optimizing water and nutrient usage (Verma et al., 2023). To achieve higher-quality production, formulations containing bacteria with biopesticidal, biostimulant, or biofertilizer properties are being utilized. These bacteria not only enhance plant productivity but also reduce plant residues, contributing to consumer safety. Additionally, these bacteria are known for their ability to produce lytic enzymes that inhibit plant pathogens and promote plant growth (Basu et al., 2021; Chojnacka, 2015).
Soil salinization has emerged as a significant challenge, severely impacting agricultural productivity and posing a threat to agricultural development worldwide (Litalien & Zeeb, 2020; Singh, 2021). To combat this issue, harnessing the potential of plant growth-promoting rhizobacteria is paramount. By leveraging these beneficial microorganisms, it can effectively restore degraded soils and sustain soil health, thus fostering sustainable agricultural practices worldwide (Kumar et al., 2023).
Utilizing organic sources of manures and fertilizers, along with proper management practices, can significantly reduce the excessive reliance on chemical fertilizers. This approach enables smallholder farmers to save costs for subsequent cropping seasons. Furthermore, the market demand for inorganic fertilizers surpasses that of organic ones due to their availability and concentrated nutrient content. In chemically fertilized soils, essential nutrients are quickly utilized and often leached out by various meteorological factors. In contrast, organic fertilizers and manures degrade slowly, allowing for the retention of nutrients over an extended period. This results in a continuous supply of soil nutrients, promoting sustainable soil health and agricultural productivity (Praharaj, 2007).
The metabolism of bacterial communities has shown significant benefits by enhancing existing soil microorganisms and improving nutrient assimilation. These positive outcomes can have notable implications for tomato growth, potentially resulting in a remarkable increase in tomato yield by up to 36.82%. These practices hold promise for improving agricultural productivity and sustainability (Dragomir & Hoza, 2022.; Wang et al., 2024).

MATERIALS AND METHODS

The experiment occurred in Scărișoara, Olt county, spanning 2022 and 2023. It followed a randomized block design, with three replicates for each experimental variant and six plants per replicate, covering an area of 200 m^2. Two variable factors were examined: soil characteristics and effects on tomato hybrids.
The primary objective of the experiment was to assess soil characteristics and the performance of hybrids when cultivated alongside radicular biostimulating products: Bactilis 5 L/ha and Rizobac 10 L/ha. The control group did not receive any biostimulant application.
Bactilis is a microbial inoculant which enhance root development, overall plant growth, and vigor. Additionally, these metabolites enhance the resilience of the root system against stressful conditions induced by a variety of biotic and environmental factors (Retrieved from https://www.humofert.gr/en/product/2015-05-29-12-29-20/bactilis-detail.html).
Rizobac is a microbial inoculant abundant in nutrients and beneficial microorganisms, designed to enhance rooting and facilitate the rapid establishment of transplanted crops in soil (Retrieved from https://www.humofert.gr/en/product/2015-05-29-12-29-20/biostimulants-1/rooting/rizobac-1-detail.html).
The biological material utilized consisted of three F1 hybrids: Bucanero, Buffalosun, and Kingset.
By combining the 2 factors, 9 variants resulted, each variant having 3 repetitions and 6 plants per repetition, as follows:

V1 - Kingset F1 Unfertilized; V2 - Bucanero F1 Unfertilized; V3 - Buffalosun F1 Unfertilized; V4 - Bucanero F1 + Rizobac; V5 - Buffalosun F1 + Rizobac; V6 - Kingset F1 + Rizobac; V7 - Buffalosun F1 + Bactilis; V8 - Kingset F1 + Bactilis; V9 - Bucanero F1 + Bactilis.

In order to produce seedlings, the seeds were sown in the last decade of January, followed by transplanting after 14-16 days from germination. Greenhouse planting occurred in the last decade of April, with spacing set at 0.8 m by 0.4 m, resulting in a density of 3.2 plants per square meter and 32,000 plants per hectare. The seedlings were approximately 60-65 days old at the time of planting. Throughout the vegetation period, specific care practices were implemented.

Soil assessments were conducted, samples were taken from 0-20 cm depth. Soil samples have been collected in plastic bags, then dried in the laboratory at room temperature for determination of Electrical Conductivity (EC) was analyzed according with FAO 2008 potentiometric method for pH in aqueous suspension at soil /water ratio of 1/5, pH level analyzed according with FAO 2008 potentiometric method for pH in aqueous suspension at soil/water ratio of 1/2.5., apparent density, total porosity, compaction degree analysed, according to the ICPA methodology, 1987 volume I, which is our national standard. Total nitrogen (N) was determined by using the modified Kjeldahl method as well as the count of bacterial colonies before and after inoculation using the method of dilutions up to 10^{-6} and after sowing by the "in the lawn" method (Benson 1990). Additionally, root measurements included weight (in grams) with PS R2 balances, length (in centimeters) with roulette, and the volume of the root system of the plants, using a 1 L Class A graduated cylinder (in cubic centimeters).

The determinations were carried out in the laboratories of the Research Center for Studies of Food Quality and Agricultural Products at the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

Results were interpreted statistical F-test ($p \leq 0.05$) and T-test ($p \leq 0.05$).

Data values were collected from six replicates and subjected to an F test to determine whether variances were equal ($P > 0.05$) or unequal ($P < 0.05$). Statistical significance of differences was established using the T test, with significance levels interpreted as follows: $p > 0.05$: not significant, $p < 0.05$: weakly significant, $p < 0.01$: moderately significant, $p < 0.005$: highly significant, $p < 0.001$: very strongly significant, $p < 0.0001$: extremely significant and Correlation Coefficients.

RESULTS AND DISCUSSIONS

The application of biostimulating products on the three analyzed hybrids notably improved the EC (Table 1).

Table 1. The impact of biostimulating products on pH and EC

	Before treatment application		Control		Rizobac		Bactilis	
	EC (µS/cm)	pH	EC (µS/cm)	pH	EC (µS/cm)	pH	EC (µS/cm)	pH
Kingset F1	4.19	7.82	2.33	7.95	2.20	7.93	2.22	7.97
					p=0.465	p=0.722	p=0.614	p=0.616
Bucanero F1	4.19	7.82	3.38	7.91	1.98	7.91	2.57	8.04
					p=0.023	p=1	p=0.142	p=0.053
Buffalosun F1	4.19	7.82	2.18	7.98	1.99	7.83	2.05	8.08
					p=0.001	p=0.081	p=0.643	p=0.064

$p > 0.05$: not significant, $p < 0.05$: weakly significant, $p < 0.01$: moderately significant, $p < 0.005$: highly significant, $p < 0.001$: very strongly significant, $p < 0.0001$: extremely significant.

Before the application of treatments, the following values were recorded for EC 4.19 (µS/cm) and pH 7.82. After applying the Rizobac treatment, the EC values decreased significant for Bucanero F1 (p=0.023) and Buffalosun F1 (p=0.001) with values of 1.98 dS m^{-1} and 1.99 (µS/cm) would be optimal to create a more conducive environment for microbial activity during tomato cultivation (Maltas et al., 2022). EC remained stable for Kingset F1 compared to the control group.

pH levels generally remained stable or increased slightly for all hybrids.

Regarding Bactilis treatment, the EC values range from 2.05 to 2.57 (µS/cm), very similar with control value. The pH values range from 7.97 to 8.08.

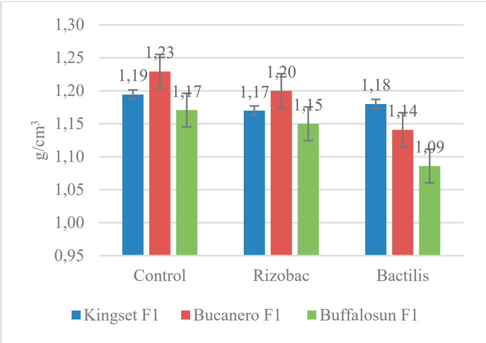

Figure 1. The influence of treatments on the apparent density of soil, g/cm^3

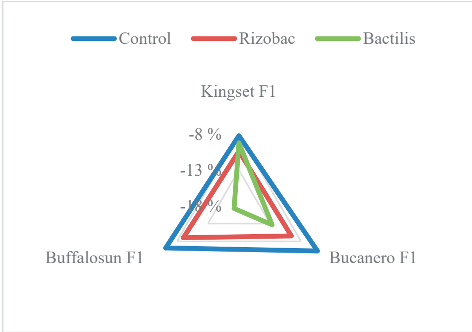

Figure 3. The influence of treatments on the compaction degree of soil, %

For the Kingset F1 hybrid, it can be observed that the application of both Rizobac and Bactilis treatments resulted in an improvement in apparent soil density compared to the control group (Figure 1.). The substantial decrease in apparent soil density with the Bactilis treatment for the Bucanero F1 hybrid suggests that this treatment had a particularly positive effect on soil structure.

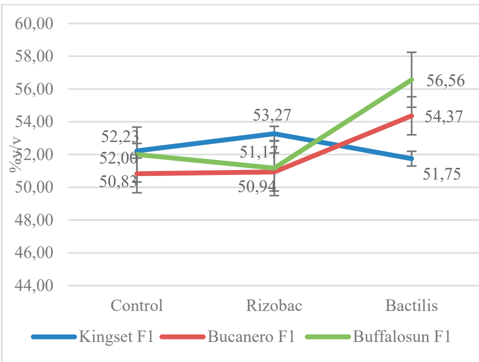

Figure 2. The influence of treatments on the total porosity, % v/v

The application of products had a significant impact on the porosity of the soil, particularly with the Rhizobac product, as evidenced by a significant increase (p=0.029) observed specifically in the Kingset hybrid (Figure 2.) Bucanero and Buffalosun hybrids exhibited notable positive responses to the application of the Bactilis product, showing values that were a few percentage points higher compared to control.

The application of the products led to a shift in soil settlement classes from Small, indicating slight loose soil with values between -9 and 1 (Mihalache et al., 2013), values between which the control falls -8.22 to -5.33 (Figure 3), to Very Small, indicating moderately loose soil with values between -17 and -10, observed for the application of Bactilis and Rizobac for the hybrids utilized.

To emphasize the relationship between the total nitrogen content and the number of bacterial colonies, it was conducted a "Correlation Coefficients" test (Figure 4) illustrates a robust, inversely proportional relationship.

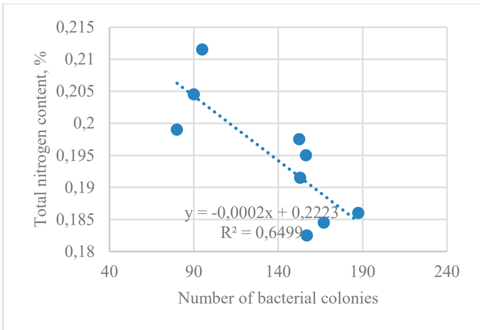

Figure 4. The correlation between the total nitrogen content and the number of bacterial colonies

In summary, based on the statistical correlation test, it conclude that the relationship between the total nitrogen content and the number of bacterial colonies (Figure 5) is as follows: as the total nitrogen content decreases, the number of bacterial colonies increases proportionally.

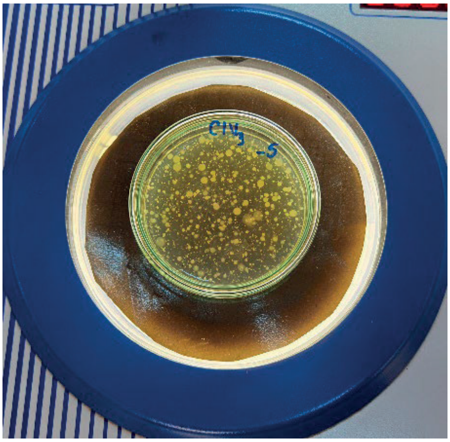

Figure 5. Bacterial colonies

By correlating roots weight with the number of bacterial colonies, a beneficial effect was observed. The correlation coefficients suggest a moderate to strong positive correlation between root weight and the number of bacterial colonies, indicating that as root weight increases, the number of bacterial colonies tends to increase as well (Figure 6).

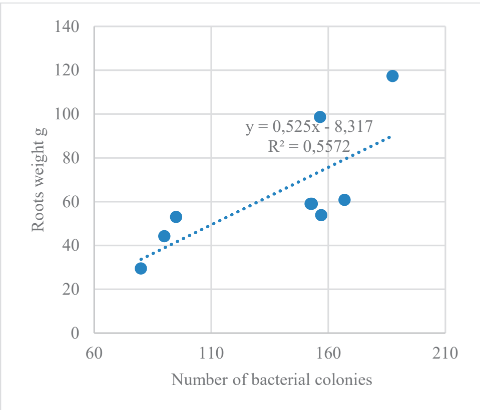

Figure 6. The correlation between roots weight and the number of bacterial colonies

Regarding root growth parameters, Rizobac fertilizer notably demonstrates positive and statistically significant effects on both analyzed parameters: root length and root volume. Specifically, the root length is significantly greater for the Bucanero F1 hybrid by approximately 5 cm compared to the non-fertilized version, with statistical significance (p=0.001). Additionally, it resulted in the longest roots observed in the experiment, measuring 25.42 cm.

It can also be observed that all the hybrids react very well to the application of treatments, showing positive values, when Rizobac and Bactilis were applied (Figure 7.).

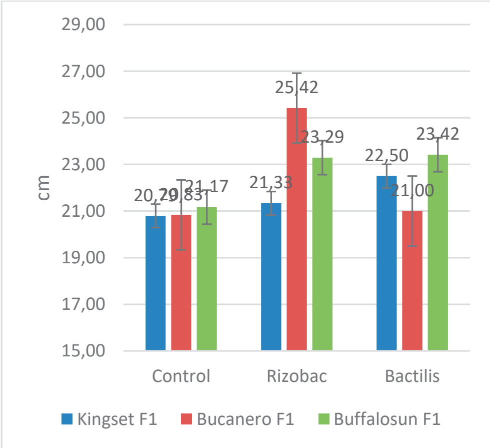

Figure 7. The impact of treatments root length, cm

The volume of the roots was significantly higher when applying the Rizobac biofertilizer compared to the non-fertilized version for all the hybrids from 35% to 98% (Table 2.).

Table 2. The impact of biostimulating products on roots volume

Roots volume, cm^3			
	Control	Rizobac	Bactilis
Kingset F1	32.33	52.83	37.50
		p= 0.006	p= 0.248
Bucanero F1	51.25	101.30	76.60
		p= 0.039	p= 0.123
Buffalosun F1	46.00	62.00	66.60
		p= 0.027	p= 0.036

$p > 0.05$: not significant, $p < 0.05$: weakly significant, $p < 0.01$: moderately significant, $p < 0.005$: highly significant, $p < 0.001$: very strongly significant, $p < 0.0001$: extremely significant

Appliction of Bactilis lead also to a biger volume for Buffalosun hybrid with 45%.

CONCLUSIONS

The present research highlights the significant influence of genotype on various traits, particularly evident in traits related to pH and EC. Fertilization with Rizobac yielded the best results for the Bucanero and Buffalosun hybrids compared to the control, maintaining an ideal level of 2.0 (μS/cm) for tomato plants.

Furthermore, the application of Rizobac and Bactilis improved soil apparent density, total porosity, and compaction degree. In summary, based on statistical correlation tests, it can be concluded that as the total nitrogen content decreases, the number of bacterial colonies increases proportionally. Additionally, there is a moderate to strong positive correlation between root weight and the number of bacterial colonies, indicating that as root weight increases, the number of bacterial colonies tends to increase as well.

Regarding root growth parameters, Rizobac biofertilizer significantly and positively influenced all analyzed parameters, including root length and root volume. These findings demonstrate that the application of biofertilizers on tomato crops serve as a sustainable and organic technology, potentially reducing or even eliminating the need for conventional chemical applications.

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STUDY REGARDING THE INFLUENCE OF SOME TECHNOLOGICAL FACTORS ON THE QUALITY OF SWEET POTATO PLANTING MATERIAL

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Abstract

The preliminary study was conducted at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, on various sweet potato varieties obtained at the SCDL Dăbuleni, Romania. The sweet potato tubers were rooted in different types of substrates, in various combinations (100% peat, 100% perlite; 25% peat+75%perlite; 50% peat + 50% perlite; 75% peat + 25% perlite), with the aim of obtaining shoots necessary for establishing the crop. Observations were made regarding the growth of the obtained seedlings and the rooting time.
The root volume and root mass were determined, and correlations were made regarding the influence of the type of substrate and the fertilizers used.

Key words*: Ipomea batatas, substrate.*

INTRODUCTION

The sweet potato (*Ipomoea batatas* (L.) Lam) belongs to the *Convolvulaceae* family and is native to South America. It is currently cultivated on all continents, but China is the world leader in terms of production.
Sweet potatoes are rich in carotene, fiber, minerals, and vitamins, making them recommended for diabetics, heart patients, and those with digestive disorders, as they have beneficial effects on immunity.
Recent analyses regarding the areas cultivated with sweet potatoes in Spain indicate a significant decrease of approximately 60% over the past two years. This reduction has been caused by several factors, including rising production costs, such as high prices for agricultural inputs (energy, fertilizers, labor), as well as increasing competition from Egypt in European markets.
The production of sweet potatoes in Egypt has grown due to lower production costs, favorable climate, and access to European markets through large-scale exports. As a result, the competitive prices of Egyptian sweet potatoes have negatively impacted the competitiveness of Spanish producers, leading to a reduction in the cultivated areas in Spain.
This trend highlights the challenges faced by European farmers in the face of globalization and international competition, underscoring the need for new strategies to maintain their competitiveness in the European market (https://www.freshplaza.com/europe/).
Sauti et al. (1994) demonstrated that sweet potato is a crop that can bring substantial benefits when intercropped with maize or sorghum.
Tadda et al. (2022) showed in their studies that sweet potato shoots can also be obtained through in vitro multiplication.
Lyu et al. (2020) mention that light is a very important factor both for obtaining high-quality seedlings and for the growth of sweet potato plants.
The experiments conducted on the Pumpkin and Chestnut varieties at the Banu Mărăcine station in Craiova showed that sweet potato yields can reach 53.3 tons/ha and 35.6 tons/ha, respectively (Dinu and Soare, 2015).
In Romania, sweet potatoes are cultivated on limited areas, and experiments conducted at the Research Institute in Dăbuleni on sandy soils in southern Oltenia have yielded remarkable

results, with good quantitative and qualitative production (Croitoru et al., 2019).

In Romania, there have been efforts to create sweet potato varieties with very good results under production conditions. Thus, at USAMV of Bucharest in 1991, the variety Victoria IANB was created, followed by the variety Crux in 1997. Given that the climatic conditions in our country are favorable for sweet potato cultivation, and market trends have been positive, the research team at the Research Development Station for Plant Culture on Sands (SCDCPN) Dăbuleni identified, selected, created, and registered two additional varieties, “DABU 23” and “KORETTA,” in 2023.

In Romania, the Official Catalog of Crop Plant Varieties for 2024 lists four varieties, including KSC1 and KSH1, which have been registered since 2015, and Dabu 23 and Koretta, which were registered in 2023.

The planting material for establishing the sweet potato crop consists of shoots emitted from the sweet potato roots, which are rooted using various methods, such as water, soil, or other types of substrate. Once the shoots have grown to about 10-15 cm in length, they are detached from the sweet potato root and placed for rooting in water or other substrates. After roots have developed, the cuttings are ready for planting.

The process of producing cuttings takes place about two months before field planting. Rooting cuttings requires temperatures of 20-25°C. They are planted in a substrate (such as compost or garden soil) or in water. The cuttings are then acclimatized before being planted in the field.

The quality of sweet potato seedlings can be affected by the fertilization regime (Solano, 2018), photoperiod, and light intensity.

Yong (2008) states that sweet potato shoots exhibited very good growth at a photoperiod of 16/8 hours, with an intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and at a temperature of 23°C during the day and 20°C at night. Yarmento and Meamea (2019) mention that applying treatments to limit apical shoots resulted in increased vegetative growth as well as larger root yields.

Sasaki (1991) and Velumani and Raju (2012) note that planting sweet potatoes at high densities influences growth as well as the formation of branching in the plant.

Velumani and Raju (2012), Crop Physiology of Sweetpotato, Fruit, Vegetable and Cereal Science and Biotechnology, Global Science Books, http://www.globalsciencebooks.info/Online/GSBOnline/images/2012/FVCSB_6(SI1)/FVCSB_6(SI1)17-29o.pdf.

The level of fertilization influences sweet potato production (Esan et al., 2021).

Research conducted by Hlerema in 2021 on sweet potatoes focused on optimizing cultivation practices by analyzing the horizontal placement of cuttings during planting.

To maximize the production of sweet potato cuttings (Ipomoea batatas), it is essential to pay attention to the size and quality of the tuberous roots, as these directly influence both the number and vigor of the cuttings. Obtaining high-quality planting material requires a rigorous evaluation of the shoots developed from tubers, considering criteria such as uniformity, vigor, and regeneration capacity.

Thus, a fundamental objective of this study was to identify the number of vigorous shoots produced by each plant and to determine the optimal timing for their emergence. Additionally, the evaluation of the cultivation substrate and fertilization regime aimed to identify the most effective conditions for obtaining a high number of quality shoots suitable for planting.

The selection of planting material was based on the vigor of the shoots, ensuring a biologically homogeneous and agronomically optimal material adapted to the cultivation conditions.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse of the Research Center for Quality Control of Horticultural Produce, Faculty of Horticulture, UASMV of Bucharest, from 25 October to 10 November 2023.

The biological material used in the experiment consisted of four sweet potato varieties: V1 - Dabuleni 23; V2 - Hayanmi; V3 - Koretta; and V4 - Ro-CH-M.

“Hayanmi” is a Korean variety with white flesh, included in the SCDCPN Dăbuleni collection since 2015. The sweet potato line “DCh19/3” was obtained at SCDCPN Dăbuleni through selection and has purple flesh.

The substrates used for rooting the sweet potato tubers were: 100% peat; 100% perlite; 25% peat + 75% perlite; 50% peat + 50% perlite; and 75% peat + 25% perlite.

The statistical program for analysis was STATISTICA, StatSoft software (version 10) to perform ANOVA analysis at $p \leq 0.05$, 0.01, or 0.001 levels, and Tukey HSD was used to compare the significant difference of each dependent variable at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

In the case of most varieties, substrates with a combination of peat and perlite (especially those with equal or higher percentages of perlite) led to a higher number of shoots compared to using either 100% peat or 100% perlite. For V1 - Dăbuleni 23, the highest number of shoots (20.3) was obtained from the substrate composed of 50% peat and 50% perlite, while the lowest number (14.7) was recorded with 100% perlite (Figure 1).

For V2, the Hayammi variety, the highest number of shoots (20.7) was achieved with the 100% perlite substrate and the combination of 25% peat and 75% perlite, suggesting that this variety responds better to substrates with a higher perlite content. The lowest number of shoots was identified in the substrate made of 75% peat and 25% perlite (17.3 shoots per tuber) (Figure 1).

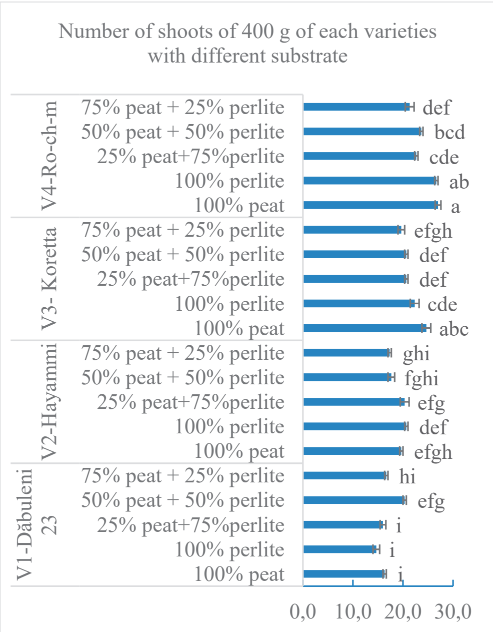

Figure 1. Influence of substrate type on the number of shoots formed on sweet potato tubers

For V3, the Koretta variety, the highest number of shoots (24.7) was identified in the 100% peat substrate, suggesting that this variety develops better in predominantly peat substrates. The lowest number of shoots was observed in the substrate combination of 75% peat and 25% perlite (19.7 shoots per tuber) (Figure 1).

In V4, the Ro-ch-m variety, a high tolerance for shoot emergence was noted, with the highest number of shoots (27.0) obtained in the 100% peat substrate and 26.7 shoots in 100% perlite. It was also observed that the number of shoots slightly decreases as the percentage of peat increases in the mixtures with perlite (Figure 1).

The varieties V4 - Ro-ch-m and V2 - Hayammi responded better in terms of producing a high number of shoots in perlite substrate, while V3 - Koretta thrived in peat substrate. V1 - Dăbuleni 23 exhibited balanced growth across all substrate combinations, with a slight preference for the 50% peat and 50% perlite mix.

It can be appreciated that the substrate played a crucial role in shoot emergence, and the optimal mix varies between varieties. Additionally, each variety shows different responses to substrates, suggesting that the optimal substrate mix for shoot production depends on the specific characteristics of each variety.

Analyzing the number of cuttings based on the variety, a gradual increase in the number of shoots on the tubers can be observed. Thus, the Ro-CH-M variety (V4) showed the highest average number of shoots across all substrate variations, suggesting that it may have a superior potential for shoot emergence compared to the other varieties.

A consistent increase in the number of shoots was noted from V1 to V4, which could reflect genetic or environmental factors influencing shoot formation in the studied varieties (Figure 2).

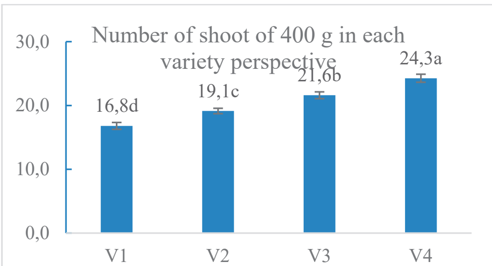

Figure 2. Average number of shoots formed per plant for 400 g tubers

The best results regarding the number of shoots emerging from 400 g sweet potato roots were observed in substrates containing 100% peat (21.9 shoots/tuber) or 100% perlite (21.1 shoots/tuber). As the proportion of perlite increased in the mixtures (from 25% to 75%), there was a noticeable decrease in the average number of shoots per tuber. The mixture of 75% peat and 25% perlite yielded the poorest results (18.8 shoots/tuber), suggesting that a balance between these two components can significantly influence root development.
The mixture of 75% peat and 25% perlite yielded the poorest results (18.8 shoots/tuber), suggesting that a balance between these two components can significantly influence root development (Figure 3).

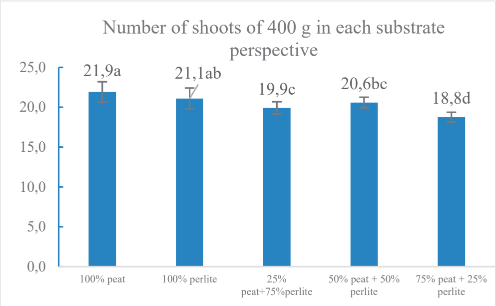

Figure 3 Number of 400 g shoots observed in each substrate type

Figure 4. The appearance of sweet potato plants

In the case of 400 g tubers, for the Dăbuleni 23 variety, the correlation performed to see how the type of substrate influenced the number of shoots indicated that it did not significantly affect it ($R^2 = 0.2239$), as shown in Figure 5.

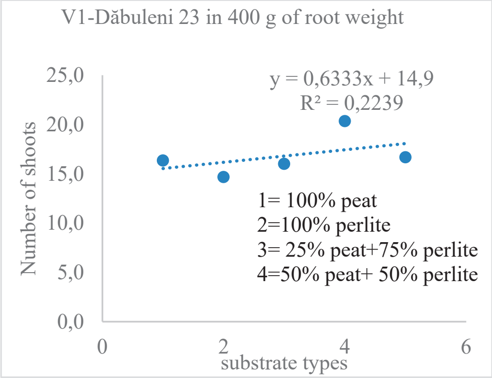

Figure 5. Influence of Substrate Type on the Number of Shoots for Variety V1 - Dăbuleni 23

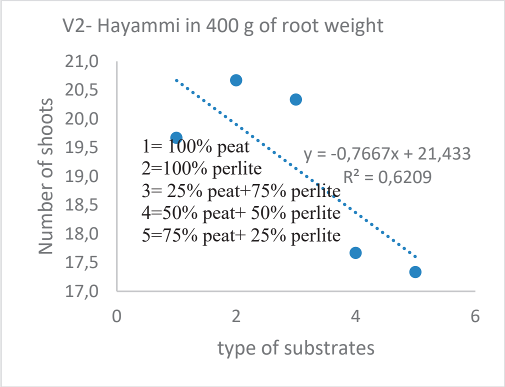

Figure 6. Influence of Substrate Type on the Number of Shoots for Variety Hayammi Using 400 g Roots

In the case of the Hayammi variety, the number of shoots per plant was influenced by the type of substrate ($R^2 = 0.6209$), as shown in Figure 3. This observation was also noted for the Koretta and Ro-ch-m varieties, where the correlation coefficients were highly significant: $R^2 = 0.8826$ for V3 - Koretta and $R^2 = 0.8299$ for V4 - Ro-ch-m, as illustrated in Figures 6-8.

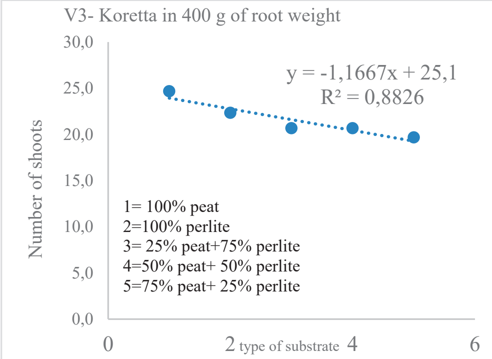

Figure 7. Influence of Substrate Type on the Number of Shoots for Variety V3 - Koretta Using 400 g Roots

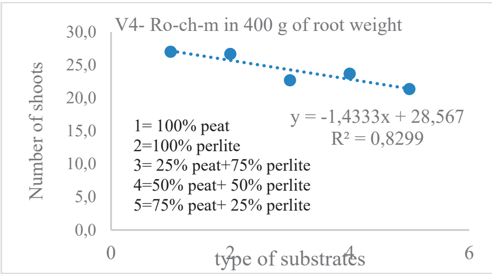

Figure 8. Influence of Substrate Type on the Number of Shoots for Variety V4 - Ro-ch-m Using 400 g Roots

The appearance of sweet potato roots and shoots is shown in Figure 9.

Figure 9. Appearance of sweet potato roots and shoots: a. Dabuleni 23 (V1); b. Hayanmi (V2); c. Koretta (V3); d. Ro-CH-M (V4)

Analyzing the number of shoots formed from sweet potato roots weighing 200g, it was observed that the Dăbuleni 23 variety (V1) had the highest average number of shoots on the 100% peat substrate (11.3 shoots/tuber) , and the smallest on 100% perlite substrate (8.7 shoots/tuber). In the variety Hayammi (V2), the highest number of shoots was obtained on the 100% perlite substrate but also with a mixture of 25% peat and 75% perlite (17.3 shoots). The lowest number of shoots of only 15.7 shoots was obtained on the 100% peat substrate. In the case of the variety Koretta (V3) it was found that it responded best to the 100% perlite substrate, with 21.7 shoots, while on peat it formed only 17.7 shoots. In the variety Ro-ch-m (V4) constant results were noted on all substrates, ranging between 15.7 and 17 shoots, with a slight increase on the mixture of 50% peat and 50% perlite (Figure 10).

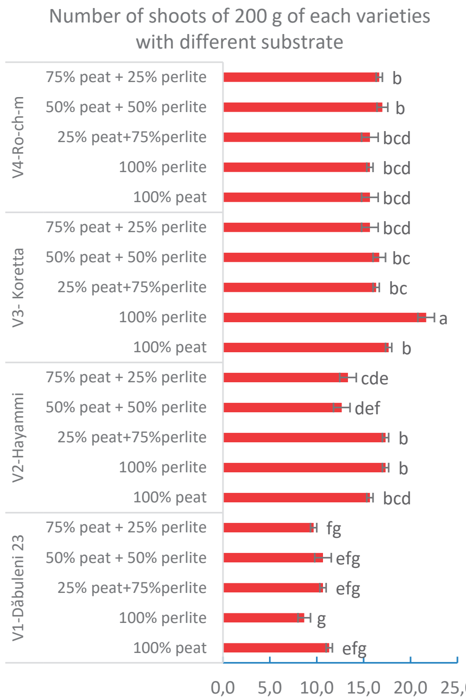

Figure 10. The influence of the substrate type on the number of shoots formed on sweet potato tubers - 200 g tubers

The average number of shoots appearing on the plant in the case of 200 g tubers was on average 17.6 shoots at V3 and the lowest at V1 - the

Dăbuleni 23 variety of 10.2 shoots/plant (Figure 11).

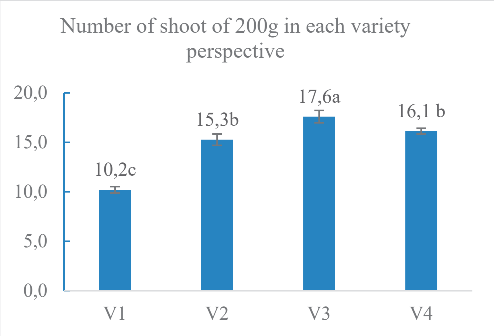

Figure 11. The average number of shoots that appeared depending on the cultivated variety, in the case of 200 g tubers

Analyzing how the type of substrate influenced the number of shoots appearing per tuber, it was found that, on the perlite substrate, in the case of 200 g tubers, an average of 15.8 shoots were obtained and, on the substrate, composed of 75% peat and 25 % perlite, the lowest number of only 13.8 shoots per tuber was recorded (Figure 12).

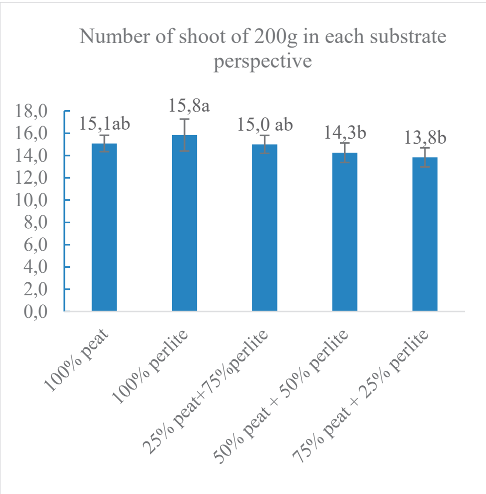

Figure 12. The number of shoots that emerged from the sweet potato tuber weighing 200 g

It was found that in the case of the Dăbuleni 23 variety, no significant relationship was identified (R^2=0.0408), regarding the correlation between the type of substrate and the number of shoots formed from sweet potato roots (Figure 13).

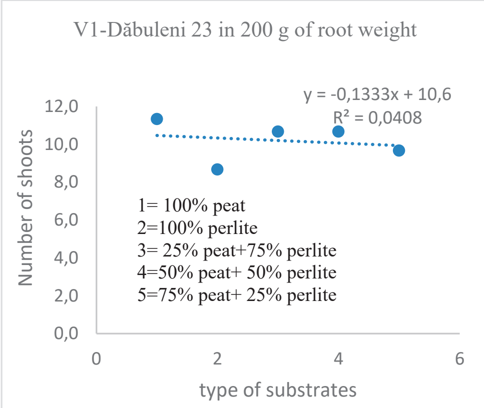

Figure 13. The influence of the type of substrate on the number of shoots in the variety V1 - Dăbuleni 23 in the case of roots of 200 g

The correlation carried out in the case of the Hayammi variety indicates that the substrate had a slight influence on the emergence of shoots (R^2 = 0.4537) (Figure 14).

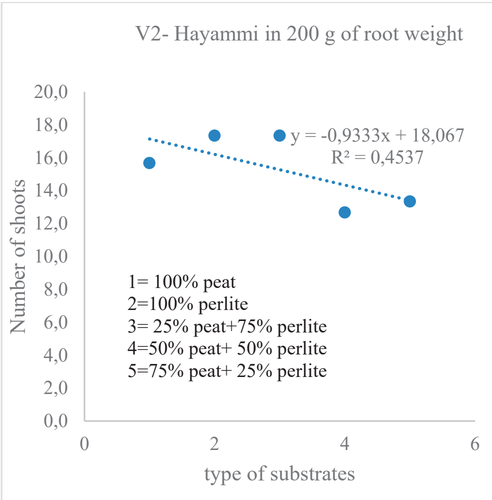

Figure 14. The influence of the type of substrate on the number of shoots in the variety V2- Hayammi in the case of roots of 200 g

In the case of the variety Koretta, it was found that the substrate had a weak influence on the emission of shoots (Figure 15), but in the variety Ro-ch-m, it had a significant influence (R^2 = 0.6579) (Figure 16).

The correlation performed to see how the type of substrate influenced the appearance of shoots showed that the substrate significantly influenced their appearance, the correlation coefficient being $R^2 = 0.6579$ (Figure 16).

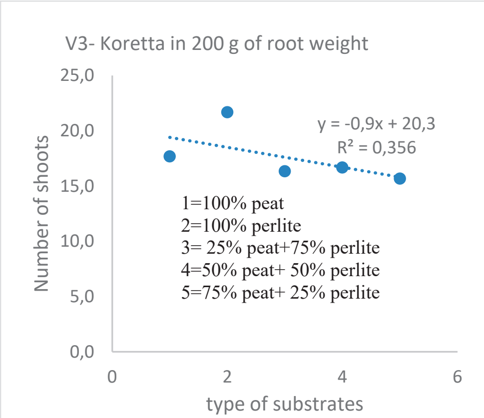

Figure 15. The influence of the type of substrate on the number of shoots in the variety V3 - Koretta in the case of roots of 200 g

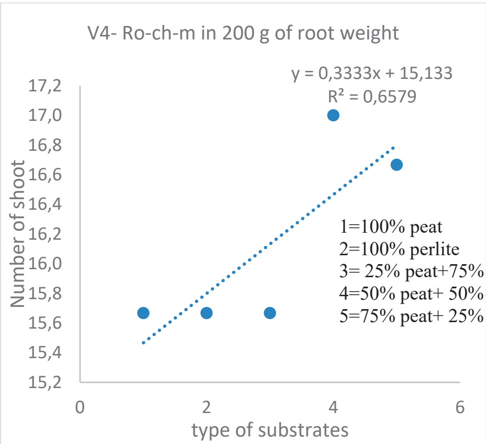

Figure 16. The influence of the type of substrate on the number of shoots in the variety Ro-ch-m (V4) in the case of roots of 200 g

Analyzing how the variety had an influence on the number of shoots, a distinctly very significant relationship was found.

Varieties V3 - Koretta and V4 - Ro-ch-m produced the most shoots, while V1-Dăbuleni 23 had the fewest. The differences between varieties are highly significant ($p < 0.001$), with varieties V3 and V4 not having significant differences between them, but having superior results compared to V2 and V1.

Substrate type has a significant effect on the number of shoots, with 100% peat and 100% perlite roots generating the highest number of shoots (18.5 ± 1.0 shoots) and reducing the percentage of peat resulted in a decrease in them. Root weight also significantly influences shoot production, with plants with 400 g roots having a significantly higher number of shoots compared to 200 g (20.5 ± 0.4 vs. 14.8 ± 0.4). There are significant interactions between varieties, substrate and root weight, suggesting that the effect of each factor depends on the others. The interactions performed provide important insights for optimizing shoot production.

Table 1. The interaction between the analyzed parameters

Varieties (V)	Number of shoots	Significant level %
V1 - Dabuleni 23	13.5 ± 0.7 c	
V2 - Hayammi	17.2 ± 0.5 b	***
V3 - Koretta	19.6 ± 0.5 a	
V4 - Ro-ch-m	20.2 ± 0.8 a	
Substrate(S)		
100% peat	18.5 ± 1.0 a	
100% perlite	18.5 ± 1.1 a	
25% peat+75%perlite	17.5 ± 0.7 b	***
50% peat + 50% perlite	17.4 ± 0.9 b	
75% peat + 25% perlite	16.3 ± 0.7 c	
Root weight (RW)		
200 g	14.8 ± 0.4 b	***
400 g	20.5 ± 0.4 a	
V x S		***
V x RW		***
S x RW		**
V x S x RW		***

CONCLUSIONS

Tubers weighing 400 g or 200 g can be used to produce sweet potato shoots. The study shows that 100% peat and 100% perlite substrates produce the highest shoot emergence in sweet potato tubers with 21.9 and 21.1 shoots/tuber, respectively. Increasing perlite content leads to a reduction in shoots per tuber, with the mixture of 75% peat and 25% perlite resulting in the lowest yield (18.8 shoots/tuber). In the case of 200 g tubers, substrate composition significantly influenced shoot formation in sweet potatoes. A higher content of perlite increased the number of shoots, especially for the variety Koretta.

Dăbuleni 23 prefers peat and Hayammi grows well in mixtures of peat and perlite. Ro-ch-m shows consistent but moderate results on different substrates.

ACKNOWLEDGMENTS

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EFFECT OF GRAFTING ON TOMATO DEVELOPMENT AND PRODUCTIVITY IN THE ECOLOGICAL CULTURE UNDER GREENHOUSE CONDITIONS

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Abstract

Tomato grafting can be considered an important strategy, especially in organic production. This work aimed to evaluate the performance of plant biometric indicators, fruit production quality, and quality indicators of five local tomato varieties Ştefăneşti 30, Ştefăneşti 67, Siriana F1, Costate 23, Ştefăneşti 24 grafted on the rootstock of Solanum sisymbriifolium sp., cultivated in an ecological system in a protected space. The rootstock used in this study increased both the weight of the roots and their number and improved the quality indicators in all the varieties analyzed. The rootstock induced a production increase of 1.4 kg/plant in the Ştefăneşti 22 variety, 1.25 kg/plant in the Costate 23 variety and 2.07 in the Ştefăneşti 30 variety. The use as rootstock of sp. Solanum sisymbriifolium proved to be a good choice to achieve good performance in organic culture. Future, it is recommended to continue additional studies for several years regarding the evaluation of the influence of the rootstock and on the tolerance of the varieties to the attack of the main pests.

***Key words**: Solanum sisymbriifolium sp., Lycopersicum L., scion, rootstock, organic culture.*

INTRODUCTION

Grafting has become an environmentally friendly agronomic practice and is widespread even in vegetables. It is known that in ecological agriculture the production of tomatoes is limited compared to that shown in conventional culture. Studies in the field claim that the use of appropriate rootstocks tolerant to biotic and abiotic stress (Rivero et al., 2003) can improve the quality of vegetable plants Caradonia et al., 2020, their tolerance to very high temperatures, to salinity with positive impact on growth, physiological parameters and yield in an ecological cropping system. Currently, tomato grafting can be considered an important strategy especially in organic production, improving fruit yield and processing quality of tomato plants when they are grown in an ecological system under greenhouse conditions (Keatinge et al., 2014). Numerous studies support that the rootstock induces a strong root system that improves tomato production (Ruiz et al., 1997; Vitre, 2002) compared to ungrafted plants. Such studies were observed in watermelon (Ruiz Yetisir and Sari, 2003), cucumber (Pavlou et al., 2002), eggplant (Passam et al., 2005), and tomato quality (Roberts et al., 2005). Numerous studies claim that the well-chosen rootstock can help tomato crops overcome biotic and abiotic stresses (Santa-Cruz, 2001; Ntatsi et al., 2001; Huang et al., 2010; Savvas et al., 2017).

For these reasons, the right choice of rootstock is very important in the success of ecological crops of tomatoes grown in protected space. The right choice of rootstock is expected to address the specific problems and be beneficial for the cultivation of local varieties, with practical utility in ecological agriculture. This study investigates the efficacy of tomato grafting using as rootstock *Solanum sisymbriifolium*, a vigorous wild species considered resistant to biotic and abiotic factors to improve plant biometric parameters, physiological parameters, quality and yield of five native cultivars grown in a culture system ecologically.

MATERIALS AND METHODS

Collect experimental data

The entire experiment was located at INCDBH Ștefanești, in a protected space of 300 m^2 and followed the influence of the rootstock on plant growth and development indicators, fruit quality and fruit production. 5 local tomato varieties *Ștefănești 30, Șfefănești 67, Stefănești 24, Costate 21* and *Siriana F1* were studied, the first 4 recently approved in our institute. All 5 cultivars were grafted onto *Solanum sisymbriifolium* rootstocks and compared with those grown on their own roots. The whole experience was organized based on organic fertilization. At planting, 0.5 l/plant of Biohumus was applied, and during the vegetation period, foliar fertilizers based on algae were applied, repeated every 14 days. At the end of the experiment, the biometric indicators of the plant were evaluated, such as the weight and number of roots, the diameter of the plants, the quality indicators regarding the fruit content in SSC (% Brix), MA (%), CA (%) and the quality of the harvest were analyzed comparatively.

Production of seedlings

Seedlings (on roots and rootstocks) were obtained in 32 alveolar plates using peat, a professional culture substrate, as substrate. For each variant (variety/portaltoi) 150 seeds were sown. The greenhouse where the observations were made is equipped with an irrigation system, with 16 mm diameter tubes, the emitters being arranged 40 cm apart from each other, recording a flow rate of 1.2-1.5 l/hour.

Control and maintenance of grafted tomato seedlings

As a method of grafting, grafting in simple copulation was used, which involves the sectioning of both symbionts at an angle of 45 degrees. For fixing I used silicone clips with a diameter of 2.1 mm. Therefore, both partners must be approximately 2-2.2 mm in diameter to be able to use 2.1 mm clips (Figure 1). Once the seedlings used as rootstocks and grafts were suitable for grafting, the actual grafting proceeded. Seedlings of wild tomato species reached the optimal size for grafting after 40 days from sowing. The seedlings of the five varieties chosen for grafting reach the optimal size for grafting after about 30 days. Successful tomato grafting was conditioned by a climatic chamber, which would maintain the temperature and humidity at a high level so that the graft would not dehydrate and initiate the process of callusation-welding-vascularization at the grafting point. For the success of a good percentage of grafting in the growth chamber, the temperature was maintained at 24°C and 95% RH during the first 7 days after grafting.

Statistical processing of results

The statistical processing regarding the effect of the experimental factors (varieties and rootstocks) and the effect of the rootstock on the main indicators of quality and productivity indicators was completed by applying the Duncan test, for a level of assurance of 5%. The statistical processing was carried out using the data was done using IBM SPSS 14 software and MS Office Excel 2010. The difference is in the way of calculation and in the fact that the Duncan test is more severe in terms of significance limits.

Figure 1. The programmers for the temperature and RH of the air in the climate room pallet with eight cells planted with grafted seedlings in the growth chamber (original)

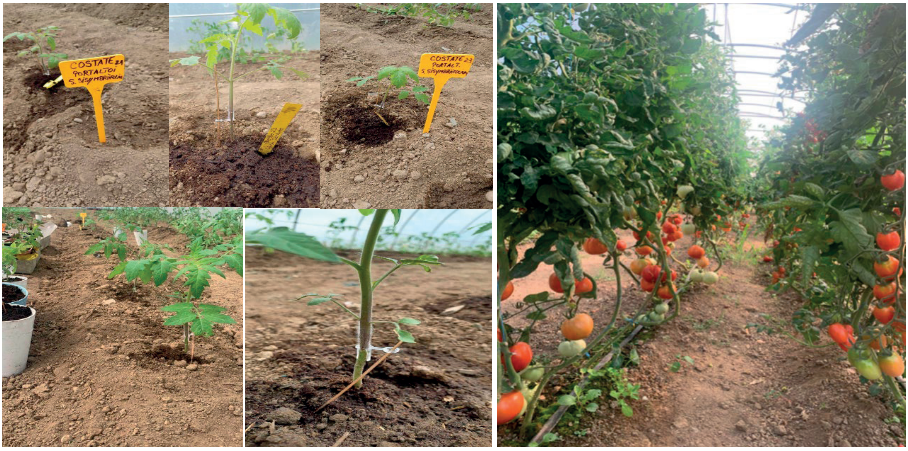

Figure 2. Experimental field image (original)

RESULTS AND DISCUSSIONS

Evaluation of morphology parameters of tomato plants grown in an ecological system

Grafted plants reflect their advantageous properties in organic culture. In this study, total yields and plant stem diameter, plant fresh, and dry weight increased by using rootstock. These results are consistent with studies that show that the effect of grafting changed depending on the other portal genotype (Santa Cruz et al., 2002; Khah, 2005; Öztekin, 2009). In the case of grafting the studied varieties on the *S. sysimbrifolium* rootstock, the root mass recorded significant increases in all the grafted varieties, compared to the control (plants grown on their roots). The highest root mass was recorded in grafted plants, regardless of the variety, the values significantly exceeding the root mass indices in the case of varieties grown on their roots. The rootstock induced the Ştefăneşti 30 variety a weight of the roots by 61.32 g higher than in plants grown on their own roots (Table 1). The same significant differences are also observed in the case of the number of roots formed, the rootstock significantly influenced this indicator in all the grafted varieties, *Solanum sisymbriifolium*, being known to be a very vigorous plant.

Regarding the diameter of the root collar, respectively of the grafting point, the highlighted values are very different and significant, regardless of the variety. The diameter of the plants was influenced by the rootstock, and it varied between 40.73 mm in the Ștefănești 22 variety grafted on *S. sysimbrifolium* rootstock and 29.68 mm, as was evident in the Costate 23 variety, in the case of control plants. All 5 varieties grown on the rootstock recorded significantly higher values than those grafted on their own roots (control) (Table 1). The research results showed that the rootstock *S. sysimbrifolium* rootstock influenced the morphology of the studied genotypes for plant height, diameter, root weight and their number. Rootstock also induced the greatest increases in plant height. The tallest plants were evident in the variety Ştefăneşti 30 (175.45 cm) in plants grafted on sp. *Solanum sisymbriifolium* which exceeds the height of plants grown on their own roots by 37.00 cm, the differences being significant. For all the varieties analyzed, the plant height was greatly improved in the case of grafting, the differences being significant (Table 1).

Table 1. Effects of grafting on biometric parameters of tomato plants grown in an ecological system

Factor's	Root weight (g)	Root number	Diameter (Rootstock/Scion) (mm)	Plant height (cm)
Ştefăneşti 30 cv.				
Grafted plant	130.10±2.01[a]	87±1.12 [a]	44.78±0.88 [a]	175.45±2.15a
Non-grafted plant (scion)	68.78±1.75[b]	58±0.33 [b]	34.58±0.46 [ab]	138.45±1.38[b]
Ştefăneşti 67 cv.				
Grafted plant	98.85±6.87[a]	88±1.19 [a]	48.41±0.62 [a]	177.59±3.15[a]
Non-grafted plant (scion)	56.93±1.39[b]	49±0.66 [b]	31.28±1.20 [b]	149.78±1.45[b]
Ştefăneşti 24 cv.				
Grafted plant	88.89±[a]	73±2.07[a]	38.89±0.78[a]	152.27±1.25[a]
Non-grafted plant (scion)	64.45±[b]	33±2.51 [b]	31.78±1.25[ab]	138.22±1.99[b]
Costate 21 cv.				
Grafted plant	114.12±1.05[a]	69±1.74[a]	38.13±1.14[a]	159.77±2.01[a]
Non-grafted plant (scion)	90.67±0.98[b]	18±2.25[b]	29.68±2.60[ab]	138.44±2.21[b]
Siriana F1 cv				
Grafted plant	91.45±5.14[a]	48.45±0.45[a]	46.89±2.25[a]	149.45±1.78[a]
Non-grafted plant (scion)	64.56±3.45[b]	34.89±0.15[b]	34.56±1.65[b]	134.89±3.45[b]

Evaluation of the quality parameters of the yields of grafted tomato plants grown in an ecological system

The influence of rootstocks resistant to the abiotic and biotic stress factors of grafting on production and fruit quality indicators have become topics of great interest in recent years (Patanè et al., 2015; Rahmanq et al., 2021). It can be concluded that *S. sysimbrifolium* sp. as a rootstock positively influenced the yield per plant confirming the good performance of the grafted plant, regardless of variety (Table 2). This result confirms the ability to use the *S. sysimbrifolium* rootstock to achieve the productive performance of genotypes from organic crops. The results showed significant differences between the varieties studied and the analyzed parameters, thus allowing growers to select varieties based on consumer requirements and establish the optimal culture technology. The rootstock induced an increase in fruit weight compared to the values shown in the control, the differences being statistically significant. The greatest increases in the weight of fruit were evident in the Ștefănești 67 variety, where the rootstock induced a fruit weight of 114 g higher than in the case of control fruits (Table 2).

The fruit size influenced the fruit weight, the differences being significant regardless of the variety analyzed (Table 2). To obtain good harvest quality, good management of the main technological links is important (Gruda et al., 2018; Sumedrea et al., 2021). During the experiment, it can be observed that the rootstock induced a production increase of 2.2 kg per plant in the Ștefănești 30 variety, 1.0 kg per plant in the Ștefănești 67 variety and 1.43 kg in the Ștefănești 24 variety. The Ștefănești 30 variety recorded productions of 6.6 kg/plant in the case of plants grafted on the *S. sysimbrifolium* rootstock, compared to 4.4 kg/plant, as shown in the control variant. The same significant differences were evident in all the grafted varieties, compared to the control (Table 2). Numerous studies claim that the use of a well-chosen rootstock can improve the quality of the harvest and fruits (Rouphael et al., 2018). The use of *S. sysimbrifolium* sp. as a rootstock increased the weight and number of root roots and the production of tomatoes. These can be due to the development of the roots, but also to a good supply of water and minerals (Mauro et al., 2020). The rootstock taken in the study obviously had a positive effect on the varieties studied, in all cases it increased both the root mass and the number of roots, and induced a higher production, regardless of the variety.

Table 2. The effects of grafting on the quality parameters of the yield of tomato plants grown in an ecological system

Factors	Mean fruit weight (g)	Total fruit number (no plant^{-1})	Production of plant (kg)
Ștefănești 30 cv.			
Grafted plant	380.12±4.30^{a}	17±0.18^{a}	6.6±0.45^{a}
Non-grafted plant (scion)	310.14±3.78^{a}	16±0.21^{a}	4,4±0.12^{b}
Ștefănești 67 cv.			
Grafted plant	371.12±2.50	18±0.33^{a}	6.00±0.42^{a}
Non-grafted plant (scion)	257.12±2.14	20±0.42^{a}	5.0±0.10^{b}
Ștefănești 24 cv.			
Grafted plant	367.13±3.47^{a}	15±0.15^{a}	5.68±0.12^{a}
Non-grafted plant (scion)	257.00±2.89^{b}	16±0.14^{a}	4.25±0.31^{b}
Costate 21 cv.			
Grafted plant	371.48± 3.44^{a}	15±0.22^{b}	5.63±0.45^{a}
Non-grafted plant (scion)	275.33± 3.78^{b}	19±0.68^{a}	5.26±0.54^{a}
Siriana F1 cv.			
Grafted plant	194±2.78^{a}	29±0.89^{a}	5.6±0.24^{a}
Non-grafted plant (scion)	178±2.96b	27±0.78^{a}	4.9±0.33^{b}

The effects of plant grafting on the quality parameters of varieties grown in an ecological system

From Figures 3, 4 and 5 it can be seen that the rootstock did not significantly influence the quality indicators, these being rather a characteristic of the variety and less influenced by the rootstock. Results similar to our study on reported in a recent study on the effects of rootstock on quality indicators and certain organic acids were reported by Doltu (2017). Tartaric acid was reduced in grafting, regardless of the variety, while the content of dry matter and malic acid was high, although the differences are not significant.

According to studies reported by Fernandez-Garci et al. (2004), grafting could be a useful tool for increasing tomato fruit quality by increasing the sugars and acid composition of tomatoes.

Some studies claim that the right choice of the rootstock used in tomato culture can lead to significant increases in the content of sugar and titratable acids in the fruit (Flores et al., 2010; Schwarz et al., 2013).

In this study, even if in the case of the grafted plants the SSC (% Brix) values were higher than in the case of the control variant, they were insignificant.

A higher content of SSC was evident in the case of the Costate 21 variety in the grafted variant (4.91% Brix). These increases could be more due to organic fertilization and less could be attributed to the rootstock.

These increases could be due, on the one hand, to organic fertilization, but it is obvious that they can also be attributed to the rootstock. János Ágoston (2017), states that tomatoes intended for consumption in a fresh state should register values of content in dry matter between 3.5-4.5% Brix, and tomato varieties intended for industrialization must exceed the value of 5% Brix.

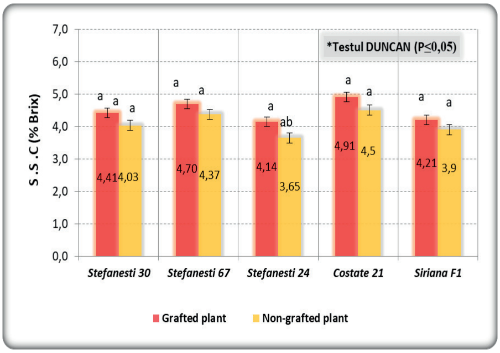

Figure 3. Rootstock influence on SSC (% Brix)

Of the total soluble solid content, which represent 75% of the total dry substance, 15% are organic malic and tartaric acids and approximately 10% are represented by sugars, the major constituents providing the nutritional value (Sima et al., 2010; Sumedrea et al., 2021). In general, fruit organic acid content was similar in grafted and ungrafted tomatoes.

Regarding malic acid (%), it was higher in the grafted plants, except for the Ștefănești 30 and

Ştefăneşti 67 varieties, which presented higher values in the control variant (plants grown on their own roots), although the differences are not significant.
Those studies highlight that certain rootstocks used for grafting tomatoes can affect the characteristics of fruit quality Savvas et al. (2011). Tomato cultivar and fruit harvest maturity were the main factors affecting the nutritional value of tomatoes (Erba et al., 2013).
Studies developed by Huitrón-Ramírez (2009) concluded that portaltoi induced a greater firmness in tomatoes without changing the quality of the fruits (Huitrón-Ramírez et al., 2009).

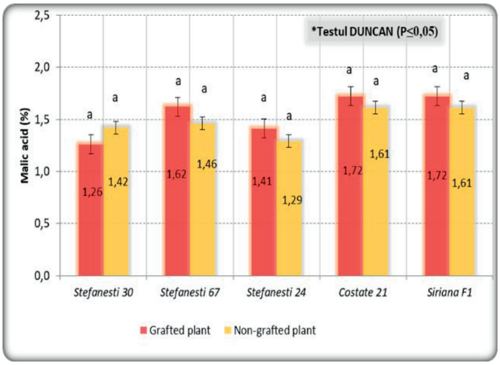

Figure 4. Rootstock influence on MA (%)

Table 5 shows the influence of grafting on the citric acid content of the fruit. As in the case of the other organic substances, citric acid had similar and insignificant values between grafted and non-grafted plants, regardless of the variety.

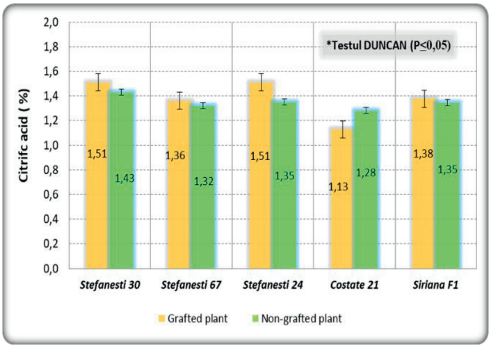

Figure 5. Rootstock influence on CC (%)

Higher CA (%) values were evident in the case of the Ştefăneşti 30 and Ştefăneşti 67 varieties grown on rootstocks (Figure 5). Results similar to our study were also reported by Sayvas, in a study in which he used several valuable tomato genotypes as rootstocks (Sayvas et al., 2017).

CONCLUSIONS

The rootstock induced greater vigor in the cultivars analyzed, more vigorous roots, and a greater number of roots and positively influenced the yield quality in the cultivars grown in the greenhouse in organic culture.
S. sysimbrifolium can be recommended for organic production due to its higher performance. We can conclude that grafting could be used in organic culture, in our study the rootstock induced a production increase of up to 2.2 kg per plant.
The rootstock induced an increase in both average fruit mass and fruit production. During the experiment, the rootstock induced a production increase of 2.2 kg per plant in the Ştefăneşti 30 variety, 1.0 kg per plant in the Ştefăneşti 67 variety, and 1.43 kg in the Ştefăneşti 24 variety. The Ştefăneşti 30 variety recorded productions of 6.6 kg/plant in the case of plants grafted on the S rootstock, compared to 4.4 kg/plant, as shown in the control variant.
We can state that the rootstock used in our study for the 5 autochthonous varieties of tomatoes positively influenced both the quality of the fruits, by increasing the values of SSC and malic acid. The results showed that the grafting of tomatoes on a suitable rootstock, such as *S. sysimbrifolium*, has positive effects on the morphological characteristics of the varieties, the quality of the yields and their quality, in ecological culture, compared to the ungrafted ones.
Nevertheless, it is recommended to continue the study for several years, to evaluate the influence of the rootstock on the tolerance of the varieties to the attack of the main pests, as well as on the quality and productivity indicators, cultivated in other environments.

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INFLUENCE OF OPAQUE WALL GREENHOUSE MICROCLIMATE ON MELON GROWTH AND FRUITING

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Abstract

Melon (Cucumis melo) belongs to the Cucurbitaceae family and is suitable for cultivation in various systems, such as cultivation in the field, solariums and greenhouses. The present study refers to the behavior of 3 hybrids grown in protected spaces, in the Chinese solarium of the Faculty of Horticulture in Bucharest, in the climatic and social context of 2021.3 hybrids of different origins were used: one Dutch (Antalya F1), one Chinese and one Korean. The culture was established by planting the seedlings, the technology specific to melons was applied in protected spaces. In the case of vegetative growth, but also fruiting, all hybrids had a favorable evolution, even if the temperature was higher than the limit cited in the specialized literature for Cucurbitaceae (35˚C). The plants showed very good vegetative growth, over 2.5 m high, formed between 11 and 19 shoots. From the point of view of fruiting, the hybrids had a production per plant between 0.91 kg for the Korean hybrid, and 3.22 kg for the Chinese hybrid. They stood out for their high SUS content and superior organoleptic qualities.

Key words: *Chinese solarium, hybrids, production protected culture.*

INTRODUCTION

The yellow watermelon (*Cucumis melo*, Fam. *Cucurbitaceae*) is among the most highly regarded vegetable species globally due to its aromatic and uniquely flavored fruits. It is cultivated on every continent, with the most extensive cultivation found in Asia, America, and Europe (FAO, 2022). In Romania, it is commonly grown in open fields. However, to obtain fruits earlier and ensure an extended consumption period, it is also cultivated in protected spaces, albeit on a much smaller scale. It is generally consumed fresh as a dessert fruit, in fruit salads, or combined with other foods; it can also be processed into juices, syrups, or jams and has health benefits due to its complex composition, especially carotenoid substances (Esteras et al., 2018). The fruits are rich in nutrients, especially provitamin A and vitamin C (Lester, 2008; Mohamed and Maha-Mohamed, 2016), and the content of phytonutrients is influenced by the variety and cultivation area (Singh et al., 2022). Carotenoid substances, besides their important role in human health, also play a role in plant development, photosynthesis, and photoprotection (Maoka, 2020). The accumulation of phytonutrients in fruits is influenced by the genetic characteristics of the variety, the stage of development, and environmental factors (Batkat et al., 2017; Bernillon et al., 2013). Light, in terms of quantity and quality, significantly influences the accumulation of phytonutrients in watermelons, especially carbohydrates (Yang et al., 2019). The type of soil, fertilization, and irrigation also influence the quality of watermelons (Jifon et al., 2012). The quality of the fruits is influenced by the cultivation technology, requiring the limitation of the use of pesticides and chemical fertilizers harmful to the environment. In this regard, Soare et al. (2018) have shown that using the biofertilizer Lignohumat at a rate of 200 g/ha resulted in yellow watermelon fruits with higher total dry matter content (TDS), acidity, and reducing sugars, while at a concentration of 100 g/ha, antioxidant activity increased. The potassium content in the soil influences the postharvest quality of the fruits, commercial appearance, and phytonutrient content (Lester et al., 2010). Additionally, to produce fruits with a low potassium content, demanded by consumers with kidney diseases, potassium can be reduced in the nutrient solution for soilless culture (Asao et al., 2013). Mulching the soil in greenhouses with biodegradable film improved the yield and quality of watermelons produced in the cold season (Wang et al., 2022). Using the natural predator *Phytoseiulus persimilis* to reduce mite infestation in yellow watermelons reduced the infestation rate to less than 1% (Calin et al.), resulting in superior quality fruits. To stimulate plant growth and root system, seed coatings from Brassica species have been tested with good results, whose effect can be enhanced by adding microorganisms from the genus Trichogramma (Galleti et al., 2015). The quality of watermelons also depends on the harvest time. To achieve acceptable quality, the soluble dry matter content at harvest should be above 9% (Calixto et al., 2022). Storing them while maintaining quality is quite difficult because they do not tolerate temperatures below 5-10°C depending on the variety, and during storage, firmness and carbohydrate content decrease significantly, posing a challenge for researchers (Saltveit, 2011).

MATERIALS AND METHODS

The experimental research was conducted in the Chinese-style greenhouse within the University of Agronomic Sciences and Veterinary Medicine, while the physico-chemical analyses and tasting session were carried out in the Vegetable Cultivation Laboratory of the Faculty of Horticulture, Bucharest. The cultivation of yellow watermelons was established on April 2, 2022, using three hybrids of yellow watermelon: V1 - Antalya F1, a vigorous hybrid with high resistance to Fusarium wilt and moderate resistance to powdery mildew.

The fruits have a medium size, with a yellow, textured skin covered by a network of healed cork. The flesh is crunchy, green, and has a specific aroma. V2 - the Chinese hybrid, with medium growth vigor, forms spherical, orange, pubescent fruits with concentric rings around the stem when reaching harvest maturity.

Figure1. Antalya F1

Figure 2. Chinese hybrid

The flesh is orange-colored, juicy, and sweet, while the seeds are small. V3 - the Korean hybrid, with medium growth vigor, bears small-sized, pear-shaped fruits with pronounced stripes and yellow skin.

Figure 3. Korean hybrid

The pulp is firm, juicy, sweet, and aromatic, with a yellowish-white color, and small seeds. The experiment was organized in subdivided plots with three repetitions per variant and 5 plants per repetition.

The Chinese-style greenhouse is oriented in an east-west direction, with plant rows aligned north-south. It has a vertical, opaque wall on the northern side, while the opposite side is made of transparent polyethylene film, semi-circular and sloping towards the south, ensuring favorable exposure to sunlight for the plants. The ends of the greenhouse are fixed, with one having an access door, which also aids in air ventilation within the growing space.

The cultivation was initiated on April 2nd using seedlings produced in a warm greenhouse, aged 42 days, with a spacing of 0.8 meters between rows and 0.5 meters between plants within rows. The cultivation was mulched with gray agrotextile placed on the ground after installing the drip irrigation system and before planting the seedlings.

The land was prepared according to specific technology for establishing crops in protected spaces, with the specification that before planting, fertilization with Italpolina fertilizer at 2 t/ha was applied along the row, incorporating it into the soil. This fertilizer contains 4% total nitrogen, 4% phosphorus pentoxide, 4% potassium oxide, 0.5% magnesium oxide, and 41% organic carbon, ensuring a balanced nutrition regime for the plants in the first weeks after planting.

During the vegetation period, specific care activities for yellow watermelon cultivation in protected spaces were performed without interventions on plant height growth. Weekly measurements were taken regarding plant height and the number of shoots per plant. At harvest, the number of harvested fruits per plant, average, production, fruit pericarp firmness using the FT 327 penetrometer with an 11 mm^2 cylinder (piston, probe) on 5 fruits/hybrid from 3 points of the fruit, soluble dry matter content (ºBrix) determined with the HI96800 digital refractometer by analyzing fruit juice on 5 fruits/hybrid with 3 determinations/fruit were determined.

The obtained fruits were also organoleptically analyzed during a tasting session at the Vegetable Cultivation Laboratory. Twelve individuals, students, and faculty members participated, each completing a tasting form. Standard deviation was calculated for the performed determinations, and correlation coefficients were calculated to determine the influence of growth on fruiting.

RESULTS AND DISCUSSIONS

The comparative analysis of the three hybrids of yellow watermelons, originating from different sources, showed that they behaved differently in terms of vegetative growth and productivity under the same technological conditions. Regarding plant height, it was found that the Antalya hybrid exhibited the highest vigor, with the stem reaching 4.1 m at the end of the vegetation period and the highest weekly growth increment, ranging between 0.15 m and 0.37 m. The Chinese and Korean hybrids had a weaker vigor, with stems reaching approximately 2.6 m in height and a weekly growth increment ranging between 0.05 m and 0.10 m for the Chinese hybrid and between 0.03 m and 0.08 m for the Korean hybrid (Table 1).

Table 1 Stem Growth Dynamics in Some Yellow Watermelon Hybrids

Hibrids	Date	Stem height (m)	Growth Increment (m/week)
Antalya F1	29.05	2.90	-
	5.06	3.27	0.37
	12.06	4.20	0.93
	18.06	5.00	0.80
	26.06	5.75	0.75
	3.07	6.20	0.45
Chinese	29.05	2.25	-
	5.06	2.35	0.10
	12.06	2.40	0.05
	18.06	2.50	0.10
	26.06	2.55	0.05
	3.07	2.60	0.05
Korean	29.05	2.40	-
	5.06	2.40	0
	12.06	2.43	0.03
	18.06	2.50	0.07
	26.06	2.57	0.07
	3.07	2.65	0.08

The yellow watermelons are characterized by a high capacity for lateral shoot formation, which is a particularity of plants in the Cucurbitaceae family. In the analyzed hybrids, the number of lateral shoots ranged from 11 in the Chinese hybrid to 19 in the Antalya hybrid, with the Korean hybrid having 15 (Figure 4). The presence of lateral shoots on the plant is important because female flowers form on these shoots, and their appearance can be influenced through repeated pruning techniques in cultivation technology.

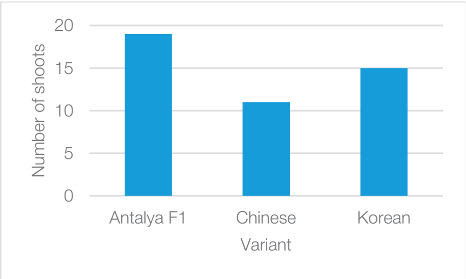

Figure 4. Lateral Shoot Capacity

In the specific conditions of the Chinese-style greenhouse, the yellow watermelon fruited well (Table 2), with the recorded productivity elements indicating this aspect. Analyzing the average weight of fruits, it was observed that the Antalya and Chinese hybrids had average fruit weights of 992 g and 946 g, respectively, while the Korean hybrid produced fruits weighing 416 g, being a hybrid with small fruits. The number of fruits harvested per plant, reaching commercial size, was similar for the three hybrids, with 2.4 fruits for the Chinese hybrid and 2.2 fruits for the other hybrids. The fruit production per plant recorded different values, being greatly influenced by the biological characteristics of the hybrids, and implicitly by the average fruit weight. The lowest production, 0.915 kg/plant, was obtained with the Korean hybrid, which has small pear-shaped fruits, compared to hybrids and varieties of yellow watermelons cultivated in Romania. For the Antalya and Chinese hybrids, the production was similar, with 2.18 kg/plant and 2.27 kg/plant, respectively. Reporting the yellow watermelon production per square meter, the Chinese hybrid was the most productive with 5.67 kg/m², followed by Antalya F1 with 5.45 kg/m², while the lowest production was obtained with the Korean watermelon, 2.29 kg/m².

Table 2. Productivity elements in the analyzed hybrids

Hibrids	Number of Harvested Fruits per Plant		Average Fruit Weight, g		Average Production per Plant		Average Production in	
	(pieces)	Standard deviation	(g)	Standard deviation	(kg)	Standard deviation	(kg/sm)	Standard deviation
Antalya F1	2.2	0.2449	992	2.4494	2.18	0.0244	5.45	0.0244
Chinese	2.4	0.3511	946	4.0824	2.27	0.0163	5.67	0.0163
Korean	2.2	0.3741	416	1.6329	0.915	0.040	2.29	0.0408

At harvest, the yellow watermelon fruits were quite firm, and the values obtained for firmness were in line with data presented in specialized literature. Determinations conducted by Haiyong Zhao et al., 2023, on yellow watermelon fruits showed that the pericarp firmness ranged between 4 and 6 kgf/cm², which is close to the values obtained in this study (4.3-5.8 kgf/cm²) (Figure 5), while the soluble dry matter content ranged between 8.64% and 10.85%, compared to a soluble dry matter content of 11.1% for the Korean hybrid and 7.6% for the Chinese hybrid cultivated in the Chinese-style greenhouse in Romania (Figure 6).

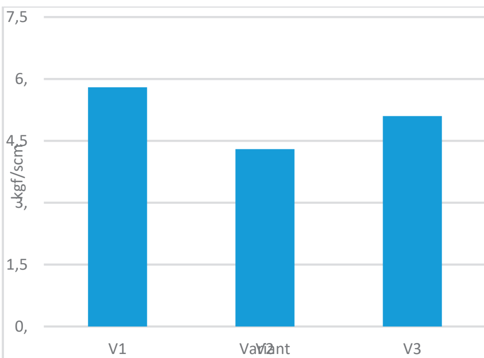

Figure 5. Pulp Firmness

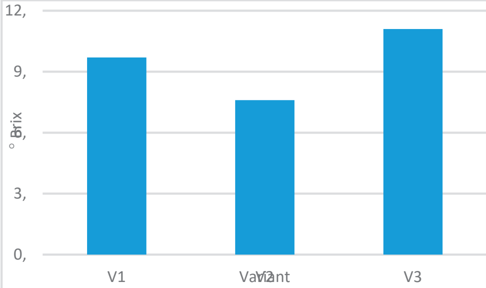

Figure 6. Soluble Dry Matter Content

The sensory evaluation of the fruits showed that the Antalya hybrid had superior quality characteristics compared to the other two hybrids, expressed through size, pulp color, taste, aroma, and soluble dry matter content (Figure 7).

Figure 7. Fruit Quality Indicators

By correlating plant growth data with those related to productivity, it was found that a higher vegetative growth slightly negatively influences production (Figures 8 and 9), but it has a beneficial effect on firmness and soluble dry matter content. Leaf area growth also increases the capacity for synthesis and the storage of organic substances (Figures 10 and 11).

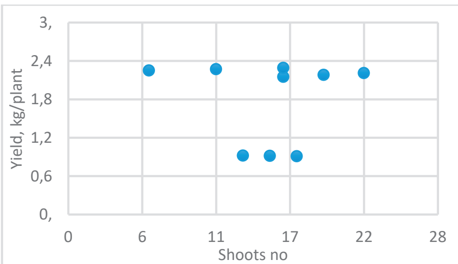

Figure 8. The correlation between the number of lateral shoots and production per plant (r = 0.029)

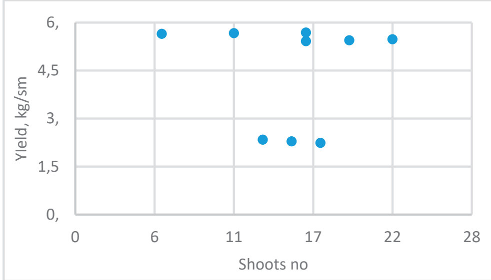

Figure 9. The correlation between the number of lateral shoots and production per square meter (r = - 0.04)

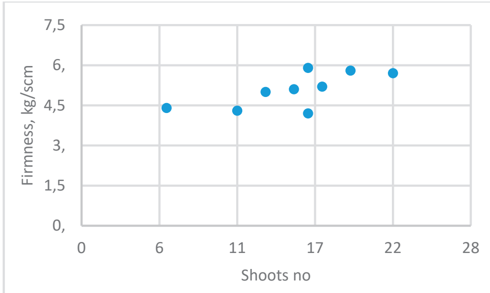

Figure 10. The correlation between the number of lateral shoots and firmness (r = 0.69)

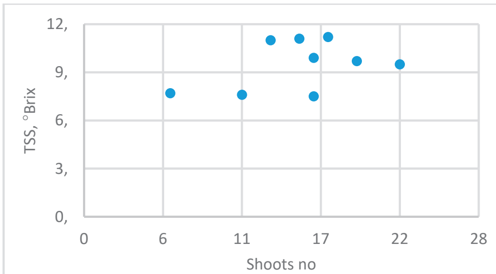

Figure 11. The correlation between the number of lateral shoots and soluble dry matter content (r = 0.41)

CONCLUSIONS

The different behavior of the yellow watermelon hybrids was due to their biological characteristics rather than the growing conditions. The hybrid with the highest vegetative growth was Antalya, while the Chinese and Korean hybrids reached similar values in terms of stem growth and number of lateral shoots. These hybrids are productive, with the highest production achieved by the Chinese hybrid at 5.67 kg/m², closely followed by Antalya F1 at 5.45 kg/m², while the lowest production was recorded for the Korean hybrid, which has smaller fruits, at 2.29 kg/m². The fruits are of very good quality, appreciated by consumers, with the highest score obtained in the tasting session by the Antalya hybrid with 32.3 points, while the Chinese and Korean hybrids scored 27.3 and 25.1 points, respectively. This recommends them to Romanian consumers for diversifying the assortment of yellow watermelons on the Romanian market, especially during the summer and beyond.

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STUDY ON THE MEDICINAL APPLICATIONS OF *MOMORDICA CHARANTIA* SPECIES: A REVIEW

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Abstract

Natural products are useful in the treatment of many human ailments as well as in the process of finding new drugs. In general, medications made from naturally occurring products are safer, less expensive, easier to get, and more effective than pure manufactured medications for treating a variety of illnesses. A bitter melon's abundance of bioactive substances, including as saponins, alkaloids, polypeptides, minerals, and vitamins, can help prevent and treat a number of illnesses, including diabetes, cancer, kidney stones, diabetes mellitus, stomach pain, tumor growth, and fever. Steroid saponins, the primary component of BM known as charantin, function similarly to peptides and certain alkaloids in regulating blood sugar levels. By controlling blood cholesterol, M. charantia's therapeutic qualities help reduce cardiovascular diseases including atherosclerosis. tannins are examples of secondary metabolites. Alkaloids, flavonoids, and tannins are only a few of the secondary metabolites with antibacterial qualities found in both fresh and dried leaf extracts of bitter melon. It was demonstrated that the phytochemical content of both leaves exhibited antibacterial action against a range of bacteria, including Bacillus, Streptococcus, E. Coli, Pseudomonas aeruginosa, and Salmonella.

Key words*: Momordica charantia, bitter melon, antidiabetic, wound healing, antimicrobial, anticancer.*

INTRODUCTION

Momordica charantia is a plant used in herbal medicine and as food. It is also known as bitter guard or bitter melon. The scientific name Momordica comes from the Latin meaning "to bite", which refers to the leaves' pointed edges. Momordicinso is a bitter chemical found in every part of the plant, including the fruits. It tastes incredibly bitter. The plant is found in tropical regions such as Thailand, tropical Africa, America, Malaya, Bangladesh, India, China, and the Middle East. *Momordica charantia* contains several physiologically active phytochemicals, including proteins, triterpens, saponins, flavonoids, steroids, alkaloids, and acids. The plant possesses anti-tumorous, anti-fungal, anti-parasitic, anti-cancer, antiviral, anti-fertility, antibacterial, and hypoglycemic properties due to its abundance of phytochemicals. Traditional medicine uses fruits and leaves to treat a wide range of conditions, including worms, gout, rheumatism, colic, and liver and spleen disorders. Because Momordica contains peptides and alkaloids that resemble insulin and charantin, a class of steroidal sapogenins, it possesses hypoglycemic qualities.

BOTANICAL INFORMATION

Momordica is a thin, climbing, monoecious plant that grows in the axils of leaves and has long, stalked leaves. It has distinct male and female flowers that are colored yellow. It belongs to the family Cucurbitaceae. The 4- 10 cm single or alternating leaves have 3-6 deeply separated lobes, and the tendrils are either unbranched or have two branches. Fruits have an oval, small-cucumber-like, ellipsoidal, or spindle shape. They are packed with flat, pulp-filled seeds that typically have a ridged surface. The seeds either dehisce irregularly into a fleshy capsule or they are indehiscent. When the fruits are young, they are green in color; as they develop, they become orange or yellow. The fruit splits into three uneven valves and folds backward, releasing a large number of white or reddish-brown seeds that are covered in reddish-purple arils. The seeds and pith of unripe fruits are white, whereas those of maturing fruits are red. Flowers: Single flowers have a shallow hypanthium, five lobed calyx, five yellow petals, one to three incurved scales at the base, and broad filaments with three stamens typically inserted toward the base of the hypanthium; pistillate flowers are usually solitary on a bracteate scape; anthers are distinct or coherent, with two of them being dithecal and the other monothecal; and curved or flexuous cells. With multiple horizontal ovules, three stigmas, two lobed stigmas, and either no staminodes or three, the hypanthium's perianth, which forms an oval to spindle, is frequently smaller than that of staminate flowers. There are a few to many ovate seeds that are usually carved.

ACTIVE CONSTITUENTS

Bitter melon has 91.8% water, 1.4% fiber, 0.20 percent fat, and 4.2% carbs. It is an edible vegetable and a naturally occurring medicinal plant. Albumin makes up 49.3% of the protein makeup, globulin 29.3%, and glutelin 3.1% (Farhan Saeed et al., 2018).

The fatty acid profile of the seeds indicates that they include 3.3% of monounsaturated fatty acids and 36.71% of saturated fatty acids. Additionally, the seeds account for 35% to 40% of the oil extracted from them. Sixty percent is the highest concentration of polyunsaturated fatty acids (PUFA) in bitter guard. The principal constituents of fruits include β-sitosterol-β-D-glucoside, momordicoside G, momordicoside F1, momordicoside F2, momordicoside I, momordicoside K, momordicoside L, momordin, stigmasta-5, momordicin, and 25-dien3-β-O-glucoside (Chekka et al., 2020).

α-eleostearic acid (54%), a conjugated linolenic acid, is remarkably important in PUFA (Grossmann et al., 2009; Liu et al., 2010).

The highest concentrations of minerals in BM may be found in the fruit and leaves, including potassium, sodium, calcium, phosphorus, and magnesium. Naturally, seeds are the best source of zinc (45.45 mg/100 g) and chromium (5.65 mg/100 g). Momordica has a wide range of physiologically active chemicals, including two groups of saponins: triterpenoids of the oleanane and cucurbitane types (Popovich et al., 2020).

Approximately 20-25 phytochemical components, accounting for 91% of the oil applied. The oil's primary constituents are cisdihydrocarveol, trans-nerolidol apiole, and germacrene (Mesia et al., 2008; Ahmad et al., 2012).

Alpha-electostearic acid is rich in oil, has potent blood fat-lowering qualities, and acts as an anti-inflammatory and anti-cancer agent by inhibiting the growth of tumor cells. Minerals, especially zinc and cr, have a significant impact on proteins or polysaccharides and can predict the development of hyperlipidemia, hyperglycemia, and hypercholesterolemia (Hsu et al., 2011).

A wide range of health-promoting phytochemicals, Leafy greens, fruits, and seeds are rich in many compounds, such as steroidal saponins, volatile oils, resins, vitamins, polypeptides, minerals, and alkaloids. Standard components include p-insulin, which is a polypeptide found in nature, momordicine alkaloid, and steroidal saponin, or charantin (Tan et al., 2014).

Bitter melon fruit pulp has no free pectic acid and only soluble pectin. Among the elements of BM include momordium, minerals, alkaloids, ascorbic acid, steroidal saponin glucosides, and carantin. Phenolic components, epicatechin, catechin, gentisic acid, chlorogenic acid, and gallic acid are all present in Momordica extract (Horax et al., 2010).

MEDICINAL USES OF *MOMORDICA CHARANTIA*

Potassium, calcium, manganese, magnesium, zinc, iron, phosphorus, and dietary fiber are among the minerals that are abundant in Momordica, as well as nutrients like betacarotene, foliate, thiamine, and riboflavin. Momordica's strong antioxidant content is attributed to its flavonoid, phenolic, anthroquinone, terpene, isoflavone, and glucosinolate content, which collectively give it its bitter flavor (Snee et al., 2012).

The beta-carotene concentration of bitter gourd juice improves vision and lessens eye diseases, while regular usage of the juice increases bodily stamina and prevents chronic weariness. Bitter melon stimulates the digestive system, which increases acid output and helps alleviate dyspepsia (Ahmad et al., 1999).

Juice from bitter melon lowers blood sugar and controls insulin levels. The hypoglycemic quality of bitter guard is attributed to a combination of phytochemicals called charantin, alkaloids, and insulin-like peptides, which also improve without increasing blood insulin levels, glucose tolerance. These living ingredients help with glucose absorption, fuel metabolism regulation, AMPK protein activation, and all other diabetic-related activity impairments. Bitter melon increases the number of beta cells in the pancreas that release insulin. Numerous research have demonstrated the anticancer, anti-inflammatory, and anti-diabetic properties of bitter gourd. Some pharmaceutical companies have included this fruit in their formulae as a result (Jayasooriya et al., 2000).

Bitter gourd juice protects against jaundice and strengthens the liver. In addition to nourishing the liver and aiding in hangover recovery, bitter melon juice detoxifies (Murray et al., 1995).

It is hypothesised that in cancer patients, bitter melon can enhance immune cell function and modify immunity. It is also used to treat cancer, psoriasis, malaria, tumors, high cholesterol, flu, and fever (Leatherdale et al., 1981).

The fresh juice of bitter guard leaves was used to treat diarrhoea and treat cholera in its early stages.

ANTIDIABETIC ACTIVITY

Momordica charantia contains a variety of phytochemicals, including charantin, polypeptide-p, plant insulin, vicine, karavilosides, and glycosides. These substances operate as hypoglycaemic agents by promoting the liver, muscles, and fat cells' production of glycogen and increasing their uptake of glucose (Chekka et al., 2020).

A polypeptide found in fruits and seeds called TP-insulin helps treated rats' blood sugar levels fall and return to normal. Bitter melon contains lectin, a bioactive substance that functions similarly to insulin. It functions similarly to insulin by connecting the two receptors for insulin. Lectin functions similarly to insulin in peripheral tissues and lowers blood glucose levels. Lectin is crucial to the hypoglycemic impact that results from eating bitter guard. Charantin's alcoholic extract it is a potent hypoglycemic medication used to treat diabetes and lower blood sugar levels. It is composed of a mixture of steroids. By encouraging the production of insulin, which in turn promotes the uptake of glucose by cells, the bitter melon fruit improves the action of insulin. In diabetic mice, fruit extract (fresh and dried) lowered blood sugar levels. In rats with alloxan-induced diabetes, bitter gourd extracts exhibit hypoglycemic, anti-diabetic, hypolipidemic, and hepato-renal protective effects. By lowering capillary permeability at the arteriolar and capillary levels, bitter melon controls microvascular dysfunction, a typical diabetes consequence. Charantin, a hypoglycemic chemical, is a member of the steroidal saponins family, which is composed of a mixture of stigmasteryl glucoside and sitosteryl glucoside (1:1). When given orally or intravenously, candantin causes hypoglycemia in rabbits (Lotlikar et al., 1966).

P-insulin, another bitter guard hypoglycemic agent, is a polypeptide made up of 166 amino acids with a molecular weight of roughly 11,000 Da. Clinical research demonstrated that polypeptide-pZnCl2 had a blood sugar-lowering impact. When administered intraperitonially, pyrimidine nucleoside, A vicine that can be found in fruits and seeds might make rats hypoglycemic. Individuals with type 2 diabetes benefit from Momordica extracts high in charantin (Banerjee et al., 2019).

ANTI-MICROBIAL ACTIVITY

The elements included in fresh leaf extracts Multiple secondary metabolites of *M. charantia* have distinct medicinal uses. Secondary metabolites include flavonoids (anticarcinogenic, antioxidant, antiviral, and antihemorrhagic); and tannins (moluscicidal, antiviral, antimicrobial, and antitumoral). The antibacterial qualities of BM leaves are effective against *Bacillus*, *Streptococcus*, *Salmonella*, and *Pseudomonas aeruginosa*. and *Escherichia coli* (Brandao et al., 2016).

Various fresh leaf extractions shown efficacy against distinct strains of *B. cereus*, *S. aureus*, and *Escherichia coli*. Both the fresh and dried leaf extracts include a large number of secondary metabolites of different types, including as flavonoids, alkaloids, and tannins, which have a variety of biological activities, including antibacterial ones. Bitter melon seed extracts have antimicrobial properties that inhibit the growth and infection of gram-positive and gram-negative bacteria and viruses, including Shigella, Salmonella, H. pylori, Escherichia coli, Pseudomonas, Streptococcus, and H. pylori and Streptobacillus, as well as parasitic organisms like Plasmodium falciparum and Entamoeba histolytica. Potential chemotherapeutic possibilities against leishmaniasis include the bioactive compounds found in bitter melon (Gupta et al., 2010).

ANTI-MALARIAL ACTIVITY

A naturally occurring medicinal herb called *Momordica charantia* is used to cure and prevent malaria. Historically, people from Asia, Panama, and Colombia have all thought that bitter
guard guards against malaria. Malaria is treated using tea made from leaves that have been boiled in water. Laboratory studies have demonstrated the antimalarial activity of some Momordica variants (Olasehinde et al., 2014).

ANTIOXIDANT ACTIVITY

Antioxidants are chemicals that help lessen or stop the harm that free radicals do to cells. High levels of antioxidant activity. are present in the ethanolic extracts of bitter melon such as phenolic substances (Aljohi et al., 2016; Qader et al., 2011).

Inhibiting stress-induced lipid peroxidation, bitter guard lowers glutathione levels and improves catalase activity. There is antioxidant action in the phenolic chemicals that are isolated from bitter melon. The antioxidant-rich *Momordica charantia* seeds successfully restore the reduced antioxidant status in streptozotocin-induced diabetic rats (Sathishsekar et al., 2005).

ANTI-TUMOR PROPERTIES

Bitter gourd inhibits the growth of tumor cells and has anti-carcinogenic properties. Anti-carcinogens or chemopreventive agents are present in BM. In a study using rat models, bitter guard water extract can stop the progression of prostate cancer. The whole plant's extract in hot water prevented the growth of tumor cells in the mammary glands of mice. Bitter melon has been shown to have anti-leukemic and anti-cancerous properties in a number of *in vitro* experiments using a wide range of cell lines. These studies include melanoma, liver cancer, solid sarcomas, and human leukemia (Fang et al., 2012) (Grover et al., 2004).

In cancer patients, bitter gourd is a potent immunomodulator that enhances immune cell function. Bitter melon extracts from the fruit and seeds stop the growth of several cancer cell lines, such as those from metastatic breast cancer, prostate adenocarcinoma, and human colon cancer. Research using MDAMB. 23140-41 *in vitro*.

HYPO-CHOLESTEROLEMIC ACTIVITY

Research on both normal and diabetic animals has shown that Momordica charantia has hypocholesterolemic effects. Rats fed sunflower for four weeks were given Momordica charantia seeds, which contained octadecatrienoic fatty acid. After four weeks, the rats' levels of nonenzymatic liver tissue lipid peroxidation, Erythrocyte membrane lipid peroxidation and plasma lipid peroxidation both decreased. For about 21 days, bitter gourd fruit and/or seeds were given to the diabetic rats. during which time their cholesterol and triglyceride levels returned to normal. Rats' blood and liver lipids are affected by bitter gourd oil (BGO) (Anilakumar et al., 2015).

ANTI-VIRAL PROPERTIES

Studies conducted *in vitro* have demonstrated the bitter gourd's antiviral properties against a variety of viruses, such as the Epstein-Barr, herpes, and HIV viruses (Bourinbaiar et al., 1995).

Bitter melon leaf extract stimulates natural killer cell activity, produces more interferon, boosts resistance to viral infections, and has an immunostimulant impact on animals. The proteins or glycoproteins that bitter gourd contains have antiviral properties. Because

bitter gourd absorbs poorly when taken orally, it may not be able to stop the spread of the virus in HIV-positive individuals. On the other hand, taking Momordica orally counteracted the side effects of anti-HIV medications. Bitter guard leaf extracts exhibit antibacterial properties against *Salmonella*, *Escherichia coli*, *Pseudomonas*, *Streptobacillus*, and *Streptococcus*. The whole plant extract exhibits antiprotozoal action against *Entamoeba histolytica* (Gupta et al., 2010).
Helicobacter pylori, the bug that causes stomach ulcers, is susceptible to the antibacterial qualities and activity of fruit and fruit juice.

LARVICIDAL ACTIVITY

Bitter Guard contains phytochemicals that may have larvicidal effects. Several investigations have been carried out targeting two mosquito vectors, namely *Anopheles stephensi* and *Culex quinquefasciatus*. (Balboa et al., 1992).

ANTI-GENOTOXIC ACTIVITY

Momordica charantia has antigenotoxic action that decreases the genotoxic effects of methylnitrosamine, tetracycline, and methanesulfonate, hence reducing chromosome breakage (Hussan et al., 2014).

ANTI-HELMINTIC ACTIVITY

M. charantia fruit, leaves, and seed extracts have been shown to have pharmacological activity against helminths. Aqueous extracts of Momordica are more effective than piperazine in treating *Ascaridia galli*. The anthelmintic saponins cause paralysis in the worms by inhibiting the function of the enzyme acetylcholinesterase, which ultimately results in death.

WOUND HEALING ACTIVITY

Momordica charantia fruit powder ointment is superior to the control group in a rat model with respect to wound contracting, wound closure time, epithelization period, tensile strength of the wound, and tissue regeneration at the wound site. It is comparable to a reference medication, povidone iodine ointment (Subhashchandra et al., 2010).
Bitter melon has a strong ability to heal wounds. Bitter melon contains phytochemicals, such as candelin, that promote the synthesis of growth factors, cause fibroblasts to proliferate, and quicken the wound's oxygenation and capillary circulation. The antimicrobial and antioxidant qualities of phytochemicals like flavonoids and glycosides speed up the healing process. Momordica has a positive impact on how quickly wounds heal. In addition, it accelerates the rate of wound contraction, accelerates wound closure, and increases wound tension.

CONCLUSIONS

For many years, *M. charantia*, a naturally occurring dietary supplement, has been utilized as an ethnomedical treatment for a range of ailments, such as diabetes, cancer, inflammation, and other conditions. Worldwide research has been done on *M. charantia's* natural therapeutic qualities, which include antioxidant, antimalarial, anticancer, antibacterial, and antidiabetic effects. A plant with various uses, bitter guard has the ability to treat practically every illness. The plant has a number of therapeutic components that can function collectively or individually to produce its therapeutic effects. Phytochemicals like charantin, insulin-like peptide, and alkaloid-like extracts have hypoglycemic properties in relation to diabetes, much like the plant or its pure extracts do. Numerous compounds present in plant extracts possess the capability to modulate various systems implicated in the supervision and therapy of diabetes mellitus. *M. charantia* is a workable and naturally occurring choice for people who are more likely to develop diabetes.

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STUDY ON THE EFFECT OF SOME PRODUCTS FOR FOLIAR APPLICATION ON THE PRODUCTIVITY AND ESSENTIAL OIL CONTENT IN CORIANDER SEEDS (*CORIANDRUM SATIVUM* L.)

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Abstract

The field trial was carried out in the period 2015-2017 on alluvial-meadow soil type on the territory of the village of Voivodinovo – Central-South Bulgaria. The experiment was carried out with coriander of Yantar cultivar. The following foliar fertilizers were included in the study at the respective rates: Variant 1 – Humustim – 1 l/ha; Variant 2 – Maxgrow – 5 l/ha; Variant 3 – Tecamine vigor – 1.5 l/ha; Variant 4 – Yara Tera kristalon blue – 2.5 kg/ha; Variant 5 – Poly Plant – 1 kg/ha. In order to follow out the effect of those products on the elements of productivity, seed yield and essential oil content, the variants were compared to an untreated control (Variant 6). The results show: the structural elements of the yield, i.e. the number of umbels per plant, the number of seeds per plant, the seed weight per plant and the 1000 seed weight in the treated variants exceeded the untreated control by 4-15%, 5-15.2%, 3.2-15.4% and 6.5-12.5%, respectively. The highest seed yield was produced under the treatment of coriander with leaf fertilizer Yara Tera kristalon blue in dose of 2.5 kg/ha. An increase in the essential oil content from 2.8 to 9.4% was established after treatment with the foliar applied products compared to the untreated control.

Key words: *coriander, fertilizer, productivity, seed yield, essential oil.*

INTRODUCTION

Coriander is one of the major essential oil crops grown in Bulgaria. The application of suitable agrotechnical practices in its cultivation is a prerequisite for the realization of the productive potential of plants (Manhart & Delibaltova, 2022; Delibaltova, 2020; Mishra et al., 2017; Panda et al., 2007; Kehayov et al., 2005). The use of growth regulators stimulating plant growth and development is of great importance for increasing the seed yield and essential oil content, as well as the ability of the crop to overcome some abiotic stress factors (Kuri et al., 2017; Vinogradov et al., 2017). Those products have an impact by enhancing metabolism, by promoting nutrient uptake and helping to redistribute them in the body (Meena et al., 2015). They stimulate or suppress the physiological processes essential for plant growth and development. Unlike other nutrients, they are not feeding the plants but they have an effect on life processes, on growth rates and development, they coordinate the activities of the separate organs (Yugandhar et al., 2016). A number of studies show that the application of foliar fertilizers and growth regulators in coriander is an appropriate method to stimulate the biological potential of plants. As a result, higher values of the elements of productivity, seed yield and essential and ordinary oil content were reported compared to the control (Singh et al., 2017; Yugandhar et al., 2017; Haokip et al., 2016; Kolev et al., 2005). The results of those studies confirmed that coriander reacts positively to the applied growth regulators and foliar fertilizers, thus motivating further investigations on the crop.

The aim of the present study was to investigate the effect of some foliar fertilizers on the elements of productivity, seed yield and the essential oil content in Yantar coriander cultivar.

MATERIALS AND METHODS

The field trial was carried out in the period 2015-2017 on alluvial-meadow soil type on the territory of the village of Voivodinovo – Central-South Bulgaria. The experiment was set by the block-plot design method in four replications with a plot size of 15 m^2, after wheat as a predecessor. The experiment was carried out with coriander of Yantar cultivar.

The following foliar fertilizers and growth regulators were included in the study at the respective rates: Variant 1 – Humustim – 1 l/ha; Variant 2 – Maxgrow – 5 l/ha; Variant 3 – Tecamine vigor – 1.5 l/ha; Variant 4 – Yara Tera kristalon blue – 2.5 kg/ha; Variant 5 – Poly Plant – 1 kg/ha. They were applied at the end of buttoning and the beginning of flowering stage. In order to follow out the effect of those products on the elements of productivity, seed yield and essential oil content, the variants were compared to an untreated control (Variant 6).

The experiment was carried out following the adopted cultivation technology. The structural elements of the yield were determined after analysing 50 plants from one square meter.

Soil cultivation included ploughing in of the stubble in July and plowing at a depth of 20-22 cm in September, twice pre-sowing cultivation with harrowing, the last being at a depth of 5-6 cm. The phosphorus fertilizer was introduced before plowing at a rate of 80 kg/ha and the nitrogen fertilizer – with the last pre-sowing soil cultivation at a rate of 10 kg/ha. Every year sowing was carried out in the period 10-20 February at spacing between the rows 12-15 cm and a seed rate of 250 germinating seeds per m^2 at a depth of 3-4 cm. Weed control was achieved by treatment with the herbicide Linurex 45 SC – 2 l/ha, applied after sowing, pre-emergence of the crop. All the steps of the adopted coriander cultivation technology were respected.

The following characteristics were reported: number of umbels per plant, number of seeds per plant, seed weight per plant, 1000 seed weight, test weight, seed yield and essential oil content in seeds.

Data obtained for the values of the structural elements, the yield and the essential oil content were statistically processed by the method of dispersion and correlation analyses.

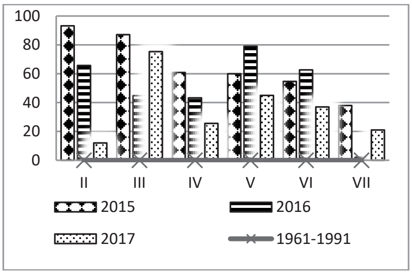

Figure 1. Rainfall, mm

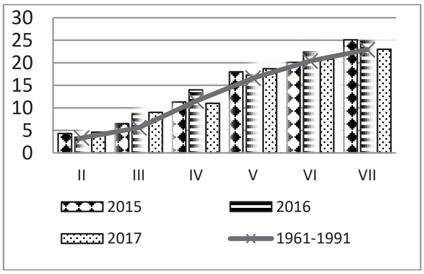

Figure 2. Average monthly air temperature, ^0C

Meteorological conditions during vegetation had an effect on the growth, development and productivity of coriander. Figures 1 and 2 present data about the amount of rainfall and the average monthly air temperature in the period February 2015 till July 2017. The results show that the air temperatures were close to or slightly higher than those established for a multiple-year period, with no significant deviations from the crop requirements. The differences between the three years of the study were established in the amount of rainfall during vegetation. The lowest amount of precipitation was reported in 2017 economic year – 215 mm versus 276.0 mm for a multiple-year period. That year was characterized by uneven distribution of rainfall, which was not enough to meet the plant requirements for water at the critical stages. In April, May and June at the stages of buttoning, flowering and fruit setting, the amount of rainfall was 107.6 mm versus 156 mm for a multiple-year period, i.e. about 31% less. That determined the third experimental year as less favorable for the productivity of coriander compared to the others.

The first year of the study (2015) was characterized by the greatest amount of rainfall during vegetation (383.9 mm). The reported amount was 107.9 mm above the climatic norm. Precipitation was evenly distributed during vegetation and in a combination with the reported temperature values, it was considered to be the most favorable for coriander cultivation of the three experimental years. In

2016, the total amount of rainfall was 295.7 mm and exceeded the values for the period 1961-1991 by 19.7 mm, however it was not very well distributed. At the beginning of vegetation (February-March) the rainfall was 33.5 mm above the norm, during the buttoning and flowering stages it was 24.5 mm more than the reported over a multiple-year period, and, during the ripening stage (July) rainfall was almost missing. This had a good effect on the seed yield and on the essential oil content.

Out of the three years of study, the most favourable for the coriander seed yield was 2015 and referring to the essential oil content, 2016 proved to be the best.

RESULTS AND DISCUSSIONS

The more favorable climatic factors in 2015 preconditioned the formation of a larger number of umbels per plant than in the other two experimental years (Figure 3). All the treated variants exceeded the untreated control by 2.1% to 12.4%. The largest number of umbels was reported in Variant 4 – 31.5, followed by Variant 2 – 30.0 and the smallest number was established in Variant 1 – 28.6. In the second experimental year, the number of umbels per plant was by 10% lower on average, compared to 2015 and the applied foliar fertilization increased the values of that characteristic to 13.5%.

The smallest number of umbels per plant was reported in the third year of the study (2017), ranging from 19.0 in the control to 25.0 in Variant 4. The treated variants exceeded the untreated control by 7.1 to 19.0%.

On average for the three-year period, the plants treated with Yara Tera kristalon blue at a rate of 2.5 l/ha formed the largest number of umbels – 28.4, followed by those treated with Maxdrow at a rate of 5 l/ha – 27.1 and the smallest number of umbels was established in the control variant – 24.7. The applied growth regulators increased the values of that indicator from 4.0 to 15%.

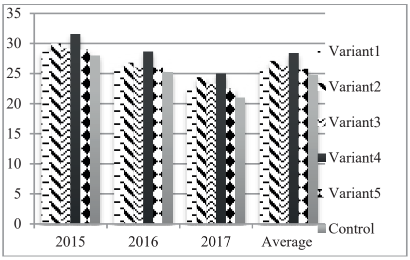

Figure 3. Effect of leaf fertilizers and growth regulators on number umbels per plant

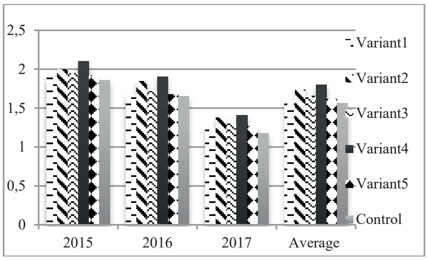

Figure 4: Effect of leaf fertilizers and growth regulators on seed weight per plant, g

The analysis of variance shows a strong statistically significant impact of both the studied variants and the years with their specific climatic conditions (Table 1). An interaction between Variant and Year was observed.

Table 1. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Variant**	126.4863	5	25.29725	43.01508	<0.001	2.38607
Year**	533.7325	2	266.8663	453.7756	<0.001	3.168246
Interactions**	23.0625	10	2.30625	3.921515	<0.001	2.011181
Within	31.7575	54	0.588102			

*F - test significant at P<0.05; **F - test significant at P<0.01; ns - non significant.

Table 2 presents data about the number of seeds per plant. The results confirm that the foliar applied products increased the values of that characteristic up to 14% in 2015, up to 13.3% and 18.4% in 2016 and 2017, respectively, compared to the untreated control.

On average for the years of study, the largest number of seeds per plant was formed in the variant of plant treatment with Yara Tera kristalon blue at a rate of 2.5 l/ha – 296 versus 257.0 in the control. All the treated variants exceeded the control by 13 to 39 seeds.

Table 3 presents the results of the analysis for the number of seeds per plant. Both the variants and the conditions during the year had the strongest effect on the variation of the characteristic. The interaction between the two factors was less expressed.

Table 2. Number of seeds per plant

	Years of study			Average for the period
	2015	2016	2017	
Variant 1	335 [b]	265 [b]	209 [b]	270
Variant 2	352 [d]	277 [d]	230 [d]	286
Variant 3	346 [c]	270 [c]	219 [c]	278
Variant 4	365 [e]	290 [e]	232 [d]	296
Variant 5	340 [b]	270 [b]	211 [b]	274
Variant 6	320 [a]	256 [a]	196 [a]	257

*Means within columns followed by different lowercase letters are significantly different (P<0.05) according to the LSD test.

Table 3. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Variant**	13883.24	5	2776.647	99.05133	0.00	2.38607
Year**	194335.4	2	97167.68	3466.262	0.00	3.168246
Interactions*	571.9722	10	57.19722	2.040396	0.05	2.011181
Within	1513.75	54	28.03241			

*F - test significant at P<0.05; ** F - test significant at P<0.01; ns - non significant.

An important characteristic determining the coriander seed yield is the seed weight per plant. The different climatic conditions during the years of the experiment, as well as the applied foliar products resulted in the formation of seed yield of different weight (Figure 4). The lowest values of that indicator were reported in the control – 1.09 g in 2017 when the climatic conditions were less favorable for the growth and development of coriander compared to 2015 and 2016. In 2017 the variants, in which the plants were treated with growth regulators, exceeded the untreated control variant by 6.8 to 19.5%.

Table 4. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Variant**	2.68844	5	0.537688	50.1709	<0.001	2.38607
Year**	13.087	2	6.543501	610.5647	<0.001	3.168246
Interactions**	2.465431	10	0.246543	23.00458	<0.001	2.011181
Within	0.578725	54	0.010717			

*F - test significant at P<0.05; **F - test significant at P<0.01; ns - non significant.

During the first and third experimental years, the weight of the seeds of the plant in the treated variants exhibited a higher value in comparison to those of the control. Specifically, there was an observed rise in seed weight from 2.2% to 12.9% in 2015, and from 1.8% to 15.1% in 2017. On average for the period 2015-2017, the highest seed weight per plant was obtained in Variant 4 – 1.80 g (Yara Tera kristalon blue at a rate of 2.5 l/ha) and in Variant 2 – 1.74 (Maxgrow – 5 l/ha) versus 1.56 g in Variant 6 (control). The rest of the treated variants exceeded the control by 3.2 to 6.4%.

The results of the analysis of variance (ANOVA) about the effect of the factors and their interaction on seed weight per plant show a clear statistical significance in the changes of the characteristic and the interaction between the two factors was statistically significant (Table 4).

The 1000 seed weight in the first experimental year (2015) varied from 7.3 to 7.7 g in the treated variants versus 6.9 g in the control. Treatment with foliar fertilizers exceeded the untreated control by 8.7% on average (Figure 5).

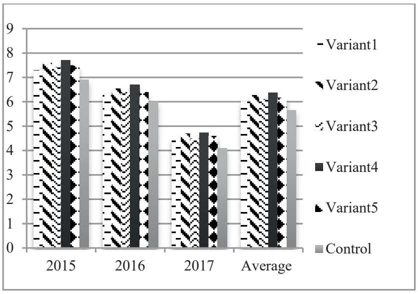

Figure 5. Effect of leaf fertilizers and growth regulators on 1000 seed weight

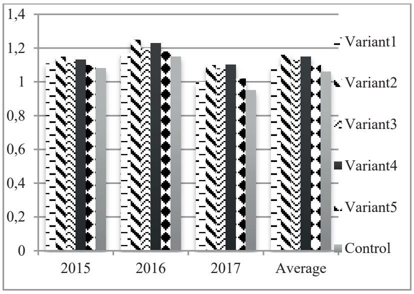

Figure 6. Effect of leaf fertilizers and growth regulators on essential oil content

In 2016, the 1000 seed weight ranged from 6.3 to 6.7 g in the treated variants versus 6.0 g in the control. On average for the study period, the highest 1000 seed weight was reported in the variant, in which the coriander was treated with Yara Tera kristalon blue at a rate of 2.5 kg/ha (6.37 g), followed by Variant 2 (6.28 g) and Variant 5 (6.17 g). An increase of 6.5% to 12.5% was reported in the treated variants compared to the control.

The results of the dispersion analysis about the influence of the factors and their interaction on the 1000 seed weight show clear statistically significant variations and the interaction between the two factors was statistically insignificant (Table 5).

Table 5. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Variant**	4.88684	5	0.977368	7.27933	0.00	2.38607
Year**	100.4047	2	50.20233	373.9014	0.00	3.168246
Interactions ^{ns}	0.438264	10	0.043826	0.326414	0.97	2.011181
Within	7.250375	54	0.134266			

*F - test significant at P<0.05; **F - test significant at P<0.01; ns - non significant.

The test weight is an indicator of the commercial quality of seeds and it plays an important role in determining the sale price. The results of the data analysis show that the values of that indicator were not affected by the applied foliar fertilizers. The effect of the year on the test weight was statistically significant but the interaction between both factors was insignificant (Table 6).

Table 6. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Variant ^{ns}	3.924444	5	0.784889	0.484001	0.79	2.38607
Year**	92.01694	2	46.00847	28.3711	0.00	3.168246
Interactions ^{ns}	12.05306	10	1.205306	0.743251	0.68	2.011181
Within	87.57	54	1.621667			

*F - test significant at P<0.05; **F-test significant at P<0.01; ns - non significant.

Data about the effect of the foliar applied products on yield seed depending on the meteorological conditions during the three experimental years, are presented in Table 7.

The results show that both the structural elements and the seed yield increased after the application of foliar fertilizers and growth regulators. The favorable combination of temperature and moisture during the vegetation period of coriander was a precondition for obtaining a higher seed yield in 2015 compared to 2016 and 2017. During the first experimental year, the values of that indicator in the treated variants ranged from 2340 to 2460 kg/ha versus 2190 kg/ha in the control. All the variants, in which the plants were treated with growth regulators, exceeded the untreated control by 6.8 to 12.3%, the difference being statistically significant. In 2016, the coriander seed yield was about 12% lower than in the previous year and the treated variants exceeded the untreated control by 9.2% on average.

In the last year of the experiment, the seed yield ranged from 1600 to 1685 kg/ha in the treated variants and in the control variant it was 1530 kg/ha.

Table 7. Seed yield, kg/ha

	Years of study			Average for the period
	2015	2016	2017	
Variant 1	2340 [b]	2070 [b]	1600 [b]	2003
Variant 2	2430 [d]	2140 [c]	1685 [e]	2085
Variant 3	2400 [c]	2120 [c]	1664 [c]	2061
Variant 4	2460 [e]	2190 [d]	1680 [e]	2110
Variant 5	2360 [b]	2100 [b]	1672 [d]	2044
Variant 6	2190 [a]	1950 [a]	1530 [a]	1890

*Means within columns followed by different lowercase letters are significantly different (P<0.05) according to the LSD test.

In the last experimental year the seed yield, obtained in the treated variants, was by 4.6 to 9.8% higher compared to the control.

On average for the study period (2015-2017), the highest yield was obtained in Variant 4 with application of Yara Tera kristalon blue at a rate of 2.5 kg/ha (2110 kg/ha); followed by Variant 2 with the application of Maxgrow at the rate of 5 l/ha (2085 kg/ha) and lowest yield of 1890 kg/ha was established in the control. All the variants treated with growth regulators exceeded the untreated control by 6.0 to 11.6%.

The results of the dispersion analysis about the effect of the factors Variant and Year, as well as their interaction, on the indicator seed yield, are presented in Table 8. The results show a statistically significant effect of the studied factors and an insignificant of their interaction.

Table 8. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Variant*	389423.6	5	77884.72	5.255084	0.00	2.38607
Year**	8072578	2	4036289	272.3389	0.00	3.168246
Interactions [ns]	232905.6	10	23290.56	1.571474	0.14	2.011181
Within	800325	54	14820.83			

*F - test significant at P<0.05; **F - test significant at P<0.01; ns - non significant.

In coriander cultivation, seed quality is determined by the essential oil content. That characteristic is influenced by both the years with different climatic conditions and the foliar applied products (Figure 6). Unlike the other studied characteristics, which were higher in the year with the highest and evenly distributed rainfall, the essential oil content is favoured by dry, hot weather, as well as by the lesser amount of precipitation during the seed-ripening stage.

The highest oil content was reported in 2016 when the temperature during the seed-ripening stage was 2.10 times higher than that for a multiple-year period and there was almost no rainfall. The essential oil content ranged from 1.17 to 1.25% in the treated variants and 1.15% in the control, i.e. by 1.7 to 8.7% higher. In the other years of the experiment, the applied foliar treatment increased the essential oil content by 2.8 to 6.5% and by 5.3 to 15.9% in 2015 and 2017, respectively, compared to the control.

On average for the period 2015-2017, the values of that characteristic in all the treated variants exceeded the untreated control by 2.8 to 9.4%. The highest effect of the foliar treatment was reported in Variant 2 and Variant 4 - when applying Maxgrow and Yara Tera kristalon blue products.

The dispersion analysis about the effect of the factors Variant and Year, as well as their interaction, on the essential oil content in seeds shows a significant influence of the factors on the changes of the characteristic and statistically insignificant effect of the interaction between them (Table 9).

Table 9. Analysis of variance ANOVA

Source of Variation	Sum of Square	df	Mean Square	F	P-value	F crit
Variant**	0.232907	5	0.046581	9.77137	0.00	2.38607
Year**	0.498003	2	0.249001	52.23298	0.00	3.168246
Interactions**	0.050831	10	0.005083	1.066272	0.40	2.011181
Within	0.257425	54	0.004767			

*F - test significant at P<0.05; **F - test significant at P<0.01; ns - non significant.

As a result of the correlation analysis between the structural elements, seed yield and essential oil content (Table 10), a very strong correlation (r > 0.9), p < 0.05 was found between the following indicators: number of seeds per plant and seed yield; number of seeds per plant and number of umbels per plant; 1000 seed weight and seed yield; 1000 seed weight and number of umbels per plant; 1000 seed weight and number of seeds per plant.

Table 10. Values of the coefficient of correlation

	Yield seed	Number umbels	Number seed	Weight seed- g	Mass 1000 seeds-g	Test weight	Essential oil
Yield seed	1.000						
Number umbels	0.867	1.000					
Number seed	0.911	0.924	1.000				
Weight seed - g	0.807	0.802	0.868	1.000			
Mass 1000 seeds	0.908	0.902	0.921	0.853	1.000		
Test weight	0.678	0.557	0.633	0.523	0.627	1.000	
Essential oil	0.297	0.372	0.218	0.278	0.344	0.084	1.000

High positive values of r (r > 0.8 and r > 0.6) were reported for seed yield and number of umbels per plant; seed weight per plant and seed yield; seed weight per plant and number of umbels per plant; seed weight per plant and number of seeds per plant; 1000 seed weight and seed weight per plant; test weight and seed yield; test weight and 1000 seed weight; test weight and number of seeds per plant. Mean correlation was found between the indicators: test weight and number of umbels per plant (r = 0.557), p < 0.05; test weight and seed weight per plant (r = 0.523). Weak correlation (r < 0.3) was observed between the essential oil content of seeds and all the other indicators.

CONCLUSIONS

The studied products for foliar application increased the productivity and the essential oil content in coriander seeds of Yantar cultivar.

The structural elements of the yield, i.e. the number of umbels per plant, the number of seeds per plant, the seed weight per plant and the 1000 seed weight in the treated variants exceeded the untreated control by 4-15%, 5-15.2%, 3.2-15.4% and 6.5-12.5%, respectively.

The foliar fertilizers applied to coriander did not exert an effect on the test weight.

On average for the experimental period (2015-2017), the increase in seed yield after the foliar application of the products, ranged from 6.0 to 11.6% compared to the control. The highest yield was reported in the variant treated with Yara Tera kristalon blue at the rate of 2.5 kg/ha – 2110 kg/ha.

An increase in the essential oil content from 2.8 to 9.4% was established after treatment with the foliar applied products compared to the untreated control. The highest values were obtained when using foliar fertilizers Maxgrow – 1.16% and the Yara Tera kristalon blue – 1.15%.

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STUDY ON THE EFFECT OF NUTRIENT CONCENTRATION ON QUALITY PARAMETERS FOR LETTUCE GROWN ON VARIOUS SUBSTRATE TYPES

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Abstract

The study was carried out in the research greenhouse, within the Horticultural Products Quality Research Center in 2021. On the mattresses filled with perlite, 2 varieties of lettuce were grown, oak leaf type, Kineta type, and Lollo Bionda type, variety Lugano. The nutrient solution was administered in 3 EC concentrations, respectively 1.5 mS, 2.5 mS and 3.5 mS. Three pH levels were used for each EC type. Differences were found between the experimental variants regarding the reaction of the varieties to these treatments. The aim of the study was to see the influence of nutrient solution concentration on some production and quality parameters of lettuce grown on perlite substrate.

Key words: *lettuce, soilless, perlite, conductivity*.

INTRODUCTION

Lettuce (*Lactuca sativa*) is an annual plant from the Asteraceae family, native to temperate regions of Europe and Western Asia. Its cultivation began in ancient Egypt, from where it spread to ancient Greece and Rome, becoming an essential part of the Mediterranean diet (Indrio and Motta, 2000; Janick, 2002). According to De Vries (1997), the species evolved from a wild plant to one of the most widely cultivated vegetables in the world. The spread of lettuce has been influenced by cultural, geographic, and agricultural factors (Doležalová et al., 2003; Lebeda et al., 2003; Chan et al., 2023). Numerous studies have investigated the direct impact of temperature on the metabolism and development of lettuce plants. For instance, recent studies by Velez-Ramirez et al. (2020) and Rana et al. (2019) have examined the physiological adaptations of lettuce plants to elevated temperatures. The optimal temperature for leaf growth and head formation is 16°C, while the formation of floral stems requires temperatures between 20 and 22°C. Under conditions of drought and insufficient light, the leaves become etiolated, heads fail to form properly, and those that do develop are of poor quality, being very loose (Ciofu et al., 2003; Drăghici, 2015; Drăghici and Jerca, 2022). Studies conducted by Park et al. (2021), Bantis et al. (2020), López-Gálvez et al. (2020), and Rivero et al. (2003) provide a detailed perspective on how temperature influences the growth and development of lettuce.

MATERIALS AND METHODS

The present study was carried out at the Faculty of Horticulture from USAMV Bucharest research greenhouses at the Research Centre for quality control of horticultural products.

The biological material used in the experiment consisted of two lettuce varieties: Kineta and Lugano. The experiment assessed plant growth in terms of height, diameter, number of leaves, plant mass and nitrate content.

The experimental design utilized a split-plot approach, consisting of 9 main plots and 27 subplots. In each pH and EC level, 3 plants were planted (Table 1).

Table 1. Experimental variants

Cultivars	EC-ul (µS/cm²)	pH		
Kineta	EC 1.5	5	6	7
	EC 2.5	5	6	7
	EC 3.5	5	6	7
Lugano	EC 1.5	5	6	7
	EC 2.5	5	6	7
	EC 3.5	5	6	7

RESULTS AND DISCUSSIONS

In Figure 1 it is observed that Kineta Variety at EC 1.5: pH 5 results in the tallest plants at 18.83 cm. As pH increases to 6, the plant height slightly decreases to 18.03 cm. At pH 7, the height significantly drops to 11.23 cm, indicating a negative effect on growth at higher pH values.

Regarding EC 2.5, at pH 5 results in a height of 19.00 cm, the highest observed in this category. At pH 6, the plant height decreases to 16.53 cm, and pH 7 sees a further decrease to 12.60 cm.

And at EC 3.5, at pH 5, height is 18.50 cm, showing stability at lower pH levels. At pH 6 results in the maximum plant height observed, 19.03 cm, and the height at pH 7 falls significantly to 10.50 cm. In general, for Kineta, lower pH levels (5 and 6) are more favorable for plant height, particularly at EC 2.5 and EC 3.5. Higher pH (7) consistently results in decreased growth.

For Lugano Variety EC 1.5, at pH 5 results in a plant height of 15.00 cm. At pH 6, the height is 15.13 cm, remaining relatively stable, and pH 7 results in a significant decrease to 10.13 cm.

It is observed that EC 2.5, at pH 5 produces a height of 13.53 cm. at pH 6, the height decreases to 12.07 cm, and pH 7 shows further reduction to 10.47 cm.

Regarding EC 3.5, at pH 5, the plant height is 14.33 cm. At pH 6, the height increases to 15.13 cm, and pH 7 results in the smallest plants at 10.37 cm. Similarly to Kineta, we can conclude that the Lugano variety follows the same trend, where lower pH values are linked to increased plant height. EC 1.5 and EC 3.5 at pH 6 seem to provide optimal conditions for growth, while higher pH (7) significantly reduces growth.

Impact of pH: Both plant varieties demonstrate reduced height at a pH of 7. The best growth seems to occur at lower pH levels (5 or 6), depending on the EC level.

Impact of EC: The data suggests that an EC level of 2.5 or 3.5, combined with a pH of 6, results in optimal plant growth for both varieties. EC 1.5 shows moderate growth but not as favorable as the higher EC levels (Figure 1).

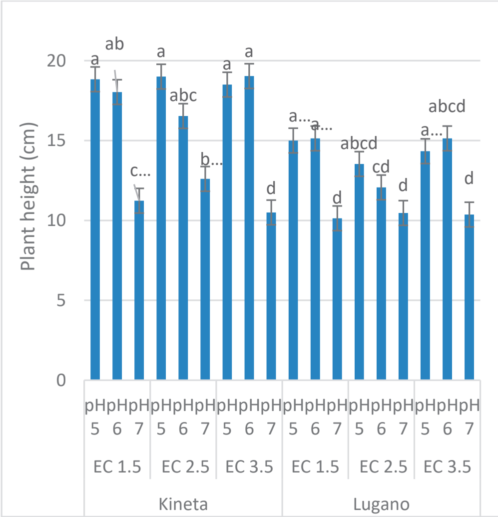

Figure 1. Influence of EC and pH on the height of lettuce plants

Regarding the Effect of EC on plants diameter can be observed higher EC values generally seem to reduce the measured diameter. For example: at pH 5: Diameters decrease from 32.63 (EC 1.5) to 30.03 (EC 3.5). At pH 6: Diameters reduce from 31.60 (EC 1.5) to 21.97 (EC 3.5). At pH 7: Diameters decrease consistently from 18.83 (EC 1.5) to 15.33 (EC 3.5). This suggests that increasing electrical conductivity negatively influences the diameter. In biological or agricultural contexts, this could imply that higher salt or nutrient concentrations may restrict growth or reduce the size of certain organisms or plants.

The analyzed effect of pH on plant diameter suggests that increasing pH (from acidic to neutral) tends to decrease the diameter. At an EC of 1.5, the diameter decreases from 32.63 at pH 5 to 18.83 at pH 7. At EC 2.5, diameter decreases from 32.87 at pH 5 to 22.30 at pH 7 and at EC 3.5: Diameter decreases from 30.03 at pH 5 to 18.07 at pH 7.

This suggests that higher pH may be less conducive to growth compared to more acidic conditions, particularly at lower EC values.

Combined Effect of EC and pH: it is observed that the largest diameters are generally at lower EC and acidic pH (around 5), indicating that organisms or materials being studied might thrive under slightly acidic conditions with lower electrical conductivity. Conversely, smaller diameters occur at higher EC values and neutral pH levels, suggesting stress or restriction of growth under these conditions.

Both higher electrical conductivity and higher pH negatively influence the diameter, likely due to the combined effects of increased salinity and reduced acidity. This pattern may reflect stress responses in biological systems, such as plants, where nutrient uptake or growth could be hindered by excessive salt concentrations or less acidic conditions (Figure 2).

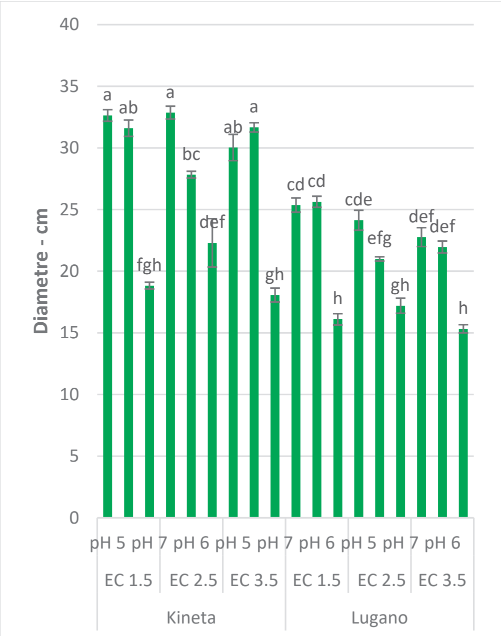

Figure 2. Plant diameter of lettuce varieties Lugano and Kineta depending on EC and pH

In Figure 3, the data shows the influence of electrical conductivity (EC) and pH on the number of leaves produced by two varieties, Kineta and Lugano.

For **Kineta**, the number of leaves tends to decrease with increasing pH at all EC levels, with the highest leaf count at EC 2.5 and pH 5 (41.33 leaves). The lowest leaf count occurs at EC 3.5 and pH 7 (13.67 leaves), indicating a negative interaction between high pH and EC.

For **Lugano**, leaf production is generally lower compared to Kineta. Similarly, the number of leaves decreases as pH increases, but the effect of EC is less pronounced. The highest leaf count occurs at EC 3.5 and pH 6 (25.33 leaves), suggesting a slight tolerance to higher EC compared to Kineta.

Overall, both varieties show reduced leaf numbers at higher pH, with Kineta responding more significantly to variations in EC and pH.

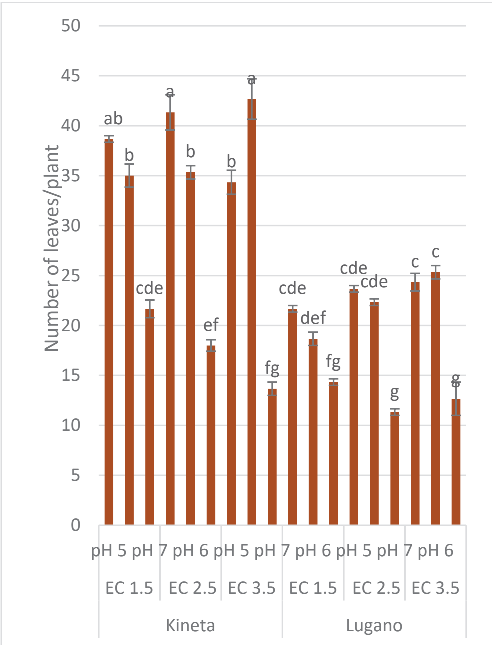

Figure 3. The influence of EC and pH values on the number of leaves per plant in the Lugano and Kineta lettuce varieties

In Figure 4, for Kineta, plant mass generally increases with EC, particularly at higher pH levels. At EC 3.5 and pH 7, the highest plant mass (150.03 g) is recorded. Plant mass production decreases slightly at lower pH values, indicating Kineta may tolerate higher EC and pH levels for optimal growth. For Lugano, the highest plant mass is observed at EC 3.5 and pH 6 (185.17 g), but there is a significant drop at pH 7 (60.00 g). Lugano shows greater sensitivity to high pH levels, particularly at EC 3.5. Kineta shows consistent plant mass production across varying pH and EC levels, with slightly higher plant mass at higher EC and pH levels. Lugano performs best at EC 3.5 and pH 6 but exhibits a sharp decline in plant mass at pH 7. This suggests that Lugano is more sensitive to extreme pH, particularly at higher EC.

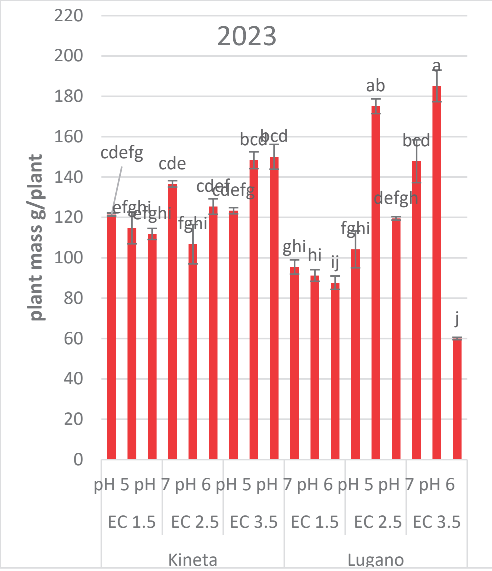

Figure 4. Mass of plants in lettuce varieties Lugano and Kineta depending on EC and pH

The data presented in Figures 5 and 6 show the effect of electrical conductivity (EC) and pH on the behavior of Kineta and Lugano on nitrate content. At lower EC (1.5), both show relatively moderate values, with Kineta decreasing as pH increases, and Lugano being higher at pH 5 and gradually decreasing. As EC rises to 2.5 and 3.5, Kineta's values generally increase at low pH but decrease at higher pH. Lugano follows a similar pattern, with higher values at lower pH but sharply reduced performance at higher pH (notably at EC 3.5 and pH 7). Overall, both EC and pH interact strongly to affect the values recorded for both Kineta and Lugano.

At low EC (1.5), Kineta decreases consistently with increasing pH, indicating that at this conductivity level, higher pH reduces Kineta's values. The drop from pH 5 (1176 mg/kg) to pH 7 (453 mg/kg) is significant, showing a 61% decrease.

At EC 2.5, Kineta starts higher at low pH (1151 mg/kg at pH 5) and then increases with pH 6 (1351 mg/kg), showing a 17% increase. However, at pH 7, there's a steep drop to 396, reflecting a significant 70% decrease relative to pH 6. At the highest EC (3.5), Kineta performs best at low pH, with the highest value (1567 mg/kg) at pH 5. It slightly decreases to 1489 at pH 6, then stabilizes at 1512 mg/kg at pH 7, showing minimal fluctuation between pH 6 and 7. Thus, at high EC, pH has less of an inhibitory effect.

Lugano follows a similar trend at EC 1.5. It starts high at pH 5 (1454 mg/kg) and decreases with increasing pH. The drop from pH 5 to pH 7 is around 30%, suggesting that Lugano, like Kineta, is sensitive to higher pH at low EC levels. As EC increases to 2.5, Lugano exhibits its highest value at pH 6 (1627 mg/kg), indicating improved performance at a mid-range pH. However, at pH 7, Lugano sharply decreases to 1000, showing a 38% reduction from its peak at pH 6. At the highest EC (3.5), Lugano experiences a peak value of 2294 mg/kg at pH 5. However, as pH increases, the values decline, with a notable drop to 508 mg/kg at pH 7 (a 78% reduction from pH 5), indicating that Lugano is highly sensitive to higher pH at elevated EC levels.

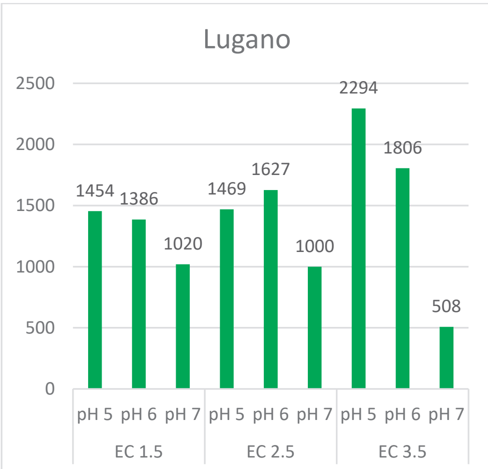

Figure 5. The nitrate content in the lettuce leaves of the Lugano variety

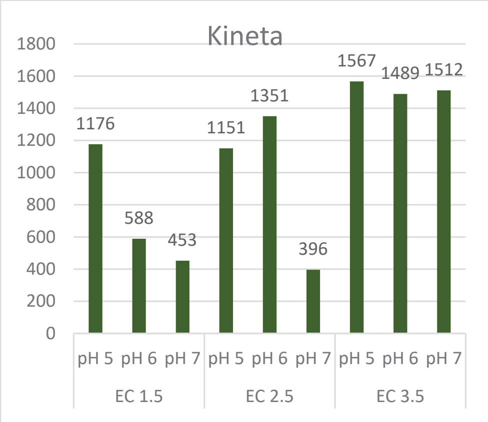

Figure 6. The nitrate content in the lettuce leaves of the Kineta variety

In the Table 2 below indicated the effect of variety on all four growth parameters is significant ($p < 0.001$ for plant height, diameter, and number of leaves; $p < 0.01$ for plant mass).
Kineta consistently outperforms Lugano in plant height (16.03 ± 0.72 cm vs. 12.91 ± 0.50 cm), plant diameter (27.31 ± 1.13 cm vs. 21.06 ± 0.75 cm), number of leaves (31.79 ± 1.99 vs. 19.37 ± 1.01), and plant mass (126.50 ± 3.20 g vs. 118.46 ± 8.0 g). These results suggest that Kineta is a more robust variety under the conditions tested.
Regarding electrical conductivity (EC) effects we can see that plant height and number of leaves show no significant effect of EC (ns), suggesting that EC variation from 1.5 to 3.5 does not influence these parameters. However, plant diameter ($p < 0.001$) and plant mass ($p < 0.001$) are significantly affected by EC. Higher EC (3.5) results in the largest plant mass (135.79 ± 9.55 g), while the lowest mass occurs at EC 1.5 (103.72 ± 3.41 g). A similar trend is observed for plant diameter, where EC 1.5 produces a significantly larger diameter than EC 3.5 (25.03 ± 1.48 cm vs. 23.31 ± 1.45 cm, respectively).
The pH also exerts a highly significant impact ($p < 0.001$) on all parameters. Plant height and plant diameter decrease significantly at pH 7, compared to pH 5 and 6, with the lowest values observed at pH 7 (10.88 ± 0.48 cm for height, 17.97 ± 0.63 cm for diameter).
Number of leaves also decreases at pH 7 (15.28 ± 0.90), compared to pH 5 and 6 (30.67 ± 1.91 and 29.89 ± 2.07 leaves, respectively).
The highest plant mass is observed at pH 6 (136.90 ± 8.81 g), while pH 7 results in significantly lower mass (109.06 ± 7.05 g), indicating that alkaline conditions (pH 7) adversely affect plant growth.
Regarding the interaction effects between variety × EC (V × EC), there was not significant different on for plant height, plant diameter, but significant for the number of leaves and plant mass at ($p < 0.01$, or $p < 0.001$). This suggests that the number of leaves and plant mass is influenced by variety differently at varying EC levels, while the other parameters remain unaffected by the interaction between these two factors.
The interaction between variety × pH (V × pH) was significant difference between variety and pH for plant height ($p < 0.05$), plant diameter ($p < 0.001$), number of leaves ($p < 0.001$), and plant mass ($p < 0.001$). These interactions indicate that the response of each variety to pH is different. For example, Kineta may tolerate extreme pH levels better than Lugano, leading to differential growth outcomes in terms of size, number of leaves, and biomass.
We observed that the interaction between EC × pH was significant difference for plant diameter ($p < 0.001$), number of leaves ($p < 0.001$), and plant mass ($p < 0.001$). The interaction between EC and pH levels highlights the complexity of these factors' combined effects on plant growth. Specifically, the optimum pH for maximizing diameter and mass may depend on the EC level, and vice versa.
We also can see three-way interaction between variety × EC × pH (V × EC × pH) for number of leaves ($p < 0.01$) and plant mass ($p < 0.001$), indicating that the number of leaves and plant mass are influenced by the combination of variety, EC, and pH. These interactions suggest that the response of plant growth to pH and EC is variety-dependent, and each combination of factors produces different growth outcomes.

CONCLUSIONS

The results indicate that variety, pH, and EC significantly affect plant growth parameters, with strong interactions between these factors. In particular: Kineta generally outperforms Lugano under all conditions. pH 7 negatively impacts plant growth, while pH 5 and 6 are more conducive to better plant height, diameter, and mass. Higher EC levels tend to increase plant mass, with a significant decline in diameter as EC increases. The interaction effects reveal that the impact of EC and pH varies depending on the variety, emphasizing the need for optimizing these factors according to the plant variety for improved growth outcomes.

Kineta shows more variability in response to both pH and EC, especially at lower pH levels and higher EC.

Lugano exhibits more stable performance at low pH, particularly at higher EC, but suffers significant declines at pH 7, especially under high conductivity conditions. Both variables display their highest values at low pH (5), particularly at higher EC levels (3.5), while pH 7 consistently results in lower values, suggesting that high pH negatively affects both Kineta and Lugano, especially under higher EC conditions.

Table 2. Interaction between the analyzed parameters in the 2 varieties in the year 2023

	Plant height (cm)	Plant diameter (cm)	Number of leaves/plant	Plant mass (g/plant)
Variety (V)	***	***	***	**
Kineta	16.03 ± 0.72 a	27.31 ± 1.13 a	31.79 ± 1.99 a	126.50 ± 3.20 a
Lugano	12.91 ± 0.50 b	21.06 ± 0.75 b	19.37 ± 1.01 b	118.46 ± 8.0 b
EC	ns	***	ns	***
1,5	14.73 ± 0.85 a	25.03 ±1.48 a	25.0 ± 2.14 a	103.72 ± 3.41 b
2,5	14.03 ± 0.76 a	24.22 ± 1.26 ab	25.33 ± 2.48 a	127.93 ± 6.11 a
3,5	14.64 ± 0.93 a	23.31 ± 1.45 b	25.50 ± 2.62 a	135.79 ± 9.55 a
pH	***	***	***	***
5	16.53 ± 0.61 a	27.97 ± 1.01 a	30.67 ± 1.91 a	121.48 ± 4.78 bc
6	15.99 ± 0.66 a	26.62 ± 1.03 b	29.89 ± 2.07 a	136.90 ± 8.81 a
7	10.88 ± 0.48 b	17.97 ± 0.63 c	15.28 ± 0.90 b	109.06 ± 7.05 c
V x EC	ns	ns	**	***
V x pH	*	***	***	***
EC x pH	ns	***	***	***
V x EC x pH	ns	ns	**	***

Low pH (5) is more favorable for both Kineta and Lugano, especially at high EC.

High pH (7) tends to suppress values for both, with Lugano being more negatively affected at high EC and pH.

The interaction between EC and pH has a complex effect on both variables, with pH 6 sometimes improving performance at moderate EC (2.5), but consistently declining at pH 7.

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THE ROLE OF THE PARENTAL FACTOR IN THE HERITABILITY OF THE BIOCHEMICAL CHARACTERS OF THE TOMATO FRUIT

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Abstract

The paper presents data on the testing results of 5 F_1 reciprocal hybrid combinations and 7 parental forms of tomato based on the content of dry matter, sugars, acidity, vitamin C, lycopene and β-carotene in fruits. The biochemical analysis of parent varieties and reciprocal F_1 hybrids was differentiated – specific to the genotype, the hybrid, the crossing orientation, the analyzed character. By cluster analysis (k-means) of the tomato F_1 parents and hybrids were identified – Vrojainii, Rufina, Flacara, L 10B, and the hybrid combinations F_1 Flacara x Vrojainii, F1 L 10B x Rufina, F_1 Rufina x L 10B, F_1 Vrojainii x Flacara, F_1 Desteptarea x Flacara, F_1 Flacara x Desteptarea, F_1 Flacara x Rufina, F_1 Rufina x Flacara, F_1 Dolgonosic x Mary Gratefully which are characterized by high indices of the biochemical characters, which provides opportunities for their use in breeding programs in quality of the initial material in improving the quality of tomato fruits. The differences in the manifestation of the characters analyzed in the reciprocal F_1 hybrids demonstrate the involvement of the parental factor in their phenotype.

***Key words**: tomato, varieties, hybrids, parental effect, dominance, biochemical characters.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) fruits are some of the most widely consumed and popular vegetables grown worldwide and particularly profitable for producers (Nasir et al., 2015).

Tomatoes are attractive due to the rich range of flavors, shapes, sizes and colors (Tieman et al., 2017), but also to the high value of the fruits, the various forms of consumption: fresh, mixed with other vegetables, sauces, pots, marinated and pickled tomatoes, stuffed tomatoes, etc., or industrially processed: paste, preserves, juices, etc. (Mihnea, 2016; Li et al., 2018).

The high quality of tomato fruits is one of the basic objectives of the improvement of this culture, being considered as important as the improvement of production characters and other characteristics of the variety. In this context, the wide use of tomato products, the intensification of research for this objective at the national level is fully justified (Mihnea et al., 2022).

The aesthetic and biochemical quality of tomato fruits is often a priority for the middle-income consumer's decision, even more important than the price (Klee & Tieman, 2013). For tomato, taste, appearance, color are decisive for fresh fruit, while consistency, sugar, acidity, dry matter are important in the tomato processing industry.

In the canning industry, varieties with a high content of the dry matter, sugar, vitamins, pigments and other properties are requested, which is often not recorded in the approved varieties (Botnari, 2015). From a biochemical point of view, the fruits must be rich in vitamins, with the tomato having the highest rate of vitamin C. Vitamins, dry matter content, acidity, sugar/acidity ratio determine the taste and aroma of the fruit. The morphological aspects as quality factors are: shape, color and size of the fruits. The shape and color depend on consumer preferences. The high diversity of shapes and colors is an attraction for consumers. Regarding the size of the fruit, the request is determined by the final destination: large fruits are preferred fresh, for the preparation of pasta and juice, and small ones are requested by the sphere of food service and the preservation of fruits in domestic conditions.

Worldwide, tomatoes are an important part of a the diverse and balanced diet (Willcox et al., 2003), providing a wide diversity of nutrients (Ilahy et al., 2016), vitamins, carotenoids and phenolic compounds (Martí et al., 2016; Li et al., 2018). Consumption of carotenoid-rich foods has been associated with decreased mortality and disease risk (Diplock et al., 1998; Giordano & Quadro, 2018). The health benefits of carotenoids are related to their antioxidant properties, being involved in reducing cancer and cardiovascular diseases, improving the immune system and bone health (Eggersdorfer & Wyss, 2018; Rodriguez-Concepcion et al., 2018). In addition, specific benefits for brain development and cognitive functions have been linked to lutein (Giordano & Quadro, 2018; Stringham et al., 2019). Carotenoids and polyphenolic compounds contribute to the nutritional value of tomatoes by improving their functional attributes and sensory qualities, including taste, aroma and texture (Martí et al., 2016).

The main carotenoids that accumulate in red ripe tomato fruits are lycopene (~90%), *β*-carotene (5-10%), lutein (1-5%) and less than 1% – other carotenoids (Ronen et al., 1999). Lycopene and *β*-carotene are the main pigments responsible for the characteristic color of the ripe fruit. These carotenoids largely determine the perception of the quality of fresh tomatoes, with consumers usually preferring red or deep orange tomatoes. According to some authors (Sahlin et al., 2004), the content of carotenoids in tomatoes largely depends on the genotype, stage of maturity, climatic and growing conditions.

Breeding is a sure way to improve the biochemical composition of tomato fruits – dry matter content, gluco-acidic ratio, sugar, vitamin C, etc. The creation of varieties, hybrids, new lines with a high level of dry matter is one of the main requirements in modern agriculture and the implementation of intensive technologies. Therefore, testing selection material based on this character is very important in improving of the tomato fruit quality (Mihnea et al., 2016).

The lack of fundamental knowledge regarding the peculiarities of the synthesis of valuable biochemical compounds, but also the genes involved in the control or regulation of metabolic pathways often hinders the correct and directed strategy of creating performing tomato genotypes that would maintain the taste and aromatic qualities in the long term after harvesting, which currently presents a major challenge (Moreno et al., 2014).

One of the sources of increasing the variability of the quantitative and qualitative characters of the plans is the parental factor, which at the moment is insufficiently investigated. According to data (Singkaev et al., 2015), cross-pollination in tomato, demonstrated different effects on the number of seeds per fruit, the weight of 1000 seeds, the content of dry matter, the gluco-acidic ratio, the proportions of fructose, glucose and sucrose.

Hybridization methods can be used to improve tomato fruit quality characters. For this purpose, it is necessary to know the peculiarities of their heredity. For example, total dry matter, acidity, vitamin C are under dominant control, and their low heritability indicates the involvement of non-additive interactions. Therefore, due to the low heritability and the lack of high selection potential, hybridization methods and the phenomenon of heterosis can be used in the first generation to improve these characters. On the contrary, the high selection potential for total phenol due to additive genes reveals the opportunity to improve the population by selecting the best genotypes (Randii et al., 2016).

Research on the genetic contribution to the variation of tomato seed quality traits at the transcriptome level demonstrated that the genetic control of plasticity in the regulation of genes expressed in seeds is affected by the maternal nutrient environment (Sterken et al., 2023).

Although the content of lycopene and *β*-carotene varies greatly depending on the environment, there is a high genotypic potential for improving the content of lycopene and *β*-carotene in new tomato cultivars. Real opportunities offer the involvement of the species *S. pimpinellifolium* in crosses (Duduit et al., 2022; Sterken et al., 2023).

The existing assortment of tomatoes in the Republic of Moldova has improved in recent years by introducing varieties and hybrids created in the country or abroad. Taking into

account the great diversity of the use of tomato production, staggering it over as long a period as possible, continuously improving the quality and broadening the current assortment with new varieties and hybrids is a topical objective.
The purpose of the research: elucidation of heritability peculiarities and the parental effect in the new tomato hybrid combinations, and the cluster organization of the F_1 parents and hybrids based on biochemical characters.

MATERIALS AND METHODS

As initial material for the intended research, 5 reciprocal F_1 hybrid combinations were used: Dolgonosic x Mary Gratefully/Mary Gratefully x Dolgonosic, Flacara x Vrojainii/ Vrojainii x Flacara, Flacara x Desteptarea/ Desteptarea x Flacara, L 10B x Rufina/ Rufina x L 10B, Rufina x Flacara/Flacara x Rufina and 7 parental forms: Rufina, Dolgonosic, Flacara, L 10B, Vrojainii, Mary Gratefully, Desteptarea.
The experiments were carried out under laboratory and field conditions. Tomatoes were grown by seedling culture in three repetitions according to the standard method (Ershova, 1978).
The dry matter was determined by drying (Murashev, 2007), the content of sugars – by the anthrone method (Severin & Solovyova, 1898), vitamin C in fruits and acidity – according to the standards in force (GOST 24556-89, 2003; GOST 25555.0-82, 2010).
The amount of carotenoids in fruits was determined by spectrophotometric method (Trineeva & Slivkin, 2016; Golubkina et al., 2017).
The degree of dominance (h_p) was calculated according to the formula proposed by Brubaker (1966):
$h_p = F_1 - 0.5 (P_1+P_2)/ Hp - 0.5 (P_1+P_2)$,
where:
F_1 – the average value of the character in the F_1 generation;
P_1, P_2 – the average value of the character in the parental forms;
H_p – the average value of the character evaluated at the best parental form.
The reciprocal effect (r_e) was calculated according to the formula: $r_e = (b - a)/(B - A)$, where A and B – character values for the parental forms involved in crossing; a – for the ♂A x ♀B hybrid; b – for the reciprocal hybrid ♂B x ♀A. The positive value r ($r > 0$) signifies the paternal effect, and the negative ($r < 0$) – maternal, the absolute value r ($|r|$) shows the relative appreciation of these effects in units, equal to the differences in the values of the character in the parental forms (B - A) (Reinhold, 2002).
Cluster analysis of tomato genotypes based on biochemical characters – dry matter content (%), sugar (%), acidity (%), sugar / acidity index, vitamin C (mg/%), licopene, mg/100 g, *β*-carotene, mg/100 g was performed according to the iterative algorithm for constructing dendrograms and the *k*-means centroid method – methods successfully used in genetics and breeding research (Lupaşcu et al., 2019; Kanavi et al., 2020).
The obtained data were statistically processed in the STATISTICA 7 software package.

RESULTS AND DISCUSSIONS

The biochemical data of the characters under study for parent cultivars and F_1 reciprocal hybrids of tomato depended a lot on the parent, F_1 hybrid, crossing orientation, analyzed character – dry matter, sugars, acidity, vitamin C, lycopene and *β*-carotene (Table 1).
Dry substance. It was found that in the fruits of the parental forms, the dry matter content varied within the limits of 6.0-7.4%, and of the hybrid combinations – 7.2-8.4%. It should be noted that for all hybrid combinations, the index recorded higher values than the best parent, except for the combination Flacara x Desteptarea which was at the level of the maternal form. Differences between mutual analogues were found in the combinations Desteptarea x Flacara, Flacara x Rufina, Dolgonosic x Mary Gratefully.

Table 1. Variability of fruit biochemical characters in the parental forms and F_1 hybrids of tomato

No.	Variety, F_1 hybrid	Dry substance, %	Sugar, %	Acidity, %	Sugar/acidity index	Vitamin C, mg/%	Lycopene, mg/100 g	β-carotene, mg/100 g
1	Rufina	7.2	6.9	0.59	11.7	30.1	0.67	3.93
2	Flacara	7.2	6.6	0.66	10.0	29.5	0.38	3.39
3	L 10B	7.4	6.6	0.53	12.5	34.0	0.19	0.71
4	Vrojainii	7.3	6.8	0.57	11.9	33.5	0.07	0.63
5	Mary Gratefully	6.7	6.0	0.57	10.5	25.8	1.22	1.38
6	Desteptarea	6.2	5.5	0.54	10.2	28.5	1.71	1.44
7	Dolgonosic	6.0	4.2	0.38	11.1	23.1	0.55	1.8
	$x \pm m_x$	6.86± 0.21	6.09± 0.37	0.55± 0.03	11.13± 0.36	29.21± 1.48	0.68± 0.22	1.90± 0.49
	σ	0.57	0.97	0.09	0.94	3.91	0.59	1.28
8	F_1 Flacara x Vrojainii	7.7	6.6	0.60	11.0	33.5	1.6	1.73
9	F_1 Vrojainii x Flacara	7.8	7.3	0.61	12.0	31.5	1.37	1.78
10	F_1 Desteptarea x Flacara	7.6	6.8	0.71	9.6	29.6	1.56	1.92
11	F_1 Flacara x Desteptarea	7.2	6.7	0.70	9.6	30.1	1.49	1.7
12	F_1 Flacara x Rufina	8.0	5.8	0.68	8.5	31.5	0.53	3.66
13	F_1 Rufina x Flacara	7.5	5.7	0.61	9.3	30.3	0.52	3.89
14	F_1 Dolgonosic x Mary Gratefully	7.8	5.3	0.53	10.0	31.3	1.37	2.73
15	F_1 Mary Gratefully x Dolgonosic	8.4	6.1	0.59	10.3	27.3	1.48	2.82
16	F_1 L 10B x Rufina	7.9	5.8	0.53	10.9	33.8	2.15	2.0
17	F_1 Rufina x L 10B	8.2	5.6	0.58	9.7	32.6	2.07	1.94
	$x \pm m_x$	7.81± 0.11	6.17± 0.20	0.61± 0.02	10.09± 0.32	31.15± 0.61	1.41± 0.17	2.42± 0.26
	σ	0.35	0.64	0.06	1.00	1.94	0.54	0.82

Sugars. In F_1 varieties and hybrid populations, differences were also established based on the sugar content in the fruits, which varied within the limits of 4.2-6.9% and 5.3-7.3%, respectively. The highest sugar content was recorded in the varieties Rufina, Flacara, Vrojainii, L10B and in the reciprocal combinations Flacara x Vrojainii/Vrojainii x Flacara and Desteptarea x Flacara/Flacara x Desteptarea (Table 1).

Sugar/acidity ratio. Of particular importance for the variety's performance is the sugar/acidity ratio, on which the taste properties of the fruit depend a lot. A variability was found within the limits of 10.0-12.5 for parents and 8.5-12.0 – F_1 hybrids. Based on this character, the varieties L 10B (12.5), Rufina (11.7), Dolgonosic (11.1), Vrojainii (11.0), and the hybrid combinations Vrojainii x Flacara (12.0) were highlighted, Flacara x Vrojainii (11.0). No differences were recorded between mutual analogues for the combinations Desteptarea x Flacara, Dolgonosic x Mary Gratefully.

Vitamin C. Ascorbic acid content is also important for fruit quality. The index registered a high variability in the researched varieties: 23.1 mg/% (Dolgonosic) –34.0 mg/% (L 10B), and in the hybrid combinations – between 27.3 (F_1 Mary Gratefully x Dolgonosic) and 33.8 F_1 L 10B x Rufina (mg/%).

Lycopene varied within the limits of 0.07-1.71 mg/100 g (Vrojainii, Desteptarea), 0.52-2.15 mg/100 g – F_1 hybrids (Rufina x Flacara, F_1 L 10B x Rufina), and *β*-carotene – in wider limits than lycopene: 0.63-3.93 mg/100 g (parents Vrojainii, Rufina); 1.28-3.89 mg/100 g – hybrids F_1 Flacara x Vrojainii, F_1 Rufina x Flacara. It should be noted that the average of the indices for the examined characters was higher in the case of F_1 hybrids, higher exceedances of the parental group being recorded for the content of lycopene and *β*-carotene: + 107.4% and +27.4%, respectively, which denotes the pronounced heterosis effect for these tomato fruit quality traits.

The correlational analysis (r) of the quality character indices of tomato fruits demonstrated that, based on the material under study, new tomato genotypes can be obtained with a successful association of the characters dry matter – vitamin C, sugar – acidity, sugar – vitamin C , for which there was a significant dependence: 0.49*-0.67* ($p < 0.05$). It should be noted that between the sugar/acidity index and β-carotene content, the dependence was inversely proportional: (-0.51*, $p<0.05$) (Table 2).

Table 2. Correlational dependencies (r) between indices of biochemical properties of tomato fruits

Dry substance, % – Vitamin C, mg/%	0.59*
Sugars,% – Acidity, %	0.67*
Sugars, % – Vitamin C, mg/%	0.49*
Sugar/acidity ratio – β-caroten, mg/100 g	-0.51*

*-p<0.05.

The distribution dendrogram of the parents and the F_1 hybrids taken together, demonstrates the existence of significant similarities or differences based on a complex of characters – dry matter content (%), sugar (%), acidity (%), sugar/acidity index, vitamin C (mg/%), licopene, mg/100 g, β-carotene, mg/100 g taken as classification criteria (Figure 1).

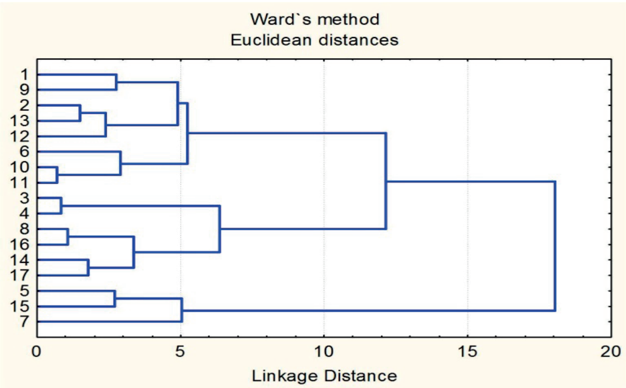

Figure 1. Distribution dendogram of F_1 tomato varieties and hybrids based on the biochemical characters
501 – Rufina, 502 – Flacara, 503 – L 10B, 504 – Vrojainii, 505 – Mary Gratefully, 506 – Desteptarea, 507 – Dolgonosic, 508 – F_1 Flacara x Vrojainii, 509 – F_1 Vrojainii x Flacara, 510 – F_1 Desteptarea x Flacara, 511 – F_1 Flacara x Desteptarea, 512 – F_1 Flacara x Rufina, 513 – F_1 Rufina x Flacara, 514 – F_1 Dolgonosic x Mary Gratefully, 515 – F_1 Mary Gratefully x Dolgonosic, 516 – F_1 L 10B x Rufina, 517 – F_1 Rufina x L 10B

For a more accurate identification of tomato F_1 parents/hybrids with valuable complex traits, the *k*-means cluster method was applied. The data obtained show that the clustering into 4 groups of similarity was more successful based on 2 characters – the content of vitamin C and β-carotene, demonstrated by the fact that the intercluster variance was much higher than the intracluster one, the ratio between them being 8.08 and 3.16, respectively (Table 3).

Table 3. Inter- and intracluster variance analysis for parent cultivars and F_1 hybrids of tomato

Quality index	Interclusterian variance (1)	df	Intraclusterian variance (2)	Ratio	df	F	p
Dry substance	2.909	3	3.815	0.76	13	3.304	0.054
Sugars	2.844	3	6.545	0.44	13	1.892	0.181
Acidity	0.040	3	0.059	0.68	13	2.921	0.074
Sugar/acidity ratio	7.464	3	11.281	0.66	13	2.867	0.077
Vitamin C	125.543	3	15.540	8.08	13	35.009	0.000
Licopen	1.880	3	5.007	0.38	13	1.628	0.231
β-carotene	12.909	3	4.081	3.16	13	13.707	0.000

It should be mentioned that the 7 parent varieties were distributed in different clusters, thus demonstrating differences according to the indices of the characters under study. Reciprocal hybrids, with the exception of F_1 Dolgonosic x Mary Gratefully/F_1 Mary Gratefully x Dolgonosic, were located together in the same cluster and also shared with both parents. This denotes the lack of differences between the reciprocal F_1 hybrids according to the whole complex of investigated characters. Regarding the content of vitamin C and β-carotene, as properties that showed the highest ability to differentiate varieties and hybrids, it can be observed that clusters 2, 3, 4 are characterized by high indices of vitamin C content – 28.9-33.2 mg/%, and cluster 2 (Rufina, Flacara, F_1 Rufina x Flacara, F_1 Flacara x Rufina, F_1 Dolgonosic x Mary Gratefully) records a high average: 30.54 mg/%, and 3.52 mg/100 g, respectively, of vitamin C and β-carotene content (Table 4).

Table 4. Descriptive cluster analysis

Cluster	Quality index	x	σ	Genotype
1	Dry substance, %	6.35	0.50	Mary Gratefully, Dolgonosic
	Sugars, %	5.10	1.27	
	Acidity, %	0.48	0.13	
	Sugar/acidity ratio	10.8	0.42	
	Vitamin C, mg/%	24.45	1.91	
	Licopen, mg/100 g	0.89	0.47	
	β-carotene, mg/100 g	1.59	0.30	
2	Dry substance, %	7.54	0.13	Rufina, Flacara, F_1 Flacara x Rufina, F_1 Rufina x Flacara, F_1 Dolgonosic x Mary Gratefully
	Sugars, %	6.06	0.44	
	Acidity, %	0.61	0.00	
	Sugar/acidity ratio	9.90	1.40	
	Vitamin C, mg/%	30.54	0.71	
	Licopen, mg/100 g	0.69	0.15	
	β-carotene, mg/100 g	3.52	0.21	
3	Dry substance, %	7.35	0.92	Deșteptarea, F_1 Desteptarea x Flacara, F_1 Flacara x Desteptarea, F_1 Mary Gratefully x Dolgonosic
	Sugars, %	6.28	0.60	
	Acidity, %	0.64	0.08	
	Sugar/acidity ratio	9.93	0.38	
	Vitamin C, mg/%	28.88	1.25	
	Licopen, mg/100 g	1.56	0.11	
	β-carotene, mg/100 g	1.97	0.60	
4	Dry substance, %	7.72	0.33	L 10B, Vrojainii, F_1 Flacara x Vrojainii, F_1 Vrojainii x Flacara, F_1 L 10B x Rufina, F_1 Rufina x L 10B
	Sugars, %	6.45	0.64	
	Acidity, %	0.57	0.03	
	Sugar/acidity ratio	11.33	1.01	
	Vitamin C, mg/%	33.15	0.94	
	Licopen, mg/100 g	1.24	0.91	
	β-carotene, mg/100 g	1.47	0.62	

The analysis of the obtained results demonstrates that the varieties and hybrid combinations evaluated differ essentially also based on the content of lycopene and carotene. If in varieties the lycopene content fell within the limits of 0.10-1.71 mg/100 g, then in reciprocal hybrids – between 0.53-2.15 mg/100 g wet mass. The highest lycopene content was recorded by the varieties Mary Gratefully, Desteptarea and most of the reciprocal combinations except for the mutual combination Flacara x Rufina/ Rufina x Flacara where the lycopene content was 0.53 and 0.52 respectively. The varieties Rufina, Flacara and the mutual combinations Flacara x Rufina and Dolgonosic x Mary Gratefully stood out for their high β-carotene content, which recorded the highest values.

The research of the manifestation of the genetic factors involved in the manifestation of biochemical characters at the level of F_1 reciprocal hybrids, demonstrated that the degree of dominance of biochemical characters varied within wide limits – from positive overdominance to negative overdominance (Table 5), which denotes that the inheritance of biochemical characters it depends on the combination and can be dominant or recessive.

Table 5. The influence of the parental factor on the degree of dominance of biochemical indices in tomato fruits

Hibrid F_1	Dry substance	Sugars	Acidity	Vitamin C	Licopene	β-carotene
Flacara x Vrojainii	+5.0	-1.0	-0.5	+1.0	+9.7	-0.2
Vrojainii x Flacara	+11.0	+6.0	-0.25	0.0	+8.1	-0.2
Desteptarea x Flacara	+1.8	+1.4	+1.8	+1.2	+0.8	-0.5
Flacara x Desteptarea	+1.0	+1.2	+1,7	+2.0	+0.7	-0.7
Flacara x Rufina	0.0	+6.3	+1.7	+5.7	0.0	0.0
Rufina x Flacara	0.0	-7.0	-0.8	+1.7	0.0	+0.9
Dolgonosic x Mary Gratefully	+4.1	+0.2	+0.6	+5.2	+1.5	+5.4
Mary Gratefully x Dolgonosic	+5.9	+1.1	+1.2	+2.2	+1.8	+5.9
L 10B x Rufina	+6.0	-6.3	-1.0	+0.9	+7.2	-0.2
Rufina x L 10B	+9.0	-7.7	+0.7	+0.3	+6.8	-0.2

Notes: $-0.0<h_p<-1$ – negative overdominance; $-1<h_p<-0.5$ – negative dominance; $-0.5<h_p<+0.5$ – intermediate dominance; $+0.5<h_p<+1$ – positive dominance; $+1<h_p<+0.0$ – positive overdominance (Bryubeyker, 1966).

It should be noted that there were significant differences in the degree of dominance of biochemical indices in all reciprocal hybrids. For example, for the dry matter, vitamin C and lycopene characters, dominance and positive superdominance were recorded, and mutual combinations differed only based on values.
Both reciprocal combinations Flacara x Rufina/Rufina x Flacara did not record higher or lower values than one of the parents for dry matter and lycopene content. As for the sugar and acidity content, the mutual combinations differed both based on the values of the degree of dominance and its orientation.
For example, in the hybrids Flacara x Vrojainii and Rufina x Flacara h_p = - 1.0; -7.0, respectively, and for their counterparts – h_p = + 6.0; +6.3, denoting that the change in crossing direction determined the dry matter content. Differences between the homologous hybrids were also observed based on the acidity content. No differences were recorded based on the β-carotene character in the homologous hybrids Flacara x Vrojainii/ Vrojainii x Flacara and L 10B x Rufina/ Rufina x L 10B. Positive overdominance was found in the reciprocal combination Dolgonosic x Mary Gratefully/Mary Gratefully x Dolgonosic.
Based on the data obtained, we can mention that the F_1 combinations Flacara x Rufina, Dolgonosic x Mary Gratefully, Mary Gratefully x Dolgonosic, which showed incomplete dominance of the parent with high values or positive overdominance of the evaluated characters, are promising and through repeated selections can be obtained lines with high taste properties.
Calculation of the reciprocal effect demonstrated its dependence on the combination and the analyzed character. Thus, significant paternal influence on: i) the content of dry matter, sugar, lycopene was found in Flacara x Vrojainii hybrids; ii) dry substance content, vitamin C – Desteptarea x Flacara; iii) acidity, β-carotene – Flacara x Rufina; iv) dry matter content, sugar – Dolgonosic x Mary Gratefully. A strong influence of the maternal parent was recorded in the case of vitamin C content in the combinations Flacara x Vrojainii, Flacara x Rufina, Dolgonosic x Mary Gratefully (Table 6).

Table 6. Reciprocal effect for some biochemical characters of tomato fruits

Reciprocal hybrid F_1	Dry substance	Sugars	Acidity	Vitamin C, mg/%	β-carotene	Licopene
Flacara x Vrojainii	+1.00	+3.50	-0.11	-0.50	-0.02	+0.82
Desteptarea x Flacara	+4.00	-0.09	-0.08	+0.50	-0.11	+0.21
Flacara x Rufina	0.0	-0.33	+1.00	-2.00	+0.43	-0.03
Dolgonosic x Mary Gratefully	+0.86	+0.44	+0.32	-1.48	-0.21	+0.16
L 10B x Rufina	-1.5	-0.67	+0.05	+0.31	-0.02	-0.17

Study of a the complex of valuable biochemical characters of the fruit in some parent varieties and reciprocal tomato hybrids demonstrated their genetic determinism, the influence of the parental factor on the degree of dominance in the F_1 generation, the cluster organization of genotypes, the differentiated role of maternal and paternal factors in the phenotypic manifestation of quality attributes.

CONCLUSIONS

Research on the quality characteristics of tomato fruits – dry matter content (%), sugar (%), acidity (%), sugar / acidity index, vitamin C (mg/%), lycopene (mg/100 g), β-carotene (mg/100 g) in 7 parent varieties and 10 reciprocal F_1 hybrids demonstrated the dependence of the indicators on the plant genotype.

Through cluster analysis (dendrogram, *k*-means) the distribution of parents and F_1 hybrids was established in clusters according to the degree of similarity/difference of the indicators, and the high capacity of vitamin C and β-carotene for their differentiation was identified. The cluster formed by Rufina, Flacara, F_1 Rufina x Flacara, F_1 Flacara x Rufina, F_1 Dolgonosic x Mary Gratefully recorded an average of 30.54 mg/% and 3.52 mg/100 g, respectively, of vitamin C and β-carotene content.

The choice of hybridization components as maternal or paternal parent influenced the degree of dominance of dry matter content, sugar, acidity, sugar / acidity index, vitamin C content in most F_1 tomato hybrids. The paternal factor was more important for the content of dry matter and sugar, and the maternal factor – for the content of vitamin C.

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RHIZOPLANE AND RHIZOSPHERE MICROBIAL ACTIVITY IN ECOLOGICALLY CULTIVATED VEGETABLES AND OTHER CROPS

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Abstract

The purpose of the article is to present data from a comparative study of the composition and activity of the microflora from the rhizoplane and rhizosphere in leeks, red beets, chard, spinach, dill, parsley, and common vetch, as well as control sample without vegetation. The experiment was conducted at the training field at the University of Forestry, Sofia, Bulgaria. Microorganisms from the rhizoplane (the surface of the roots) and the rhizosphere, near the roots, were studied. Growing leeks increases the biogenicity of roots and soil to the highest degree. The main share in the composition of the total microflora is occupied by non-spore-forming bacteria in all variants. In the vegetated variants, the catalase value was highest when growing leek, followed by parsley. Cellulase activity is highest in red beets and leeks. The studied microbiological and enzymatic indicators are sensitive indicators of soil fertility and qualitative development of the analyzed crops. The activity of the rhizosphere and rhizoplane microflora depends on the humidity in the root zone, and the quantity of microorganisms is not the only and independent prerequisite for their activity.

Key words: *catalase, cellulase, legume, microflora, spices.*

INTRODUCTION

On the roots surface (rhizoplane) and the root-inhabited soil layer (rhizosphere), an amazing number of microorganisms develop, participating in the cycles of nutrients, the improving soil fertility, quality and yield of agricultural crops. Plant characteristics influence soil fertility, yield, soil organic decomposition processes, soil nutrient cycling and soil organic matter levels (Bray et al., 2012; Cornwell et al., 2008; Hobbie, 2015; Sofo et al., 2020). Key soil processes are governed by interactions between plant roots, C and N content, and microbial metabolism, which drive decomposition reactions, soil fertility, and plant net primary production (Sofo et al., 2020). The complex composition and interaction of root-associated microbes, detailed characterization of microbiomes from three rhizocompartments (rhizosphere, rhizoplane, and root), are critical to plant health and performance (Wang et al., 2021).

In the present study, rhizoplane and rhizosphere microbiological activity was analyzed in organically cultivated vegetables and other crops: leek, dill, parsley, spinach, chard, red beet, common vetch.

Dill (*Anethum graveolens* L.) is the only species of the genus Anethum, family Apiaceae. It is used as an herb, spice and essential oil crop. The plant is annual. Green parts reach 90-160 cm in height, with thin stems and alternate leaves with strongly cut petioles. (Jana & Shekhawat, 2010). The root is spindle-shaped, slightly branched and relatively shallow (Daskalov and Kolev, 1958).

Parsley (*Petroselinum crispum* Mill. Nyman) belongs to the Apiaceae family and is grown in temperate and subtropical climates throughout the world. There are two varieties, leafy - convar. Crispum and root - convar. Tuberosum. All parts of the plant are usable, including leaves, stems and main roots. (Marthe, 2020). Leaf parsley is a biennial plant, which in the first year forms leaves and a strongly branched root, and in the second passes into a generative phase.

Spinach (*Spinacia oleraceae* L.), family Chenopodiaceae, is a long-day plant. The culture is annual for the conditions of Bulgaria, and under unfavorable conditions (drought, waterlogging, other stress or in conditions of a long day), instead of forming a product part (leaves), it quickly passes into a generative phase (Pandey & Kalloo, 1993). It is grown in early spring or late autumn with the greatest success due to its biological characteristics (Murcia et al., 2020). It has a dense root system composed of a central root and lateral branches of various orders. The central root grows rapidly and can reach a depth of 180 cm. Lateral roots develop initially at a depth of 15-26 cm and later up to 90 cm (https://www.nottingham.ac.uk/hiddenhalf/herbs/spinach.aspx). The "Large winter" variety is widespread and preferred in the country. It is very early and highly productive. It forms a large rosette with tender and juicy leaves.

Chard (*Beta vulgaris* ssp. *cicla* f. *hortensis* Alef.), family Chenopodiaceae, is a leaf chard/beet, spinach beet. Forms a very highly developed leaf rosette. The leaves are over 20 cm long. The main root is thickened in its upper part and forms a branched woody root crop, the lateral roots are stronger and strongly branched than in beetroot (Daskalov and Kolev, 1958).

The leek (*Allium ampeloprasum* L.), family Alliaceae (Amarilidaceae) has a root system typical for the family Alliaceae (Amarilidaceae) - bearded, composed of many unbranched or slightly branched string-like roots, very strongly developed. It is mainly located in the soil layer up to 30 cm deep. Individual roots reach up to 70-75 cm. Roots do not die until late in the fall (Shaban et al., 2014). The "Starozagorski 72" variety is preferred in the country because of the larger product part it forms compared to other varieties (Pidov et al., 1995).

Red beet (*Beta vulgaris* L. var. *esculenta*), family Chenopodiaceae, is a biennial plant that forms a rosette of leaves and a root crop in the first year of sowing, and in the second it goes into a generative phase. The product part can be round, oval or conical in shape depending on the variety and which parts and organs take part in its formation. The product part of the "Bordeaux" variety is formed with the participation of the sub- and supra-coelomic knee and its shape is round (Daskalov and Kolev, 1958). The root system is extensive and well branched (Greenwood et al., 1982). .

Common vetch (*Vicia sativa* L.), family Fabaceae, genus Vicia has a well-developed root system and as a nitrogen-fixing legume, a process localized in small root nodules formed by the symbiosis with *Rhizobium* bacteria, is considered as an improver of soil fertility and a valuable source of protein and minerals for farm animals.

A high bacterial abundance and a lower fungal development were found in dill cultivation (Sofo et al., 2020). Spore-forming bacteria, fluorescent *Pseudomonas* bacteria, diazotrophs, Actinomycetes, microscopic fungi have been isolated from parsley rhizosphere soil (Trzciński et al., 2018). *Rhizobium laguerreae* colonizes the roots of spinach plants, forming microcolonies typical of biofilm initiation, significantly increases several vegetative parameters such as leaf number, size and weight, as well as chlorophyll and nitrogen content, an excellent plant probiotic that increases yield and quality of spinach (Jiménez-Gómez et al., 2018). According to a study by Nunes da Rocha et al. (2013) the rhizosphere is a common habitat for Acidobacteria, subdivision 8 (class Holophagae), with Holophaga 16S rRNA gene counts being more abundant in leek rhizosphere than in bulk soil and grass and potato rhizospheres. These authors propose to monetize strain CHC25 *Candidatus Porrumbacterium oxyphilus* (class Holophagae, phylum Acidobacteria), the first cultured representative with rhizosphere competence. Tubercles are formed on the roots of the common vetch, in which tuberous bacteria live symbiotically, for example from the genus *Rhizobium*. When analyzing the quality of 15 red beet varieties, Nizioł-Łukaszewska and Gawęda (2015) found that the 'Chrobry' variety showed the most favorable quality characteristics - characterized by high antioxidant activity, high content of dry matter, soluble sugars and betalain pigments. Continuous cultivation of sugar beet (*Beta vulgaris* L.) showed lower bacterial diversity and higher fungal diversity in the root-inhabited soil layer compared to intermittent cultivation (Cui et al., 2022).

Promoting organic farming can build an ecologically, nutritionally, and economically sound nation in the near future (Wang et al., 2021). Wang et al. (2021) determined carbon and enzyme activity of microbial biomass in a six-field crop rotation (tomato, cucumber, celery, dill, cauliflower, and eggplant) using

three management practices: low-input, conventional, and organic systems. These authors found that soil microbial biomass and enzyme activities (urease, catalase, protease) in the organic farming system were significantly higher in samples collected in December compared to those obtained from soil that had been cultivated with conventional practices with low input resources. Certain red beetroot clones produce abundant levels of the peroxidase enzyme with good thermal stability (Rudrappa et al., 2005).
The aim of the research is to analyze the effect of the biological cultivation of leaf and root agricultural crops on the occurrence and activity of beneficial groups of microorganisms in the microbiomes of three rhizocompartments of the root-inhabited soil layer (rhizosphere, rhizoplane and root). The literature review on the subject showed little research in the study area for the crops studied in the present material: dill, parsley, spinach, chard, leek, common vetch, red beet, especially for chard and common vetch.

MATERIALS AND METHODS

The experiment was carried out at the training field at the University of Forestry - Sofia, Bulgaria, during the vegetation period of the studied crops in 2022. Microorganisms from the rhizoplane (on the surface of the roots) and rhizosphere were studied, and soil particles near the roots were covered. The roots, together with the particles stuck to them, were ground and analyzed for microbiological and enzymatic parameters.
The studied variants are the following:
- V0 – control, soil without vegetation;
- V1 – dill (*Anethum graveolens* L., family Apiaceae, variety "Mesten");
- V2 – parsley (*Petroselinum crispum* Mill., family Apiaceae, variety "Festival");
- V3 – spinach (*Spinacia oleraceae* L., family Chenopodiacea, variety "Large winter");
- V4 – chard (*Beta vulgaris* ssp. *cicla* f. *hortensis* Alef. family Chenopodiaceae, variety “Mageneta sunset”);
- V5 – leek (*Allium ampeloprasum* L., family Alliaceae (Amarilidaceae), variety "Starozagorski 72");
- V6 – common vetch (*Vicia sativa* L., family Fabaceae, genus *Vicia*, variety “Tempo”)
- V7 – red beet (*Beta vulgaris* L. var. *esculenta*, family Chenopodiaceae, variety "Bordeaux").
The distribution of crops by product part is presented in Table 1.

Table 1. Distribution of crops by product part

Leaf crops	Leaf-stem crops	Mixed (leaf-stem and foliar crops)	Root crops	Fruit crops
Petroselinum crispum Mill.	*Allium ampeloprasum* L.	*Anethum graveolens* L.	*Beta vulgaris* L. var. *esculenta*	*Vicia sativa* L.
Beta vulgaris ssp. *cicla* f. *hortensis* Alef.				
Spinacia oleraceae L				

Microbiological analyzes include the determination of heterotrophic, aerobic, mesophilic groups of microorganisms: non-spore-forming bacteria, spore-forming bacteria (bacilli), actinomycetes, micromycetes (mold fungi), and bacteria assimilating mineral nitrogen. The method of limiting dilutions and subsequent inoculations on specific solid nutrient media (meat-peptone agar, Actinomycete isolation agar, Chapek-Dox agar – supplier: “Ridacom”, Bulgaria) was used (Mishustin and Emtsev, 1989; Gushterov et al., 1977). The colony-forming units for each group of investigated microorganisms were listed and the results were recalculated for 1 g of absolutely dry substrate. Total microflora and mineralization coefficient (MC) were calculated according to the formula: bacteria assimilating mineral nitrogen/(non-spore-forming bacteria + bacilli) (Mishustin and Runov, 1957; Malcheva and Naskova, 2018).
Catalase activity of the rhizoplane and rhizosphere microorganisms was determined by the titration manganese-metric method (Khaziev, 1976).
Cellulase activity of the rhizoplane and rhizosphere microorganisms was determined dynamically over a period of 60 days, and during 15 days the percentage of degraded area of the cellulose filter sheets (50/10 mm) placed on the surface of the roots and the soil adhering to them in petri dishes was calculated. A 60%

marginal field moisture content is maintained (Khaziev, 1976).

Moisture content of the samples was determined on a moisture balance, model DBS 60-3, KERN, Germany.

Statistical processing of the data from the microbiological indicators included the calculation of the mean of three replicates and the coefficient of variation (CV). A correlation analysis was applied for some of the investigated indicators. Data were statistically processed using Excel 2010.

RESULTS AND DISCUSSIONS

The composition and mineralization activity of the microorganisms from the rhizoplane and rhizosphere are presented in Table 2.

Table 2. Quantity (log_{10} cfu/g), composition, and mineralization activity of rhizoplane and rhizosphere microorganisms

Variants	Total microflora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-mycetes	Bacteria assimilating mineral nitrogen	Minerali-zation coefficient
V0 Control (without vegetation)	6.28	6.04	4.88	5.84	4.73	6.67	4.063
V1 Dill	6.75	6.71	5.44	4.85	4.85	6.50	0.585
V2 Parsley	5.46	5.28	4.85	4.04	4.22	6.27	7.104
V3 Spinach	6.54	6.42	5.79	5.07	4.89	6.51	0.985
V4 Chard	6.52	6.25	6.17	4.08	4.86	6.11	0.401
V5 Leek	6.86	6.79	6.01	4.32	4.76	6.62	0.584
V6 Common vetch	6.64	6.54	5.46	5.42	5.53	6.53	0.894
V7 Red beet	5.93	5.61	5.57	4.70	4.23	6.54	4.453

*CV up to 10% for all variants (low dispersion).

The biogenicity of the samples compared to the control (V0) increases when growing leeks (V5 - 3.8 times), dill (V1 - 2.9 times), common vetch (V6 - 2.3 times), spinach (V3 - 1.8 times) and chard (V4 - 1.7 times). Cultivation of parsley (V2) and red beet (V7) did not increase the total microflora compared to the control, without vegetation. In the variants with vegetation, biogenicity decreases in the order: leek (V5) > dill (V1) > common vetch (V6) > spinach (V3) > chard (V4) > red beet (V7) > parsley (V2). However, the mineralization activity is highest in the two variants with the lowest biogenicity - in the cultivation of parsley (V2) and red beet (V7), higher than in the control (V0). In the remaining variants, the values of the mineralization coefficient are lower than in the control. The rate of decomposition of organic matter in the variants with vegetation decreases in the order: V2 (parsley) > V7 (red beet) > V3 (spinach) > V6 (common vetch) > V1 (dill) > V5 (leek) > V4 (chard). Therefore, the activity of microorganisms does not depend solely on their quantity, which was also established in other studies (Yankova et al., 2016, Malcheva, 2021). One of the main factors for their development is soil moisture in the root layer. The moisture content of the tested samples is presented in Figure 1.

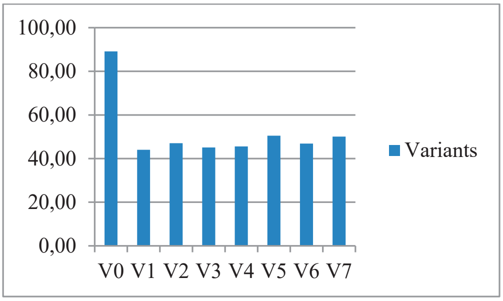

Figure 1. Rhizosphere and rhizoplane soil moisture (%)

The soil moisture in the control is about 2 times higher compared to the other variants. The moisture values of the roots with adhering soil

in the vegetated variants are close, with the highest moisture in leek (V5) and red beet (V7) and the lowest in dill (V1).
In the vegetated variants, non-spore-forming bacteria take the main share, followed by bacilli, which play a major role in the initial stages of decomposition of organic matter. In the red beet (V7) and chard varieties (V4), the percentage of non-sporing bacteria and bacilli is more evenly distributed: 48% non-sporing bacteria and 44% bacilli in red beet (V7); 53% non-sporing bacteria and 44% bacilli in chard (V4). While in the other variants, the percentage of non-sporing bacteria varies from 66% in parsley (V2) to 93% in dill (V1). Less represented are actinomycetes and mold fungi, which participate more actively in the final stages of organic destruction. In the control (V0), the quantity of actinomycetes was higher than that of bacilli. The percentage distribution of the groups of microorganisms in the composition of the total microflora in the control (V0) is as follows: non-spore-forming bacteria 57%, bacilli 4%, actinomycetes 36%, micromycetes 3%. The quantity of bacteria is higher than that of fungi when growing agricultural crops (Yankova et al., 2016; Plamenov et al., 2016; Malcheva et al., 2018; Malcheva et al., 2019; Malcheva, 2021). The highest percentage in the composition of the total microflora when growing vegetables is occupied by the ammonifying bacteria (non-spore-forming bacteria and bacilli), and the least represented are micromycetes and actinomycetes (Malcheva et al., 2020). A high bacterial abundance and a lower fungal development were found in dill cultivation (Sofo et al., 2020). Spore-forming bacteria, fluorescent *Pseudomonas* bacteria, diazotrophs, Actinomycetes, microscopic fungi have been isolated from parsley rhizosphere soil (Trzciński et al., 2018). *Rhizobium laguerreae* colonizes the roots of spinach plants (Jiménez-Gómez et al., 2018), and according to a study by Nunes da Rocha et al. (2013) the rhizosphere is a common habitat for Acidobacteria. Tubercles are formed on the roots of the common vetch, in which tuberous bacteria live symbiotically, for example from the genus *Rhizobium*. However, continuous cultivation of sugar beet (*Beta vulgaris* L.) showed lower bacterial diversity and higher fungal diversity in the root-inhabited soil layer compared to intermittent cultivation (Cui et al., 2022). Fungi are more closely related to environmental factors than bacteria (Cui et al., 2022).
The catalase activity of rhizoplane and rhizosphere microorganisms is presented in Figure 2.

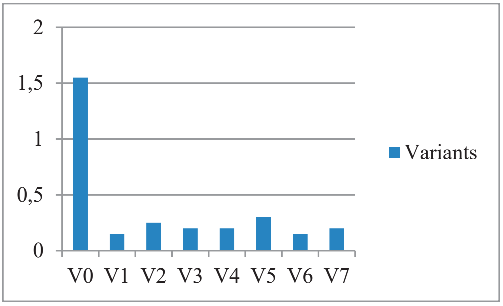

Figure 2. Catalase activity of the rhizoplane and rhizosphere microflora (ml O_2/30 min)

The results showed that catalase activity was significantly lower in the vegetated variants compared to the non-vegetated control. In the variants with vegetation, the value of the catalase enzyme is highest when growing leeks (V5), followed by parsley (V2), and the lowest is with dill (V1) and common vetch (V6). In leek (V5) cultivation, catalase activity correlates with the total quantity of microorganisms. While with parsley (V2) no dependence was established with a higher quantity of microorganisms, but with the highest mineralization activity. Therefore, catalase activity does not depend independently on the quantity of microorganisms. Influence is exerted by a complex of factors: type of soil and root system, soil temperature and humidity, pH, type of vegetation and other factors. When growing rapeseed, a lower catalase activity was also found in the variants with vegetation compared to the control without vegetation (Malcheva et al., 2019).
The cellulase activity of rhizoplane and rhizosphere microorganisms is presented in Figure 3.
Cellulose degradation is fastest in red beets (V7) and leeks (V5) – 100% degraded area on the 45th day.

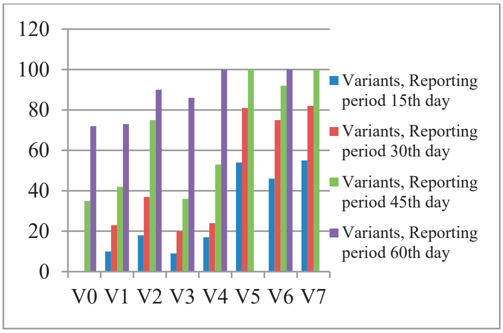

Figure 3. Cellulase activity of the rhizoplane and rhizosphere microflora (% degraded area)

Up to 100% degradation of the cellulose was also found in two other variants – chard (V4) and common vetch (V6), but on the 60th day of reporting. For a period of two months, the percentage of decomposed area for dill (V1) is 73%, for spinach (V3) 86% and for parsley (V2) 90%. Cellulase activity was higher in all variants with vegetation compared to the control (V0 no vegetation), with the cellulase value in dill (V1) being close to that of the control.

The correlation dependences between some of the studied indicators are presented in Table 3.

Table 3. Correlational dependencies

Indicator	Total microflora	Humidity	Catalase	Cellulase	MC
Total microflora	1				
Humidity	-0.25404	1			
Catalase	-0.24256	0.99322	1		
Cellulase	0.053794	-0.51287	-0.57085	1	
MC	-0.83879	0.310997	0.297103	-0.12264	1

A very high positive correlation was established between the humidity of roots and soil, and the catalase activity of the rhizoplane and rhizosphere microflora. The cellulase activity of microorganisms from the rhizoplane and rhizosphere is weakly positively dependent on the total quantity of microorganisms. A moderate correlation was found between humidity and mineralization coefficient values, as well as between the rate of decomposition of organic matter (MC) and catalase activity. In general, the results show that the activity of the rhizosphere and rhizoplane microflora depends on the humidity in the root zone, and the quantity of microorganisms is not the only and independent prerequisite for their activity.

CONCLUSIONS

Organic cultivation of leeks (V5), dill (V1), common vetch (V6), spinach (V3) and chard (V4) increases biogenicity on the roots and the root-inhabiting soil layer closest to the roots, to the highest extent in leeks (V5) and to the lowest extent in chard (V4). While the values of the total microflora in parsley (V2) and red beet (V7) are lower compared to the value of this indicator in the control (V0), without vegetation. However, the mineralization activity is highest in the two variants with the lowest biogenicity - in the cultivation of parsley (V2) and red beet (V7), higher than in the control (V0). A moderate correlation was found between humidity and mineralization coefficient values. The activity of microorganisms in the root layer does not depend solely and independently on their quantity.

The main share in the composition of the total microflora is occupied by non-spore-forming bacteria in all variants. In the vegetated variants (V1-V7), regrouping was found relative to the control (V0). In the control (V0), the actinomycetes, and in the variants with vegetation (V1-V7), the bacilli occupy the second place in the composition of the total microflora. In the red beet (V7) and chard (V4) varieties, the percentage of non-sporing bacteria and bacilli was more evenly distributed compared to the other varieties. Mold fungi and actinomycetes are least represented in cultivated crops. Non-spore-forming bacteria and bacilli are most actively involved in the initial stages of organic matter decomposition. While actinomycetes and mold fungi are more active decomposers of organic matter in the final stages of organic matter degradation in the variants with the studied agricultural crops.

Catalase activity was significantly lower in the vegetated variants (V1-V7) compared to the non-vegetated control (V0). In the variants with vegetation (V1-V7), the catalase value is the highest when growing leeks (V5), followed by parsley (V2), and the lowest is with dill (V1) and common vetch (V6). A complex of factors, including the type of vegetation and the presence of plant-derived catalase, are likely to be influential. Catalase is highly dependent on moisture in the root layer and is moderately dependent on the rate of mineralization of organic matter (MC).

Cellulase activity was highest in red beet (V7) and leek (V5), followed by chard (V4) and common vetch (V6), and lowest in dill (V1) and the control (V0). The results show that the activity of the enzyme depends on the type of vegetation. Correlation analysis showed that cellulase depends weakly positively on the total microflora on the roots and the nearest root-inhabiting soil layer.

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THE INFLUENCE OF CERTAIN AGROTECHNICAL MEASURES APPLIED IN CARROT TECHNOLOGY UNDER PEDOCLIMATIC CONDITIONS OF VEREŞTI COMMUNE, SUCEAVA COUNTY

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Abstract

Carrots are not particularly sensitive regarding cultivation technology but they require increased attention at certain stages. Some of these stages include the preparation of the seed bed, sowing and fertilization. These factors are: the timing and method of seedbed preparation, the sowing scheme, and the method of fertilization. The study was conducted over three years under the pedoclimatic conditions of Veresti Commune, Suceava County. The aim was to develop an innovative technology to increase production. Following the analysis of the data from the three years of study (2016, 2017, 2018) and the comparative analysis of the experimental variants, a significantly positive difference in production was highlighted in the experimental variant where the seedbed was prepared in the fall, the land was shaped, the sowing scheme was 15+15+75+15+15 and fertilization was done with NPK. In this variant, the production increase was 19786 kg/ha, compared to the variant where the seedbed was prepared in the spring, the land was not shaped, the applied sowing scheme was 15+15+75+15+15s, and fertilization was with NPK.

Key words: *fertilizers, seedbeds, quality, production, sowing scheme.*

INTRODUCTION

The focus of Scientific Research has shifted towards development and efficiency to increase productivity. Optimizing agricultural production is a very important and widely discussed aspect, as it is the only sustainable solution to ensure the food supply for the population (Gamez-Vazquez et al., 2022).
Food insecurity has increased in densely populated, urbanized areas with rising incomes. It is expected that these increases in insecurity will be particularly recorded in developing countries with rapid economic growth. All these changes create a favourable environment for the development of farms worldwide, and the food crisis can only be overcome through the involvement of the scientific community.
One of the crops that play an important role nutritionally and has health benefits is the carrot (Stoleru et al., 2016). This plant is among the top ten vegetables cultivated worldwide and represents a significant source of vitamin A (Barbu et al., 2022). After plants in the *Solanaceae* family, such as the potato, carrot roots are the most cultivated for their taste and nutritional properties (Omenn et al., 2005). The carotenoids are must influenced by technological factors as cultivars, fertilizers and irrigation regimes (Stoleru et al., 2020)
Carrots are a plant of significant importance from a dietary, medicinal and industrial perspective. This crop has a high economic yield because it is very productive, and the selling price is attractive for farmers (Mităluț et al., 2021).
The factors that influence production include pedo-climatic conditions, the variety or hybrid, the cultivation technology applied, etc. For example, for producing one ton of vegetable product requird to apply 3 kg N s.a., 1.5 kg P_2O_5 s.a., 5 kg K_2O. The amount of manure recommended for organic fertilization, is 30 t/ha for carrots crop (Dușan & Stan, 2023).
The study aims to observe the influence of innovative elements in carrot cultivation technology with positive effects on production. The experimental factors studied are the type and timing of seedbed preparation, the sowing

scheme, and fertilization (Merlin & Bertrand, 2020).

The purpose of this paper is to study the influence of the period and method of seedbed preparation, the sowing scheme, and fertilization on production.

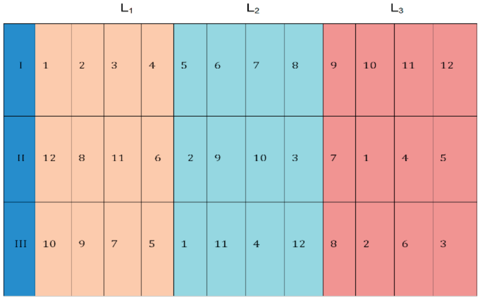
MATERIALS AND METHODS

The study aims to observe the influence of innovative elements in carrot cultivation technology with positive effects on production. Three experimental factors were studied: the first two factors with two levels and the third with three levels, as presented in Table 1.

Table 1. Experimental Factors

Experimental factors	Graduations of the experimental factors
Experimental factor A: Germinal bed preparation	A1 – Conventional seed bed preparation system (the spring, unshaped land)
	A2 – Unconventional seed bed preparation system (the autumn, shaped land)
Experimental factor B: Planting scheme	B1 – Conventional system (planting rows forming strips of 150 cm, distance between rows of 15 cm);
	B2 – Unconventional system (planting, 15 cm+15 cm+75 cm+15 cm +15 cm)
Experimental factor C: Fertilization	C1 – Fertilization with NPK C2 – Fertilization with manure C3 –Fertilization with manure and NPK

The method used to set up the experiment is the Latin rectangle, developed in 1952 by Mudra. This method was employed to study a large number of variants without increasing the number of replications. Mudra divided the columns into sub-columns, which together form a complete column. The number of variants must be divisible by the number of replications. The randomization of variants across columns does not depend on the randomization of variants across blocks; the only condition is that a variant must appear only once per column and also per block. Complete replications allow, within the method, uniform working conditions both in terms of blocks and columns. The large number of variants reduces the value of errors in obtaining results.



Replications: I, II, III; Sub-columns: L1, L2, L3
Experimental Variants: 1, 2, 3, ... , 12

Figure 1. Experimental Plot Layout

The biological material sown over the three years of the experiment is the Laguna F1 hybrid (Figure 2). This is a Nantes-type hybrid with a smooth, somewhat cylindrical root, uniform and very rapid growth. The colour is pleasant, intense orange, and has a commercially appealing appearance.

Figure 2. The hybrid F1 Laguna

In order to determine the physical, chemical, agronomic and morphological properties of the soil, soil samples were collected.

The soil samples were taken along the diagonals of the experimental plot, and laboratory analyses were performed at different depths (0-20 cm, 20-40 cm and 40-60 cm, respectively). Samples were collected on April 6th 2016, April 6th 2017 and April 6th 2018 using a soil auger, from 5 different points along each diagonal of the plot (Rusu, 2005).

The following agrochemical indices were determined: granulometric analysis by the pipette method with result interpretation; pH by the potentiometric method; humus (%) by the Walkley-Black method; total N (%) by the Kjeldahl method; potassium availability by the flame photometric method; phosphorus availability by the colorimetric method; and base saturation degree (V% = SB/T x 100) (Table 2).

Table 2. Agrochemical indices of the Soil

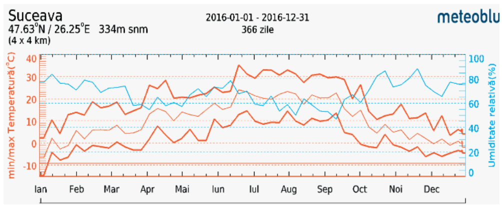
The bottom (cm)	0-20	20-40	40-60
The coarse sand	0.10	0.09	0.047
The fine sand	46.67	36.03	35.62
The dust I	10.40	18.03	22.79
The dust II	14.92	13.49	8.84
The clay	27.91	32.36	32.70
The interpretation of soil texture	The sandy loam	The loam	The loam
pH	7.68	7.80	7.92
The interpretation	Slightly alkaline	Slightly alkaline	Slightly alkaline
The carbons $CaCO_3$	1.5	1.8	1.80
The interpretation	Slightly carbonated	Slightly carbonated	Slightly carbonated
The humus %	2.15	2.39	1.38
The interpretation	Slightly	Medium	Slightly
Total N %	0.174	0.219	0.187
The interpretation	Moderate	Good	Moderate
The mobile $P_2 0_5$ ppm	40	29	30
The interpretation	Good	Moderate	Moderate
The mobile $K_2 0$ ppm	140	116	110
The interpretation	Good	Moderate	Moderate

Carrots are a plant that develops in a temperate climate. Seeds germinate at minimum temperatures of 3-4°C, with a germination period of 20 days at these temperatures. At optimal germination temperatures, between 20-25°C, the germination period shortens to 10-12 days (Apahidean and Apahidean, 2000). The optimal temperature for root growth is in the range of 18-22°C. High temperatures favour more vigorous leaf development and result in smaller roots. Root shape is influenced by temperature; lower temperatures and high precipitation favour the formation of sharp, long, and poorly coloured roots. Carrot plants can withstand temperatures as low as -5°C. The vernalisation process occurs at temperatures of 5-10°C over a period of 36-70 days (Indrea et al, 2012).

For normal carrot crop development, soil moisture should be between 65-75% of field capacity during the initial growth period, and then increase to 75-80% during root thickening. The most sensitive periods to moisture are germination, leaf formation, and root thickening (Bender et al, 2009). The climatic parameters in Verești commune are similar to those in Suceava city, considering the geographical position relative to the city and the distance of approximately 21 km. Minor differences may be recorded due to the presence of the two rivers, Siret and Suceava.

The year 2016 was climatically erratic, with alternating drought months and months of abundant precipitation. In June and August, reduced precipitation and high temperatures favoured the occurrence of soil drought, while the abundant precipitation in October led to significant losses in agricultural production.

As shown in Figure 3, the maximum temperature from January 1 to December 1, 2016, was recorded at the end of June and exceeded 35°C. The minimum temperature was below -10°C and was recorded in January (Figure 3).

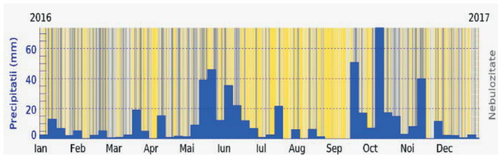


The maximum/ minimum temperature

The relative humidity

Figure 3. The evolution of air temperature and humidity during January-December 2016
Source: www.meteoblue.com

Precipitation from January 1, 2016, to December 31, 2016, can be observed in Figure 4. In mid-October, the recorded values were over 65 mm, while the smallest amounts of precipitation were recorded in September (20 mm).

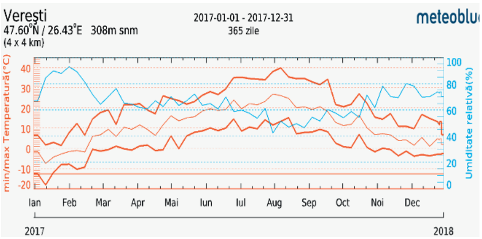


the amount of precipitation

Figure 4. The precipitation evolution during January-December 2016
Source: www.meteoblue.com

In 2017, from the perspective of the most important climatic parameters - temperature and precipitation - the year was relatively erratic.

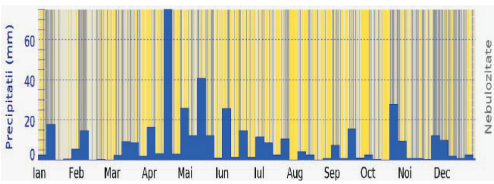
The amount of precipitation was low and uneven. Monthly average temperatures fell within the multiyear averages (8°C), but they varied only in March, July, and August. Large temperature differences between day and night had negative effects on agricultural production. In the spring, we faced temperature fluctuations and recorded late frosts that affected the carrot crop. During the summer, precipitation was low, and temperatures were high (Figure 5).
The maximum temperature from January 1 to December 1, 2017, was recorded in mid-August and was approximately 40°C. The minimum temperature was recorded in January and was approximately -20°C.



The maximum/ minimum temperature
The relative humidity

Figure 5. The evolution of air temperature and humidity during January-December 2017
Source: www.meteoblue.com

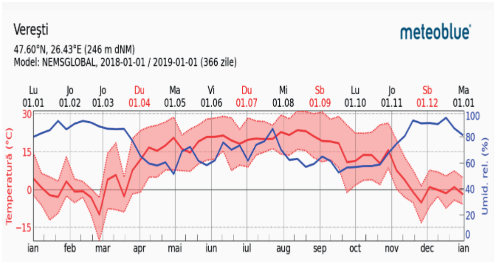
Precipitation from January 1, 2017, to December 31, 2017, can be observed in Figure 6. The maximum amount of precipitation was recorded in mid-April, exceeding 65 mm, while the minimum amount of precipitation was below 5 mm in mid-October.



the amount of precipitation

Figure 6. The precipitation evolution during January-December 2017
Source: www.meteoblue.com

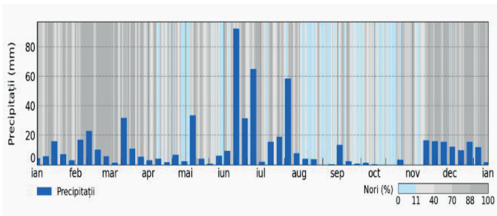
In 2018, the year was not as erratic as 2017, and the climatic conditions were favourable for carrot cultivation. Precipitation was adequate and temperatures were moderate. In the spring, we faced reduced precipitation and high temperatures, which was a critical period for soil water availability. There were also temperature fluctuations during this time. In the summer, precipitation was balanced and temperatures were moderate.
The maximum temperature from January 1 to December 1, 2018, was recorded in the latter part of August and was around 35°C. The minimum temperature was recorded at the end of February and was below -15°C (Figure 7).



The maximum/ minimum temperature
The relative humidity

Figure 7. The evolution of air temperature and humidity during January-December 2018
Source: www.meteoblue.com

Precipitation from January 1, 2018, to December 31, 2018, can be observed in Figure 8. The maximum amount of precipitation was recorded in mid-June, exceeding 89 mm, while the minimum amount of precipitation was 0 mm from the end of September to the end of October (Figure 8).



the amount of precipitation

Figure 8. The precipitation evolution during January-December 2018
source: www.meteoblue.com

Observations were made on the development stage of the crop in different phenophases of the carrot plants (Figure 9). The uniformity of the crop and the development of the roots in length and diameter were monitored. The height of the carrot leaves was measured at 10 points of each experimental plot, and samples consisting of 10 carrots were taken to measure the diameter and length of the roots.

Figure 9. The observations in experimental field

The evaluation of agricultural production was determined to establish the quantity. This action was carried out periodically, in phenophases specific to the species, especially after root thickening, 10-20 days before harvest and after harvest.

Production was evaluated through gravimetric measurements, and the obtained results were processed in Microsoft Excel.

The interpretation of the results was carried out using the multi-comparison test method, specifically the Duncan test. This type of test is especially applied in multifactorial experiments. Data processing was conducted using the DSAASTAT.xls database.

RESULTS AND DISCUSSIONS

The results obtained from the experimental field from 2015-2018 are presented in Table 3, where differences in production under the influence of experimental factors can be observed.

Table 3. The productions obtained in 2015-2018

Experimental variants	Repetition I kg/ha	Repetition II kg/ha	Repetition III kg/ha	Arithmetic average kg/ha
a1b1c1	39866	46523	41900	42763
a1b1c2	46400	48113	42166	45560
a1b1c3	34600	35520	36100	35406
a2b1c1	38300	38646	38166	38371
a2b1c2	44916	42450	43066	43477
a2b1c3	37066	37280	46116	40154
a1b2c1	44500	43500	46116	44705
a1b2c2	38836	37906	39233	38658
a1b2c3	63403	44113	43100	50205
a2b2c1	65286	54380	54806	58157
a2b2c2	56833	39336	44723	46964
a2b2c3	46700	43263	42533	44165

As observed in the graphical representation of the yields obtained (Table 3) during the period 2015-2018, the best results were achieved in the experimental variant where the seedbed was prepared in the fall, the soil was ridged, seeds were sown according to scheme 15+15+75+15+15, and fertilization was done with NPK. In contrast, the experimental variant V3 yielded the lowest average production per replication, at 35520 kg/ha. In this experimental plot, the seedbed was prepared in the spring, the soil was not ridged, seeds were sown in bands of 150 cm with 15 cm between rows, and fertilization was done with NPK and manure. Good results were also obtained in experimental variants where the seedbed was prepared in the fall, the soil was ridged, seeds were sown according to scheme 15+15+75+15+15, and fertilization was done with NPK and manure. It can be seen that average yields of over 40000 kg were also obtained in experimental variants where the seedbed was prepared in the spring, seeds were sown in bands of 150 cm width with a distance of 15 cm between rows, and fertilization was done with manure.

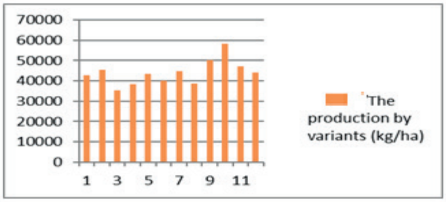


Figure 10. The production by variants, 2015-2018

In Table 4 we can observe the difference of production between variants due to the influence of experimental factors.

As we can observe (Table 4), the best results in terms of production were obtained in the variant where the seedbed was prepared in the fall, the soil was ridged, seeds were sown according to scheme 15+15+75+15+15, and fertilization was done with NPK. Compared with the variant where the seedbed was prepared in the spring, while the sowing scheme and fertilization were carried out in the same manner as in the previously mentioned variant, a production increase of 19786 kg was recorded. Distinctly highly significant positive results were also obtained in the variant where the seedbed was prepared in the fall, the soil was ridged, seeds were sown in bands of 150 cm with a distance of 15 cm between rows, and fertilization was done with NPK during the growing season, with manure applied during seedbed preparation.

Table 4. The comparative interpretation of data - influence of factor A to B and C (The planting seed scheme, the preparation of seeds bed and fertilization)

Variant	Production	%	Difference	Difference of significance
$a_1b_1c_1$	42763	100	0	Mt
$a_2b_1c_1$	44705	104	1942	*
$a_1b_1c_2$	45559	100	0	Mt
$a_2b_1c_2$	38658	84	-6901	0
$a_1b_1c_3$	35406	100	0	Mt
$a_2b_1c_3$	50205	142	14798	***
$a_1b_2c_1$	38370	100	0	Mt
$a_2b_2c_1$	58157	151	19786	***
$a_1b_2c_2$	43477	100	0	Mt
$a_2b_2c_2$	46964	108	3486	-
$a_1b_2c_3$	40154	100	0	Mt
$a_2b_2c_3$	44165	110	4011	-
DL (p 5%) = 435 kg/ha; DL (p 1%) = 898 kg/ha; DL (p 0.1%) = 1082 kg/ha;				

*positive not significant difference
0 - negative not significant difference
***distinctly highly significant positive difference
- not significant difference

In Table 5 we can observe the difference between variants generated by experimental factors.

From the comparative analysis of the experimental factors B, A, and C, we can observe that the highest yield was obtained in the experimental variant where the sowing scheme was 15+15+75+15+15, the seedbed was prepared in the fall, the soil was ridged, and fertilization was done with NPK. In this variant, the production difference is distinctly highly significant positive, with an increase of 30%, compared to the variant where the sowing scheme was in bands of 150 cm with a distance of 15 cm between rows, fertilization was done with NPK, and the same seedbed preparation method was used. Thus, we can recommend that the carrot crop be sown according to sowing scheme 15+15+75+15+15, the seedbed be prepared in the fall, the soil be ridged, and fertilization be done with NPK.

Table 5. The comparative interpretation of data's - influence of factor B to A and C (The planting seed scheme, the preparation of seeds bed and fertilization)

Variants	Production	%	Difference	Difference of significance
$b_1a_1c_1$	42763	100	0	Mt
$b_2a_1c_1$	38370	89	-4392	00
$b_1a_1c_2$	45559	100	0	Mt
$b_2a_1c_2$	43477	95	-2082	0
$b_1a_1c_3$	35406	100	0	Mt
$b_2a_1c_3$	40154	113	4747	*
$b_1a_2c_1$	44705	100	0	Mt
$b_2a_2c_1$	58157	130	13452	***
$b_1a_2c_2$	38658	100	0	Mt
$b_2a_2c_2$	46964	122	8305	*
$b_1a_2c_3$	50205	100	0	Mt
$b_2a_2c_3$	44165	88	-6040	-
DL (p 5%) = 731 kg/ha; DL (p 1%) = 1061 kg/ha; DL (p 0.1%) = 1620 kg/ha;				

*positive not significant difference
***distinctly highly significant positive difference
0 - negative not significant difference
00 - significantly negative difference
- not significant difference

Table 6. The comparative interpretation of dates - influence of factor C, A to B (The fertilization method, preparation of seeds bed and planting scheme)

Variant	Production	%	Difference	Difference of significance
$c_1a_1b_1$	42763	100	0	Mt
$c_2a_1b_1$	45559	107	2796	*
$c_3a_1b_1$	35406	83	-7356	00
$c_1a_1b_2$	38370	100	0	Mt
$c_2a_1b_2$	43477	114	5106	**
$c_3a_1b_2$	40155	115	1783	*
$c_1a_2b_1$	44705	100	0	Mt
$c_2a_2b_1$	38658	87	-6047	0
$c_3a_2b_1$	50205	112	5500	**
$c_1a_2b_2$	44165	100	0	Mt
$c_2a_2b_2$	46964	106	2798	*
$c_3a_2b_2$	58157	131,68	13992	***
DL (p 5%)= 1421 ha/kg; DL (p 1%)= 1622 kg/ha; DL (p 0.1%)= 2472 kg/ha;				

*positive not significant difference
***distinctly highly significant positive difference
00 - significantly negative difference
- not significant difference

A comparative analysis of the results obtained in the experimental variants under the influence of the gradations of experimental factor C, in relation to factors A and B, showed that the highest production was achieved in the experimental variant where fertilization was done with NPK and manure, the seedbed was prepared in the fall and ridged, and sowing was done according to scheme 15+15+75+15+15. The production differences were distinctly highly significant positive compared to the variant where fertilization was done with NPK and the seedbed was prepared in the fall, ridged, and the sowing scheme was 15+15+75+15+15. It is recommended to fertilize the carrot crop with manure and NPK, provided that the seedbed is prepared in the fall, the soil is ridged, and sowing is done according to scheme 15+15+75+15+15. Distinctly significant negative differences were recorded in the variant where fertilization was done with NPK and manure, the seedbed was prepared in the spring, the soil was not ridged, and sowing was done in bands of 150 cm with a distance of 15 cm between rows.

Table 7. The synthesis of the comparison through the Duncan test

No.	Variants	Production	Classification
1	$a_1b_1C_3$	35406	A
2	$a_1b_2C_1$	38370	AB
3	$a_2b_1C_2$	38658	ABC
4	$a_1b_2C_3$	40154	ABC
5	$a_1b_1C_1$	42763	ABCD
6	$a_1b_2C_2$	43477	ABCD
7	$a_2b_1C_3$	44165	BCD
8	$a_2b_2C_1$	44705	BCD
9	$a_1b_1C_2$	45559	BCD
10	$a_2b_2C_2$	46964	CD
11	$a_2b_1C_3$	50205	D
12	$a_2b_2C_1$	58157	E

In the interpretation of the data using Duncan's test, it can be observed that variants with similar influences and productions are marked with common symbols in the classification column (Table 7). Thus, there are no significant differences between the variants where the seedbed was prepared in the spring, sowing was done in bands of 150 cm with a distance between rows of 15 m, and fertilization was done with manure, and the variants where the seedbed was prepared in the spring, the soil was not ridged, sowing was done according to scheme 15+15+75+15+15, and fertilization was done with NPK and manure. Comparing the results from all experimental variants, it can be observed that significant production differences were obtained in the variant where the seedbed was prepared in the fall, the soil was ridged, sowing was done according to scheme 15+15+75+15+15, and fertilization was done with NPK. On the opposite end, with a production of 35406 kg and significant negative results, is the variant where the seedbed was prepared in the spring, the soil was not ridged, sowing was done in bands of 150 cm with a distance of 15 cm between rows, and fertilization was done with NPK and manure. In recent years and especially at the U.S.A.M.V. Different studies were carried out in Cluj-Napoca with the aim of improving carrot culture technology. Bota, during three years, studied the optimal time for seeding and fertilization specific to root vegetable crops, including carrot crops. Mănuțiu, he studied the behaviour of 2 carrot hybrids (FLAKKER-3, NANTES-5), in the period 2010 – 2012, under the influence of sowing time and facial chemical fertilization. The best production results were obtained for the Flakker-3 hybrid, in the first sowing season (March) with an increase of 5.08 t. He found that the Fakker-3 hybrid reacted favourably (35.30 t), when sown in the first stage (March) and chemical phase fertilization, while Nantes-5 hybrid reacted favourably to manure fertilization (29.02 t) and sown in the same period (March).

The productions obtained in the other experimental variants are close in values. The linear regression of production, based on the interaction of the experimental factors, shows a uniformly upward trend across the entire graphical representation.

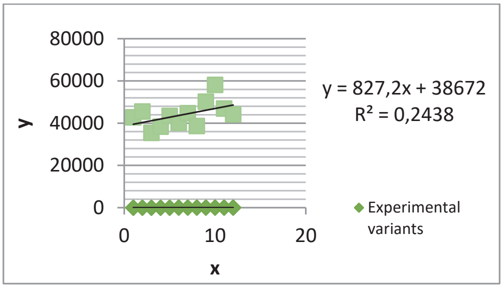


Figure 11. The linear regression of production

From the graphical representation, it can be observed that production can either increase or decrease depending on the method of seedbed preparation. It is noted that if the seedbed is prepared in the fall, the soil is ridged, sowing is done according to scheme 15+15+75+15+15, and fertilization is done with NPK, the production difference is distinctly highly significant positive, compared to the variant where the seedbed is prepared in the spring, the soil is not ridged, sowing is done in bands of 150 cm with a distance between rows of 15 cm, and fertilization is done with NPK.

CONCLUSIONS

Thus, based on the three years of studies and the comparative analysis of the experimental variants under the influence of the three experimental factors, a distinctly highly significant positive production difference is highlighted in the experimental variant where the seedbed was prepared in the fall, the soil was ridged, sowing was done according to scheme 15+15+75+15+15, and fertilization was done with NPK. In this variant, the production increase is 19786.66 kg/ha, compared to the variant where the seedbed was prepared in the spring, the soil was not ridged, sowing was done according to scheme 15+15+75+15+15, and fertilization was done with NPK. Therefore, it is recommended to prepare the seedbed in the fall, ridge the soil, sow according to scheme 15+15+75+15+15, and fertilize with NPK. The other variants yielded significant positive results with similar values.

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HEALTHY PLANTS BY INCREASING SOIL VITALITY - MONITORING RESULTS OF SOIL ANALYSES IN VEGETABLE CULTIVATION SYSTEMS

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Abstract

The production of healthy vegetables first requires a healthy soil in which to grow. The aim of the research is to evaluate the state of soil fertility in vegetable field cultivation in central Europe and to create a catalogue of measures to stabilize or even increase the soil fertility. To achieve this goal, a scientific monitoring of 13 pilot farms was taking place, start with soil analysis, the selection of area-specific measures and end with the evaluation of the effectiveness of these measures. Soil sampling was done 2021 from 0 - 30 cm and 30 - 60 cm. In one third of the fields the pH was below 6.0. Low humus contents of <2% were also found in one third. Humus enrichment is often advisable. An increase in diversity through intercrops promotes soil life in addition to organic matter. The results showed that the levels of macronutrients were in 50% of the fields very high. As a result, fertilization of P and K can be saved. The success in maintaining good soil vitality should be analysed two years after implementation.

Key words: *soil fertility, nutrient supply status, soil microbial biomass, soil organic carbon, food production.*

INTRODUCTION

Agricultural land worldwide is under increased production pressure. The soil is an important factor for effective food production. Intensive anthropogenic use as well as natural processes leads to nutrient losses and degradation over long time. Our research project "Healthy Soil - Healthy Plants - Healthy Vegetables" aims in to analysing the current state of soil fertility in different farms with divers soil properties in vegetable production areas. Soil fertility is the ability of soils to serve as a site for plants and is related to management effects and yield.

The content, quantity and quality of the humus are decisive in this respect. It influences soil physical, chemical and biological properties, such as water and nutrient balance, cation exchange capacity, pH value and mineralization (Thiel, E. and Kolbe, H., 2011).

The supply of nutrients, especially N, P, and K, from minerals or fertilizers to the soil affects the availability of other nutrients. In turn, the input of nutrients alters soil biogeochemical cycling. Biological characteristics are also used to estimate soil fertility influenced by agricultural activity. These are often soil biological parameters, such as soil organic matter, C/N value, and microbial biomass. Moreover, soil organic matter is considered an important factor of soil fertility. A major driver of SOC turnover are microorganisms. Microorganisms promote the formation of macroaggregates to physically protect carbon. Microbial biomass also plays a critical role in influencing the SOC pool (Zhang et al., 2013). Generally, up to 5% of the total organic carbon and nitrogen is in the soil microbial biomass. A change in microbial biomass can affect the cycling of soil organic matter. To date, there is no dataset on this scale from vegetable crops in Central Europe. During this project, numerous soil samples were collected at various locations in Germany, Poland and the Czech Republic. Now a large dataset of variety of soil chemical and biological parameters in Central Europe is available. Therefore, this work represents a first overview of the status of vegetable production areas. Field specific agronomic measures are recommended from the identified deficits.

One aim of the project is to develop a set of measures to increase the soil fertility and vitality in outdoor vegetable field production. After recording the actual state of the prevailing soils and management measures, the evaluation of the effectiveness of area-specific proposed measures on soil vitality will be conducted.

MATERIALS AND METHODS

The present study was conducted in fields with vegetable growing in Germany (7 farms), Poland (4 farms) and Czech Republic (2 farms). The research areas have an average annual temperature between 8.5 and 10.0°C. The average annual rainfall varies from 660 mm to 800 mm. In last years, rainfall has varied widely and occurs locally. Soils in the studied area are defined mostly as sandy loam (USDA texture classification), but differ from light to heavy soils. A total of around 50 fields with vegetable and arable land use were taken and analysed. The soil samples were collected out of the growing season 2021. Soil sampling was performed on a selected area of approximately 2 ha of the field, depending on the field size. In each field, minimum 20 samples from 0-30 cm and 30-60 cm depth were sampled with a borstick. They were mixed and homogenized for each plot and divided into two subsamples. One subsample was dried and ground before chemical and physical analyses. The other was stored in plastic bags at 4°C PLFA (phosphor lipid fatty acids) analyses. Fungal and bacterial biomass C were measured using this method. To determine the soil organic matter, we used the method of Eurofins Agro accredited by RvA (NIRS (TSC®). For grain size, the analysis ÖNORM L1061-2 (pipette method) was used. Soil pH was determined with $CaCl_2$ (DIN ISO 10390). The concentration of soil nutrient reserves was determined using an extract with ammonium lactate, pH 3.75 (Egner, H. & H. Riehm 1955). The CAT extraction of Mg, B, Mn, Cu and Zn was carried out by VDLUFA. The soluble fraction extracting agent of the nutrient fractions was a 1:5 water extract. The CaO reserve is the sum of the exchangeable Ca ions and the soluble, carbonated Ca ions in the extract. Heavy metal analysis is based on DIN EN 15763 and DIN EN ISO 17294-2:2017-01 and is performed by pressure digestion followed by determination by ICP-MS.

RESULTS AND DISCUSSIONS

The differences in soil substrates are shown by the fact that the soil properties vary greatly (Table 1). The coefficients of variation (cv-%) often assume values above 50%. Only for the parameters pH and C/N was the cv rather low, as expected, in the range between 14.5 and 17.8.

Table 1. Soil physiochemical and microbial properties

variable	Units	49 vegetable fields			
		Mean	Min	Max	Cv %
sand	%	61.1	5.1	93.0	43.7
silt	%	26.3	3.7	72.0	75.6
clay	%	12.7	1.7	43.0	75.0
SOC	%	1.17	0.50	2.30	41.9
pCEC	meq/100 g	11.90	4.40	33.90	55.4
pH	-	6.43	3.90	7.50	14.5
TN	%	0.14	0.07	0.28	33.7
C/N	-	9.1	6.5	13.7	17.8
K2O-Res	mg/100 g	23.0	7.0	65.0	51.6
P2O5-Res	mg/100 g	43.5	16.0	114.0	53.4
Mg-CAT	mg/100 g	10.1	1.8	24.7	45.1
B-CAT	mg/100 g	5.3	0.6	14.8	66.7
Mn-CAT	mg/100 g	587.3	91.0	2185.0	83.1
Cu-CAT	mg/100 g	22.1	5.3	86.8	76.3
Zn-CAT	mg/100 g	40.8	12.0	199.0	83.5
CaO-Res	mg/100 g	811	11	6766	191.4
Humic	%	2.14	0.90	4.80	38.0
MBC	mg C/kg	144.8	58.0	365.0	46.7
BBC	mg C/kg	61.0	23.0	161.0	48.8
FBC	mg C/kg	46.3	15.0	147.0	57.3
Pb	mg/kg	14.5	5.4	38.9	52.2
Cd	mg/kg	0.6	0.0	18.6	466.1
Cu	mg/kg	9.8	2.3	29.5	72.0
Hg	mg/kg	0.03	0.00	0.19	132.9

SOC- soil organic matter; pCEC- potential Cation Exchange Capacity; TN- total nitrogen; Res. –reserve, CAT- calcium chloride/DTPA method; MBC- soil microbial biomass carbon (Cmic); BBC- bacterial biomass carbon (Cbac); FBC- fungal biomass carbon (Cfungi)

pH values ranged from 3.9 to 7.5 in the soils. Maximum values of CaO reserve of 6,766 mg/100 g were observed. The minimum values were 11 mg/100 g for vegetable crop fields.

The soil results revealed deficits with regard to soil fertility. Thus, very low pH values occur, in extreme case this was 3.9 in one field. This can have numerous consequences on soil dynamics, such as ionic bonds and microorganism activities. Low pH may reduce Mg uptake and reduce available organic phosphorus. Our correlation results could not provide confirmation of this. Liming could be an effective tool to improve soil nutrient retention. Mg-CAT contents varied between 1.8 and 24.7 mg/100 g. Large differences also appear in the content of the micronutrient boron. In the fields, the values ranged from 0.6 to 14.8 mg/100 g. Our study partly revealed a deficiency of nutrients, mainly B as well as Mg, in the soil.

Very low levels of SOC (soil organic matter), reservers of CaO and various nutrients could also be observed. Due to these deficiencies, measures to improve these soils are necessary to ensure soil fertility in the long term. Corg content has roughly equal ranges of approximately 0.5 to 2.3%. C/N values are approximately 9.0. 30% of the fields have a low Corg and therefore humic substance content.

A look at the field history index provides information of the use of organic fertilizers. 25% of the companies don't use organic fertilizer but under-ploughing plant residues or straw of crops. 5 of 13 companies used manure (horse, cattle, chicken) as a fertilizer, 4 of 13 liquid manure, 3 fermentation residues and only one company compost as a long term fertilizer. As recommendation they should use more effective organic additives in most of the fields.

All companies use intercrops (e.g., yellow mustard, phacelia, Terra Life, sand oats, sudan grass, oil radish, cereal varieties). This is good for the water balance, microorganisms and soil organic matter. As recommended measure they should further cultivate them as well as use longer growing times, especially over winter time.

A theoretical calculation of the humus balance can also be carried out using the field index. This provides information on the development of soil organic matter. Over 60% of farms have too long fallow periods (>25%) and a negative humus balance (<-75 HÄQ/ha, according to VDLUFA). Our analyses revealed good values for the microbiome. The maximum values of microbial biomass in C are 365 mg C/kg in vegetable crop fields, the minimum value was 58 mg C/kg, respectively. Approximately 20% of the fields have a lack of microbial biomass (Figure 1).

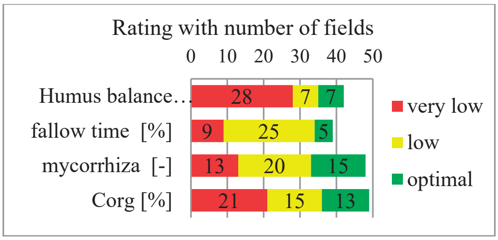

Figure 1. Evaluation of calculated parameters of the field history and soil parameters

Some relationships between soil properties are shown in Table 2. In general, sand content is negatively correlated with pH, total nitrogen (TN) and microbial parameters. However, no significant linear correlation was found between the sand content and the SOC in the soil. There is a close correlation between the total nitrogen content and soil organic matter and the microbial (especially bacterial) biomass in the soil. The revealed relationship between soil organic matter (SOC) and microbial biomass in carbon (MBC) highlights the important role of SOC in soil fertility, such as nutrient availability and the contribution of microorganisms to soil structure. Specifically, soils with SOC enrichment have more microbes to promote nutrient availability and soil structure development (Liddle et al., 2020).

Table 2. Correlation between measured parameters

	Corg	pH	MBC	BBC	FBC	TN	Clay	sand	Mg-CAT
Corg	1.0								
pH	-0.12	1.0							
MBC	0.61	0.34	1.0						
BBC	0.62	0.36	0.99	1.0					
FBC	0.51	0.38	0.97	0.96	1.0				
TN	0.87	0.25	0.81	0.82	0.74	1.0			
clay	0.4	0.62	0.78	0.78	0.72	0.74	1.0		
sand	-0.18	-0.66	-0.62	-0.61	-0.63	-0.52	-0.80	1.0	
Mg-CAT	0.37	0.36	0.61	0.59	0.62	0.57	0.62	-0.46	1.0
B-CAT	0.32	0.75	0.56	0.68	0.57	0.63	0.71	-0.71	0.45

The high correlations between microbial biomass and magnesium and boron CAT contents are also of interest. However, despite the importance of the soil microbial community for soil fertility, the effects of boron are still unknown (Vera et al., 2019). Boron has a noticeable effect on the soil nitrogen cycle. According to their study, the analysis of PLFAs showed that the effect of B on soil microbial biomass was dependent on the chemical form of fertilizers used. The correlation coefficients between boron and TN in our samples were 0.63 in vegetable crop fields. According to Vera et al. (2019), high B doses reduce soil microbial respiration, and fungal diversity is reduced. This was confirmed by our analyses. The correlation between boron and fungal biomass in carbon was between 0.54.

However, microbial biomass in vegetable fields correlates strongly (0.59-0.62) with Mg-CAT-content. According to Yang et al. (2021), who studied the short-term application of Mg fertilizers on soil microbial biomass and functions, the application of $MgSO_4$ significantly increases the C-mic content (MBC).

CONCLUSIONS

Due to frequently too low humus content and microbial biomass, the following measures are recommended for vegetable farms: more use of organic fertilizers, use of microbial soil additives and use of (new) intercrops. After consultation with the farmers of all 13 farms, a total of 71 measures were defined for the individual fields (Table 3).

Table 3. Measures resulting from the deficits

Measure	N=71
Addition of organic fertilizers	19
Calcification	18
Addition of micronutrients	16
an/ a new intercrop	10
Addition of microbial organisms	8
only observation	4

Thus, studies with altered management practices and the influence of micronutrient fertilizers, limes, and organic soil amendments are planned. The derivation of individual area-specific measures to maintain and increase soil fertility and biodiversity is to be based on the actual state determined here.

Soil organic carbon is an important parameter that determines soil fertility and maintains soil health. Chemical fertilization alters the soil nutrient status, which indirectly affects the soil carbon balance, while organic fertilization replenishes the soil carbon balance by directly adding organic carbon to the soil.

Various measures were implemented 2022 on a subplot of the pilot farms, and their success in improving and maintaining good soil vitality will be analysed and documented 2024. Maintaining and improving soil quality requires an understanding of how soils respond to agricultural and horticultural practices over time and the ability to quantitatively measure or monitor changes (Goh et al., 2001). The results are summarized with theoretical knowledge in a catalogue of measures and made available to the farmers.

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NEW VARIETY OF *SOLANUM SISYMBRIIFOLIUM* OBTAINED AT BRGV BUZĂU, ROMANIA

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Abstract

In addition to the classic tomato species of Solanum lycopersicum, the main relict genetic resources with a wild character preserved in the germplasm collection of BRGV Buzau are part of the following subspecies: Solanum torvum, Solanum caripense, Solanum melanocerasum, Solanum quitoense, Solanum mamossum, Solanum sisymbriifolium, Solanum nigrum. Of these, Solanum sisymbriifolium is the genotype that clearly expresses the authenticity of a wild tomato, in its ancestral form, as it was known since ancient times. The species was phenotypical, qualitative, and quantitative evaluated at the BRGV Buzau, obtaining a new variety being under approval and named Matilda. The plant has yellow-brown aggressive thorns with an average height of 14.5-15.4 mm on the entire vegetative part, both on the stem, as well as on the shoots, leaves, inflorescence. The berry-type fruit, with an average weight of 7.6 g, is encapsulated in a persistent calyx ornamented with thorns. It is used in intraspecific and interspecific hybridizations, using the rusticity of the species, noting that it grows and fruits well in extreme temperature conditions, having a high capacity to adapt to thermo-hydric stress.

***Key words**: biodiversity, conservation, cultivar, litchi, rusticity.*

INTRODUCTION

Solanum sisymbriifolium, originates from warm, temperate South America, is an annual or perennial erect, rhizomatous, shrubby weed with an extensive root system and spiny leaves, currently distributed throughout the world and invasive in some countries (Perpétuo et al., 2021).

It grows in ruderal locations and wild, along roadsides and in waste places, landfills and plowed fields. It is naturally pollinated by insects and widely propagated by seeds, although vegetative propagation by rhizomes is also possible. Due to its vast dispersion and rapid growth, it is also considered an invasive weed (Di Ciaccio, 2021).

The rapid spread of this species within a short span of about two years indicates the potential for this perennial shrub as a harsh invader of upland sites of the region (Shukla, 2015).

The beginnings of tomato breeding begin with Luther Burbank (1849-1926), botanist, horticulturist and the pioneer of raising breeding to the rank of science. Among his many creations, we also find wild Brazilian tomatoes, described in one of his volumes, "His Methods and Discoveries- Their practical application" (Figure 1). He tried to improve the Brazilian wild tomatoes, describing them as "relatives of the potato and tomato, with fruits similar to tomatoes" (Burbank, 1914) The fruit presented is in the transition phase, after Mr. Burbank worked on its improvement through selection for several generations, but failing to polish it. The fruit, however, increased in size and became much more aromatic. Other experiments carried out by Mr. Burbank aimed at mutual hybridization between tomato varieties producing a larger quantity of seeds. The F1 generation showed individuals with intermediate characteristics. The fruits obtained were bigger than those of the parents. Burbank then selected the most attractive plants for 6 years, obtaining the first variety that he entered in the Catalog of New Plants in 1893. The variety was described as having small, round fruit, fruiting in clusters, bright red color and pleasant, sweet aroma.

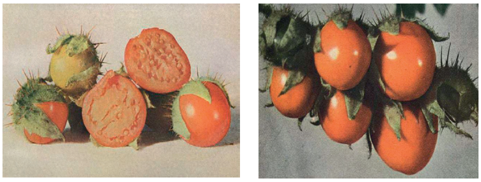

Figure 1. Original pictures with wild brazilian tomatoes used for Luther Burbank research *(Sursa/Source: Burbank, 1914)

Solanum sisymbrifolium has the popular names litchi tomato, spiny horseradish, etc.

On the African continent, more precisely in South Africa, *Solanum sisymbriifolium* is considered and declared a level 1b invasive plant in the national legislation regarding the management of biodiversity and the environment. This fact limits and even prohibits the activities related to the culture of this species.

Plants of this species were used as a protective curtain for the potato culture against the potato cyst nematode (Dias et al., 2012).

Solanum sisymbriifolium is also known to be a source of resistance, or partial resistance, to some diseases and plant pests, including fungi, bacteria, nematodes, and insects (Collonnier, 2001).

Solanum sisymbriifolium L. am. also known as "wild tomato" is a traditional medicine used by indigenous people of Central and South America, to treat both veterinary and human diseases. Various parts of the wild tomato have been widely used in prevention and treatment of numerous diseases including hypertension, diarrhoea, and respiratory and urinary tracts infections (More, 2019).

MATERIALS AND METHODS

The relict genetic resources with a wild character within the *Solanum* species come from the areas of origin of the tomato, Peru, Bolivia, and Chile. Among these, *Solanum sisymbriifolium* is the genotype that clearly expresses the authenticity of a wild tomato, in its ancestral form, as it was known since ancient times by the native populations of North America.

The germplasm collection owned by BRGV Buzau for the *Solanum* species sums up a total of 4500 lines.

Depending on the variety, the following varieties were identified: most of them belonging to the species *Solanum lycopersicum* var. *lycopersicum*, the classic tomato, registering a percentage of 57.1%, *S. lycopersicum* var. *cerasiformae* a percentage of 18.9% of the total, *S. lycopersicum* var. *pimpinelifolium* represents 8.4% of the total. From the species *Solanum cheesmanie*, 186 lines were identified and 137 lines from *Lycopersicum hirsutum*. From the species belonging to *S. peruvianum*, there are 95 lines in the collection, registering a percentage of 3.1% of the total. *Solanum spontaneum* totals 55 lines, representing 1.8% of the total and a percentage of 0.2% of the total belongs to the species *S. sisymbrifolium* with a number of 7 lines (Figure 2). Statistical analysis was performed using SPSS. Evaluation of the germplasm core collection was carried out by performing biometric measurements for the main characters based on the international UPOV and IPGRI descriptors.

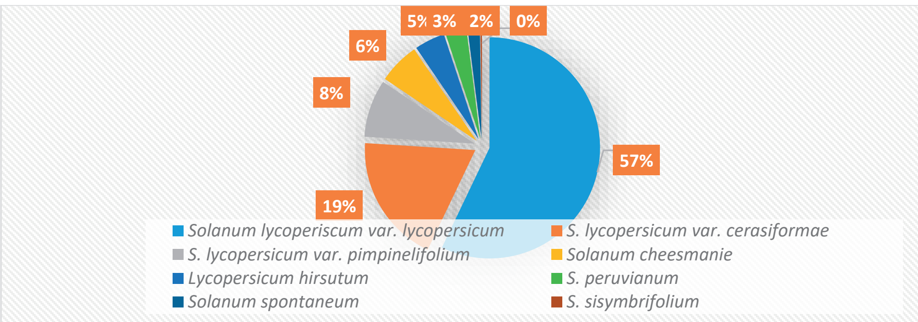

Figure 2. Germplasm collection by variety

The crop technology of *Solanum sisymbriifolium* species is similar to the classic tomato crop technology.

Through repeated individual selection works, the genetic stabilization of *Solanum sisymbriifolium* variety was succeeded, so that it was proposed for homologation and patenting. The tomato crop was planted by seedlings. Seedlings were produced in the heated greenhouse. Seedling production was carried out on 70 holes pallets using peat as substrate. Seedlings were planted 45 days after sowing. Throughout the seedling production period, purification was carried out by eliminating atypical, diseased, or non-proliferating plants. Treatments were applied to prevent seedling drop (*Pythium* spp.). Field planting was carried out in the first decade of March.

For this crop it was not necessary palisade system because the stem of the plants are strong enough to stand alone. The crop planting plan was 180 cm between strips, 60 cm between rows and 35 cm between plants per row. This crop establishment scheme allowed easy, mechanized maintenance of the strip spacing (Figures 3 and 4).

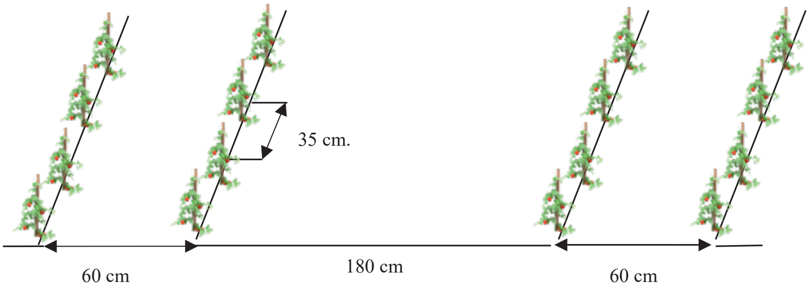

Figure 3. Crop planting plan

Figure 4. *Solanum sisymbriifolium* crop

RESULTS AND DISCUSSIONS

BRGV Buzau specialists studied this species, making qualitative and quantitative determinations. Thus, a phenotypic description of the species was made (Table 1).

The species is perennial in the area of origin, but in the temperate continental climate of our country, it behaves like an annual plant. The plants have an erect, semi-determinate habit, with a height that can reach up to 140 cm on average in the conditions of our country.

The stems do not show anthocyanin coloration and are characterized by rare pubescence. The length of the stem internodes is between 8-10 cm on average.

The plant has aggressive yellow-brown thorns, with an average height of 14.5-15.4 mm, on the entire vegetative part, both on the stem and on the shoots, leaves, inflorescence and bracts that cover the fruit (Figure 6).

The plants are semi-determinate, branching into 2-3 main shoots that bear approximately 9-10 floors. The number of leaves under the first inflorescence is 3-4 on average. The number of attached fruits per inflorescence is 6 out of 9 on average (Figure 5).

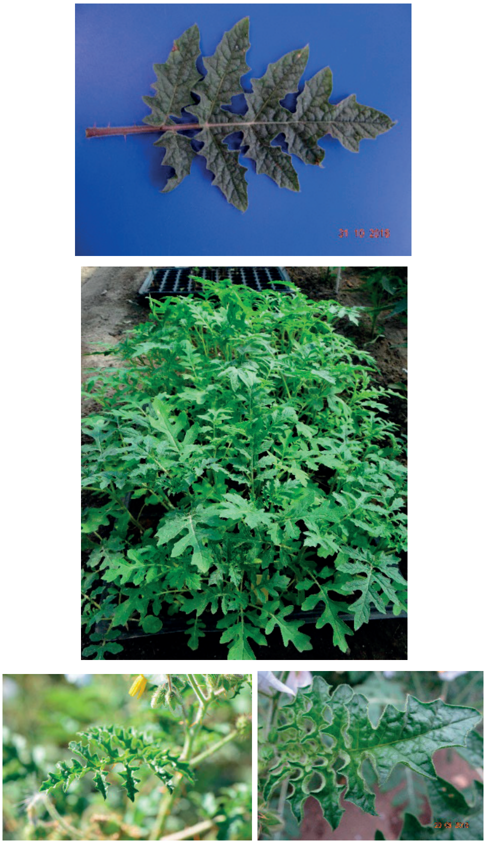

Figure 5. Leaves details

The semi-pendant leaves are arranged alternately, they are petiolate, ovate-lanceolate with deep incisions, split pinnate and the edge of the limb sinuous, pubescent on the upper side and slightly pubescent on the lower side, dark green. Both the main rib and the secondary ribs are white and decorated with aggressive spines 13.5-14.4 mm long.

Figure 6. Aggressive thorns

The length of the leaves varies between 27.4 and 42.3 cm. The shape of the leaf is oak type with an average width of 17.7 cm, weak embossing, and low gloss. The leaf has a semi-erect petiole.

The flower is similar to the potato, having a united corolla, white when immature and purple when mature. Inflorescence racem type, linear, presents 8-10 white flowers, on type 5, with a diameter between 3.5-5.8. The style is exsert and does not show pubescence (Figure 7). The fruit is cordate, berry-like and encapsulated in a persistent calyx ornamented with thorns, which opens as the fruit ripens.

Table 1. The main quantitative features

Feature	Stem lenght (cm)	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Petals length (mm)	Stamen length (mm)	Peduncle length (mm)	Fruits weight (g)	Fruit height (mm)	Fruit diameter (mm)	pericarp thickness (mm)
Average	17.5	144.2	33.5	17.7	25.3	11.6	21.4	8.7	26.6	25.0	2.8
Standard deviation	3.70	9.67	7.10	4.47	3.45	1.71	1.53	1.79	1.15	1.59	0.51
CV %	21%	7%	21%	25%	14%	15%	7%	21%	4%	6%	18%

Figure 7. Inflorescence details

The fruit has red epidermis and yellow pulp inside. The fruit has a punctate pistillate point with a pointed mucron. It easily detaches from the calyx when it reaches physiological maturity (Figure 8).

Figure 8. Fruits details

In cross-section, the fruit does not show the seminal lobes, the seeds being arranged concentrically on the edge of the pericarp and in two rows that penetrate towards the middle of the fruit. Firmness is weak.

The seeds are reniform, dark yellow, similar to those of the *Solanum melongena* species (eggplant). Flowering is late in the pedoclimatic conditions of our country, in the months of August and September.

The fruits of *Solanum sisymbriifolium* can be consumed both fresh and prepared in various jams, juices, tomato paste. *Solanum sisymbriifolium* plants have an ornamental character both through the spectacular appearance of the thorny bushes and through the white and purple flowers grouped in rich raceme-type inflorescences. Also, the intense red fruits, grouped in linear bunches, enhance the decorative appearance of the *Solanum sisymbriifolium* plant.

The species can be cultivated in an ecological system, being an alternative to countering pathogens, especially soil nematodes, being considered a trap plant. Both its rusticity and its characteristic resistance to the attack of the main pathogens but also to high temperatures, recommend it to be a plant solution in combating climate change.

Regarding breeding works, the species *Solanum sisymbriifolium* can be successfully used as a rootstock for the classic tomato. Interspecific hybridization works can also be carried out with the species of *Solanum torvum, Solanum nigrum melanocerasum, Solanum melongena* etc.

The taste of the fruit is similar to a cherry combined with the classic tomato.

The entire vegetative part, especially the leaves and stems, contain solanine, a toxic alkaloid characteristic of the species of the *Solanum* genus that confers plant resistance against pathogens.

Wild tomato plants, thanks to their characteristic aggressive thorns, can be used as a protective curtain against animals that can cause significant damage to the basic crops.

CONCLUSIONS

The germplasm collection of BRGV Buzau, as regards the species *Solanum*, has over 4,500 lines, including wild varieties.

The germplasm collection was evaluated and systematized according to varieties. Among these, the species *Solanum sisymbriifolium* attracted the attention of BRGV Buzau specialists.

This paper shows the phenotypic studies and research, the main qualitative and quantitative characters of the species.

The species can be successfully cultivated in the open field, in the pedoclimatic conditions of our country, being very resistant both to the attack of soil nematodes and to the conditions of the attack of pathogens specific to the species *Solanum* spp.

In 2024, BRGV Buzau submitted the homologation and patent documentation of the first Romanian variety of *Solanum sisymbriifolium,* under the name of the Matilda wild tomato variety.

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RESEARCH ON THE INFLUENCE OF THE IRRIGATION REGIME AND ORGANIC FERTILIZATION ON THE CUCUMBER PRODUCTION GROWN ON PERLITE SUBSTRATE

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Abstract

Food security has become an increasingly pressing global challenge, especially with the continuous growth of the world population and the reduction of arable land due to urbanization. Greenhouses, as controlled environments, provide an ideal framework for improving crop production and quality. Hydroponics is an intensive method of agricultural production that offers advanced environmental protection and increased food safety control. Cucumber production is one of the most successful crops in hydroponic systems. The study was conducted in the Automated Research Greenhouse at the Research Center for Studies of Food Quality and Agricultural Products, focusing on the cultivation of the long-fruited cucumber hybrid Peloton. The crop was established in the first cycle using a soilless cultivation system with perlite-filled mats (4 mm diameter). This system serves as an alternative to greenhouse cucumber cultivation. Three fertigation rates of 4 L/day, 3.6 L/day, and 3.2 L/day were employed. The main objective of the study is to identify the most efficient variant regarding the influence of fertigation rates on the number and weight of fruits per plant and overall cucumber production.

***Key words**: Cucumbers, hydroponic culture, greenhouse, perlite.*

INTRODUCTION

The cultivation systems employed in Romania for cucumbers, including greenhouses, solar structures, polytunnels, and open fields, along with the use of nutrient substrates in technology-equipped farms. These systems allow for year-round production but require careful management of environmental factors, especially in controlled environments like greenhouses where fertigation is crucial. This meticulous control over environmental conditions directly impacts pest and disease management, as described in the second paragraph. Controlled environments can help mitigate the risks of pests and diseases by allowing for more precise management of crop timing and yield, which are noted as key factors in pest and disease prevalence.

Soilless culture, as defined by Raviv et al. (2019), encompasses plant production methods that do not rely on mineral soil in the growing medium. This includes both liquid culture and substrate-based cultivation. In the realm of high-value vegetable crop production, soilless-grown greenhouse methods serve as a viable alternative to traditional field production (Chu and Brown, 2021a; Petre 2014 a; Petre 2016b; 2016c; Rodriguez et al., 2006; Asaduzzaman et al., 2015; Cantliffe and Vansickle, 2001; Chu and Brown, 2021). Consequently, the adoption of soilless culture has experienced a significant surge globally in recent years (Asaduzzaman et al., 2015; Chu and Brown, 2022). Perlite serves as a granular substrate commonly used in soilless cultivation, known for its high porosity and limited water-holding capacity (Goddek et al., 2019, Yang et al., 2021). Its characteristics include excellent drainage, but it retains a significant portion of water that may not be easily accessible to crops (Maher et al., 2008).

Pests and diseases pose lower risks for cucumbers compared to other crops like solanaceous or cruciferous plants, yet the success in preventing and controlling them is closely linked to agricultural practices adopted (He et al., 2022; Mahmood et al., 2021). Issues with pests and diseases are often related to the timing of cultivation and yield, and the use of resistant varieties and the implementation of proper cultural practices, such as crop rotation and moisture control, can significantly reduce their incidence (Chen et al., 2021; Bondarenko et al., 2021). For instance, cultivation in greenhouses or on nutrient substrates can facilitate the efficient use of resistant varieties and the precise application of fertigation practices, reducing the need for chemical interventions and improving the sustainability of production.

In this context, the advanced cultivation methods adopted in Romania, such as the use of greenhouses and protected structures, allow for better control of the plant environment, thereby contributing to the prevention of disease and pest outbreaks (Sorin et al., 2015)

MATERIALS AND METHODS

The study was conducted within the Research center for studies of food and agricultural products in the period 2021-2022. The biological material used was the cucumber hybrid Peloton, with long fruit, designed for a crop grown in the greenhouse. It has a fast growth, long fruits of 32-38 cm and a big productive capacity.

The crop was grown in a soilless system, on mattresses filled with perlite substrate, having the length of 1 m and a capacity of 30 l of perlite. Three plants were planted on each mattress, having a volume of 10 l of perlite substrate per plant.

The crop was established in the greenhouse, in the first cycle (January-May), the production being obtained especially from the main stem.

The seedling was produced in the greenhouse, in pots filled with perlite.

The fertilization of the seedling was conducted daily with the product Organic Grow, in dose of 1 ml/l.

We have used three watering norms as experimental variants:

- norm a (first irrigation at 7:00 and the last one at 17:00)
- norm b (first irrigation at 8:00 and the last one at 17:00)
- norm c (first irrigation at 9:00 and the last one at 17:00)

We have administrated the ecological fertilizer Gel1 before planting, in a quantity of 15 ml/plant.

Table 1. Experimental variants

Variant		Number of fertilisation/day	Norm	The quantity of solution/ fertigation	Total quantity of solution ml/ day/plant
V1	unfertilised	Unfertilised	unfertilised	unfertilised	unfertilised
V2	unfertilised	Unfertilised	unfertilised	unfertilised	unfertilised
V3	unfertilised	Unfertilised	unfertilised	unfertilised	unfertilised
V4	fertilised	20 fertilisations /day	a	200 ml/ fertilisation	4000
V5	fertilised	18 fertilisations /day	b	200 ml/ fertilisation	3600
V6	fertilised	16 fertilisations /day	c	200 ml/ fertilisation	3200

The main care works consisted of: trellising of the plants right after the planting, removing the first 10 flowers/fruits from the main stem and removing all the shoots from the main stem.

During the growing period we have ensured the optimal conditions for the growth of the plants. In the first week after planting, we have ensured a constant temperature of 24°C during daytime and night time. During the production period we have maintained a temperature of 23°C during daytime and 19°C at night. In the cloudy days we have supplemented the light, using the lamps belonging to the greenhouse, ensuring a constant intensity within 9000 lux-11000 lux. The atmospheric humidity was maintained at 75%. The content of carbon dioxide was maintained at a value of 800 ppm.

We have ensured a nutrient recipe adequate to the growing phase. In the first week, we have maintained an EC of 1.2 and a pH of 5.5. In the next weeks, we have increased the EC at 1.6-1.8, the pH being maintained at a value of 5.5 during the whole period.

We have conducted measurements regarding the height of the plants, the growth rate, the number of fruits per plant at the beginning and the end of harvesting. Biochemical determinations were also conducted regarding the content of nitrates of the cucumber fruit.

The objective of the research was to identify the most efficient variants of the cucumber production regarding the consumption of nutrient solution. The data was processed for statistical purposes using analysis of variance (ANOVA), and the differences regarding significance were determined using the Duncan test.

We have conducted correlations between the average mass of the fruits and the fertigation norm and also between the production obtained and the fertigation norm.

RESULTS AND DISCUSSIONS

In the case of applying the watering norm, analyzing the data in Table 2, it was found that at V(2) where gel was applied at planting, the average number of fruits was 16.66 fruits per plant with 2.36 more fruits than the control V1 equivalent to an increase of 16.50%. From a statistical point of view, a positive effect of the gel on the number of fruits is indicated.

Table 2. The influence of organic fertilization on the number of fruits in the case of norm a fertilized variant

Variant	Number of fruits	Difference		Significance
	no	No	%	
V(0) in average on experiment	15.48	1.18	108.25	N
V(1) Control)	14.30	0.00	100.00	Control
V(2) with Gel	16.66	2.36	116.50	*

DL5% = 2.110 DL5% in % = 14.7552
DL1% = 4.580 DL1% in % = 32.0280
DL0.1% = 15.520 DL0.1% in % = 108.5315

In the case of the fertigation norm *b*, we obtained a higher number of fruits per plant (19.7 fruits/plant) in the variant to which Gel was applied compared to the control variant to which it was not applied (17.4 fruits/plant). The difference in growth was 13.22% supported by the statistical calculation which showed a very significant increase (Table 3).

Table 3. The influence of organic fertilization on the number of fruits in the case of norm b fertigated variant

Variant	Number of fruits	Difference		Significance
	no	no	%	
V(0) average	18.55	1.15	106.61	*
V(1)	17.40	0.00	100.00	Mt
V(2)	19.70	2.30	113.22	**

DL5% = 0.740 DL5% in % = 4.2529
DL1% = 1.610 DL1% in % = 9.2529
DL0.1% = 5.480 DL0.1% in %= 31.4943

We also concluded that, in the case of the c fertilization norm, we obtained an increase in the number of fruits per plant, with a percentage of 8.15%. In the case of this variant, compared to the variant to which Gel was not applied. A significant increase in the number of fruits/plant was obtained 19.25 fruits/plant (V2) compared to 17.8 fruits/plant in V1- Control (Table 4).

Table 4. The influence of organic fertilization on the number of fruits in the case of norm c fertigated variant

Variant	Number of fruits	Difference		Significance
	No	no	%	
V(0) average	18.52	0.73	104.07	*
V(1) Control	17.80	0.00	100.00	Mt
V(2)	19.25	1.45	108.15	**

DL5% = 0.360 DL5% in % = 2.0225
DL1% = 0.790 DL1% in % = 4.4382
DL0.1% = 2.700 DL0.1% in %= 15.1685

The statistical analysis revealed that the application of both b and c fertigation norms significantly influences the number of fruits when compared to a control fertigation variant.

Table 5. The influence of fertilization norm on the number of fruits in the case of the unfertilised variant

Variant	Number of fruits	Difference		Significance
	No	no	%	
V(0) average	16.50	2.20	115.38	***
V1 – norma a	14.30	0.00	100.00	Control
V2 – norma b	17.40	3.10	121.68	***
V3 – norma c	17.80	3.50	124.48	***

DL5% = 0.130 DL5% in % = 0.9091
DL1% = 0.210 DL1% in % = 1.4685
DL0.1% = 0.400 DL0.1% in % = 2.7972

In the case of the variant to which the Gel organic fertilizer was applied, a significant increase in the average number of fruits/plant was observed (Table 6).

Table 6. The influence of fertilization norm on the number of fruits in the case of the organic fertilized variant

Variant	Number of fruits	Difference		Significance
	no	No	%	
V(0) average	18.54	1.87	111.23	***
V1 – norma a	16.67	0.00	100.00	Control
V2 – norma b	19.70	3.03	118.20	***
V3 – norma c	19.25	2.58	115.50	***

DL5% = 0.570 DL5% in % = 3.4200
DL1% = 0.940 DL1% in % = 5.6400
DL0.1% = 1.770 DL0.1% in % = 10.6200

Regarding the average weight of the obtained cucumber fruits, we noticed that, in the case of the application of norm a, the fertilized version showed a significant increase in the average weight of the fruits. Cucumber fruits recorded 302.33 g in V2, an increase of 28.78 g/fruit and 10.52% over V1 (Table 7).

Table 7. The influence of the organic fertilization on the average mass of fruits in the case of the norm *a* fertilized variant

Variant	Mass of fruits	Difference		Significance
	g/fruit	g/fruit	%	
V(0) average	287.94	14.39	105.26	***
V(1) control	273.55	0.00	100.00	Mt
V(2) with Gel	302.33	28.78	110.52	***

DL5% = 0.500 DL5% in % = 0.1828
DL1% = 1.090 DL1% in % = 0.3985
DL0.1% = 3.720 DL0.1% in %= 1.3599

In the case of *b* fertigation norm, we noticed that, from a statistical point of view, at V2 the average weight of the fruits was distinctly very significant compared to the variant to which Gel was not applied at planting. The average mass in this case being 315.75 g/fruit with 13.87% more than the control variant V1 (Table 8).

Table 8. The influence of organic fertilization norm on the average mass of the fruits in the case of the variant fertigated with norm *b*

Variant	Mass of fruits	Difference		Significance
	g/fruit	g/fruit	%	
V(0) average	296.52	19.22	106.93	***
V(1)	277.30	0.00	100.00	Control
V(2) fertilized	315.75	38.45	113.87	***

DL5% = 1.430 DL5% in % = 0.5157
DL1% = 3.100 DL1% in % = 1.1179
DL0.1% = 10.530 DL0.1% in %= 3.7973

In the case of the fertigation norm c, at V2, where Gel was used, an increase in the average fruit weight was observed according to the fertigation norm.
Thus, for norm *c*, the average weight of the fruits was 287.65 g/fruit in the control variant (V1) and 317.8 g/fruit in the variant fertilized with the organic product (Table 9).

Table 9. The influence of oganic fertilization norm on the average mass of the fruits in the case of the variant fertigated with norm c

Variant	Mass of fruits	Difference		Significance
	No	No	%	
V(0) average	302.65	15.15	105.27	***
V(1)	287.50	0.00	100.00	Control
V(2)	317.80	30.30	30.30	***

DL5% = 0.350 DL5% in % = 0.1217
DL1% = 0.770 DL1% in % = 0.2678
DL0.1% = 2.630 DL0.1% in %= 0.9148

The number of fruits obtained per 1 m² was lower in the unfertilized version when applying the 3 fertigation rules (*c*), varying from 31,777 fruits/m² in the case of the *a* norm to 39,555 fruits/m² in the case of the *c* norm.
In the case of the variant to which Gel was applied, the number of fruits/plant increased between 37,044 fruits/m^2 for norm *a* and 42,777 fruits/m^2 for norm c.
To evaluate the influence of the watering rate, we performed an analysis of the correlation between the number of fruits obtained per square meter and the watering rate. Our findings indicate that watering rate significantly influenced both variants (Figure 1).

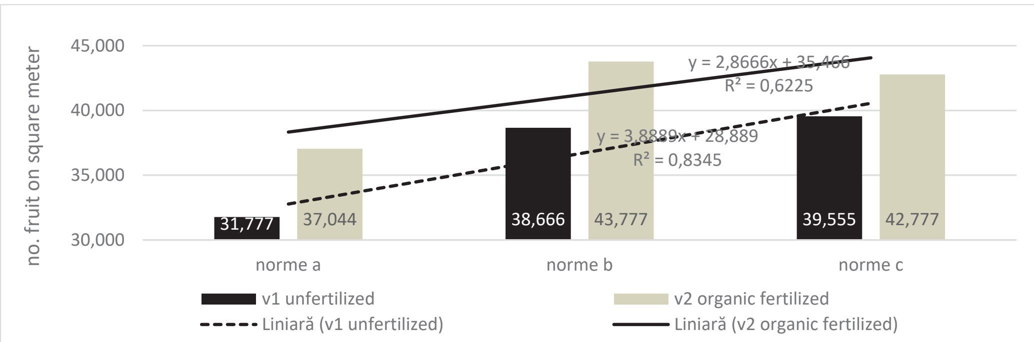

Figure 1. Total number of fruits obtained on 1 m^2

The average weight of the cucumber fruits was lower in the case of the variants without Gel, of all the watering norms (a, b and c), these being between 273.55 g and 287.5 g/fruit. The application of Gel at planting led to obtaining an increased average mass of the fruits in the case of the watering norm c. At the same time, after making the correlations between the average weight of the fruits and the fertigation rate, we concluded that the amount of nutrient solution played a significant role. The correlation coefficients were R^2=0.8474 in the case of the unfertilized variant. and R^2=0.9335 in the case of the variant where Gel was used (Figure 2).

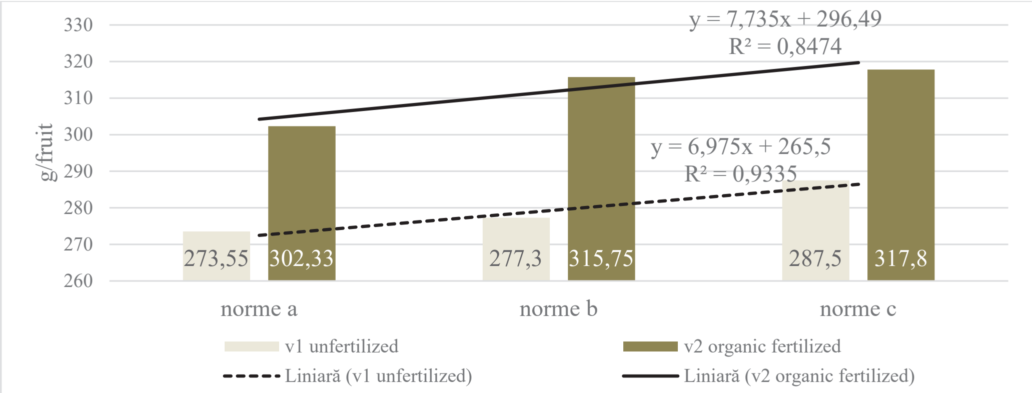

Figure 2. The average mass of cucumber fruits obtained on plant

Analyzing the production obtained per 1 m^2, we concluded that it was higher in the case of the variants where Gel 1 was used, regardless of the fertigation rate. I saw that we obtained an increased production in the case of all variants by increasing the irrigation rate. The correlation coefficients indicated that the fertilization rate has a significant role (Figure 3).

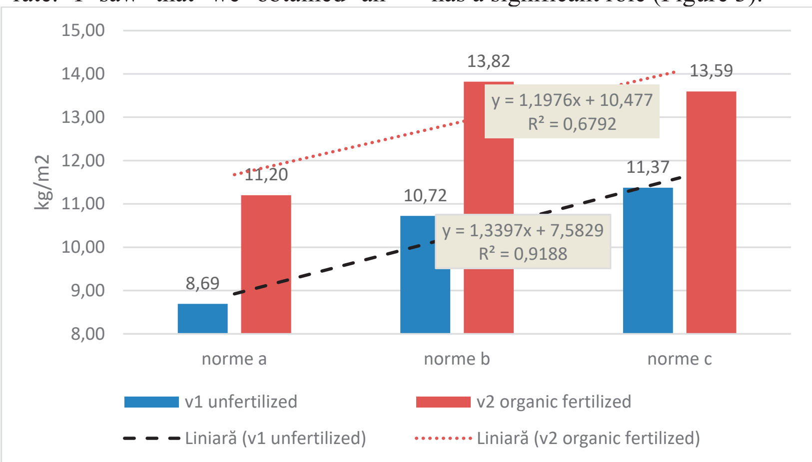

Figure 3. The cucumber production obtained on 1 m^2

CONCLUSIONS

Regarding the number of fruits/plant, in the case of the variant where Gel was used, we obtained the lowest number of fruits on the variant on which we applied the norm (14.3 fruits/plant) and the highest number on the variant on which we applied norm c (17.8 fruits); In the case of the fla variant that used Gel, an increased number of fruits/plant was obtained in the case of all the fertigation variants, between 16.66 fruits (norm *a*) and 19.7 fruits (norm *b*);
Between the fertilization norms, regarding the number of fruits/plant, significant results were obtained; The lowest number of fruits per 1m^2 was obtained in the case of the variant in which Gel was not used, norm *a*, and the highest number of fruits was obtained in the case of the

variant in which Gel was used, of 43,777 fruits/m^2 (norm *b*); The lowest production on 1 m^2 was obtained in the case of the control variant, norm *a*, of 8.69 kg/m^2 and the highest production was obtained in the case of the variant with Gel, norm *b*, of 13.82 kg/m^2.

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EFFECT OF MONTMORILLONITE BASED HYDROGELS APPLICATION ON MORPHOLOGICAL CHARACTERISTICS OF LETTUCE SEEDLINGS (*LACTUCA SATIVA*)

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Abstract

The agricultural sector and food production have witnessed persistent expansion in the past few decades. This occurrence has resulted in the excessive utilization of intensive production methodologies, leading to the unsustainable depletion of soil nutrients and water resources. Numerous research endeavors have been undertaken with the aim of examining the influence of hydrogels on the enhancement and optimization of agricultural inputs. The aim of this study is to assess the impact of four different compositions of hydrogels based on montmorillonite on the morphological characteristics of lettuce seedlings (Lactuca sativa) subsequent to the transplanting procedure, in the greenhouse conditions. The effect of the tested hydrogels on the development of the plants was regularly monitored through evaluation of the overall height, number of leaves and the relative content of chlorophyll. The results show no noticeable differences for the height parameters of the samples, meanwhile the total number of leaves and relative content of chlorophyll proved to be significantly higher for the samples cultivated using hydrogels compared to the control samples.

Key words: *hydrogels, lettuce, montmorillonite, morphological parameters.*

INTRODUCTION

Agriculture in the contemporary era encounters numerous obstacles such as the growing population, the reduction of cultivable land, and the swift process of urbanization. Consequently, the development of advanced technologies has become an essential requirement, leading to a transition from traditional farming methods to safeguarded cultivation, specifically soilless cultivation (Thomas et al., 2021).

Lactuca sativa, commonly referred to as lettuce, is a plant of considerable economic value in terms of human consumption on a global scale. This plant is cultivated primarily for its palatable flavour, its abundant nutritional content, and its potential medicinal properties. Lettuce serves as a crucial ingredient in the creation of various dishes, such as salads, soups, and vegetable curries, and serves as a vital source of phytonutrients (Güzel Murat Erdem, 2021). Furthermore, it has been extensively investigated for its pharmacological capabilities, which include its ability to combat microbial agents, its antioxidant properties, its capacity to protect neurological health, and its potential sedative effects (Naseem & Ismail, 2022). Additionally, lettuce contains indispensable components, such as vitamins, minerals, and organic substances (Shi et al., 2022). Beyond its culinary and medicinal applications, lettuce has garnered considerable attention in the realm of biotechnology and the process of *in vitro* regeneration (Farina et al., 2021).

The results obtained by Ekka et al. (2022) imply that the integration of hydrogel and inorganic manure in the cultivation of lettuce has the potential to augment the development and output of lettuce crops. The outcomes of the investigation offer significant knowledge for maximizing lettuce production in agroforestry systems based on citrus, which could potentially result in enhanced economic gain and ecological durability in lettuce farming.

Hydrogels have been used in the field of agriculture for the past five decades, demonstrating their efficacy as a reservoir for water retention and a facilitator of nutrient mobilization within the soil. The use of hydrogels composed of superabsorbent polymers has become prevalent in agricultural industry due to their significant contributions towards soil improvement, enabling plant growth in arid regions, and facilitating the process of seed germination (Palanivelu et al., 2022).

Hydrogels possess the remarkable ability to absorb water at a rate of 400 times their own weight while gradually releasing it (Darban et al., 2022), thereby reducing the leaching of herbicides and fertilizers (Kaur et al., 2023).

Montmorillonite is a type of mineral clay that is commonly found in soils and sediments. It has various applications in agriculture and horticulture due to its ability to retain water and nutrients, improve soil structure and enhance plant growth, and has recently been used in the production of hydrogels, such as the nanocomposite hydrogel with calcium montmorillonite (NC-MMt) that can enhance the growth and development of seedlings without adverse effects (Melo et al., 2019).

The growth of *Lactuca sativa* can be affected by a range of factors and technologies. A particular investigation revealed that the manipulation of the time difference between cycles of light and temperature can exert control over the development of seedlings of *L. sativa* (Masuda et al., 2022).

In this study, four different compositions of hydrogels based on montmorillonite were tested, in order to assess the effects on the morphological characteristics of lettuce seedlings (*Lactuca sativa*) subsequent to the transplanting procedure, in greenhouse conditions.

MATERIALS AND METHODS

Polymeric Material

The polymeric materials used in this study were received from The National Institute for Laser, Plasma and Radiation Physics, Măgurele. They were obtained utilizing the electron beam radiation technique and, in their composition, potassium persulfate was added as a catalyst. Four distinct compositions of hydrogels, based on montmorillonite were used in this study. Further, two methodologies for the application of the hydrogels were investigated, specifically the granular form (Hg) and beads (Hb) (Table 1).

Table 1. Sample codification used in this study

Sample code		Montmorillonite dose (%)
Bead Hydrogel	Granular Hydrogel	
Hb1	Hg1	0
Hb2	Hg2	0.25
Hb3	Hg3	0.5
Hb4	Hg4	1
C		Control sample with no hydrogel

Biological material

The lettuce (*Lactuca sativa*) seedlings placed in alveolar tray, were acquired from VDRS Buzău (Figure 1).

Figure 1. Lettuce seedling

Working protocol (Figure 2)

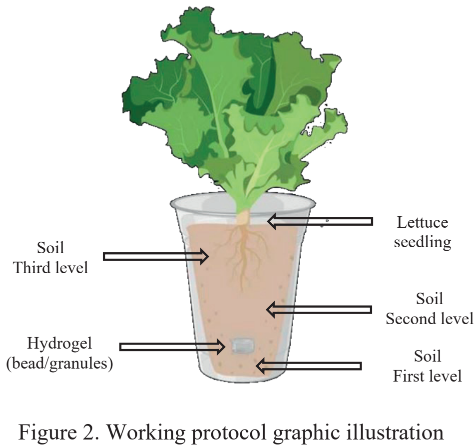

Figure 2. Working protocol graphic illustration
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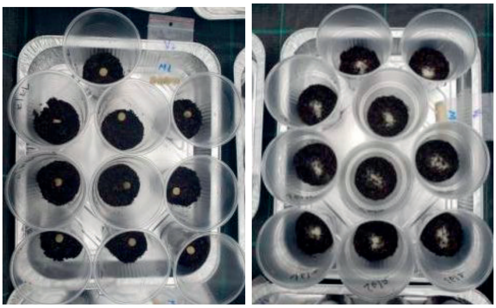
For this experiment, cups were used, in which soil (first level) and the hydrogel samples as beads (a) and granules (b), weighing approximately 0.2 g (Figures 2, 3) were placed.



(a) (b)
Figure 3. Application of hydrogel ((a) Beads and (b) Granules)

Second level of soil was then added and hydration was carried with 100 ml water/each sample to assure the maximum swelling degree of the hydrogels. Further, after 24 h, lettuce seedlings were transplanted and the third level of soil was then added (Figure 4). Ten repetitions for each sample were prepared.

Figure 4. Lettuce seedlings in cups in the presence of hidrogel samples

After 3 weeks, the seedlings were transplanted from cups into pots (Figure 5).

Figure 5. Lettuce plants in pots in the presence of hidrogel samples

The samples were maintained in greenhouse conditions: between 32 and 100% relative air humidity, at temperature between 14.1°C and 37.92°C and watered regularly. The morphological analyses: the overall height, number of leaves and relative content of chlorophyll of lettuce were further conducted for a total of 5 weeks.

Overall height

The overall height of the samples was determined weekly measuring from the ground level to the greatest height of the highest leaf of each sample (Figure 6).

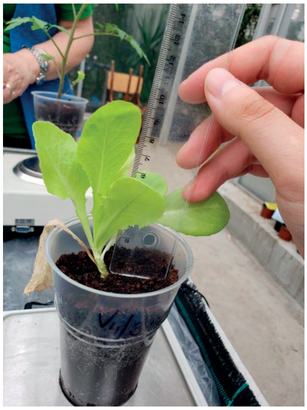


Figure 6. The determination of the height of samples

Number of leaves

The number of leaves of the samples was determined weekly by counting every leaf after the removal of the dry ones.

Relative content of chlorophyll

Relative content of chlorophyll of the samples was determined after transplanting the samples to pots and after an accommodation period of 14 days using the BIOBASE Portable Chlorophyll Meter CM-B. The chlorophyll content was determined at a specific time, in the morning, protected from direct sunlight, from the most developed leaf of each sample, and was calculated using the formula (99*SPAD)/(144-SPAD) and related to μg/cm^2 (Cerovic et al., 2012)

RESULTS AND DISCUSSIONS

The morphological analyses were conducted weekly and the relative content of chlorophyll was determined during the last 2 weeks of the experiment after the accommodation period subsiding the transplantation process.

Overall height

The evaluation of plant height is conducted in order to investigate the vertical growth of lettuce plants, as well as their overall dimensions and progress. This method provides valuable insights into the growth rate, vigor, and efficiency of nutrient absorption and utilization by the plants (Camen et al., 2022).

Overview results showed no significantly high differences between the samples containing montmorillonite and the control sample over the 5 weeks trial.

However there proved to be some differences between the different proportions of montmorillonite from hydrogel composition.

After 35 days trial, the lowest height (12.53±1.33 cm) was recorded for the the sample Hb2 (lowest concentration of montmorillonite) and the highest height value (17.06±4.27 cm) was recorded for Hb3 (medium concentration of montmorillonite) (Figure 7).

The height parameter after 35 days for lettuce in the presence of hydrogel beads was recorded as:

Hb2< Hb4< Hb1< C< Hb3

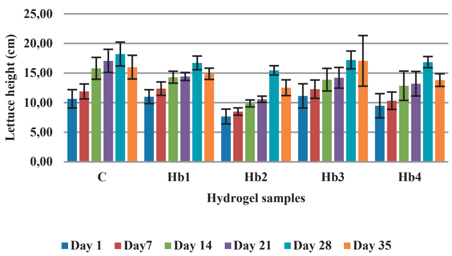


Figure 8. Overall height of the lettuce samples with bead hydrogels

Regarding the granular type of hydrogels, after 35 days trial, the lowest height (12.88±1.38 cm) was recorded for the the sample Hg4 (highest concentration of montmorillonite) and the highest height values were recorded for the control sample C and Hg2 (low concentration of montmorillonite) (Figure 8).

The height parameter after 35 days for lettuce with hydrogel granules in the presence of recorded as:

Hg4< Hg1< Hg3< Hg2< C

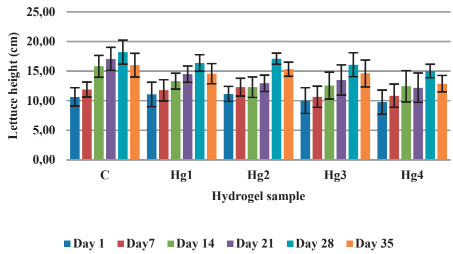


Figure 8. Overall height of the lettuce samples with granular hydrogels

The average height of *Lactuca sativa* varies depending on different factors. According to a particular investigation, an optimal crop height of 21.05cm was achieved by the application of 360 kg/ha of Ammonium Sulphate (Ponce-Lira et al., 2022). Another study showed that the height span per individual plant ranged from 15.18cm to 30.2 cm subsequent to a 60-day period of growth (Pereira de Oliveira et al., 2017).

Number of leaves

Measuring the total number of leaves present on lettuce plants provides insights into the progress and density of leaves. This process aids in assessing the general well-being and vitality of the plant, as a higher number of leaves frequently implies increased growth and productivity (Camen et al., 2022).

The number of leaves of the lettuce samples over the 35 days trial prove to increase overtime regardless of the dried base leaves that were periodically removed.

For both forms of hydrogels, the number of leaves proved to be higher then the control sample.

After 35 days trial, for the bead hydrogels, the lowest number of leaves resulted on the control sample (C) with 19.75±1.91 while the hydrogels with the lowest concentration of montmorillonite (Hb2) showed the greatest number of leaves with 25.75±1.28 (Figure 9).

The number of leaves after 35 days for lettuce in the presence of hydrogel beads was recorded as:

C<Hb1<Hb3<Hb4<Hb2

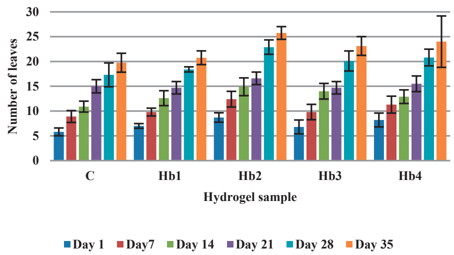


Figure 9. Total number of leaves of the lettuce samples with bead hydrogels

For the granular form, the sample with the highest concentration of montmorillonite hydrogel (Hg4) demonstrated the greatest number of leaves (24.50±3.66) after 35 days and the lowest value was recorded for the control sample (19.75±1.91) (Figure 10).

The number of leaves after 35 days for lettuce in the presence of hydrogel granules was recorded as:

C<Hg1<Hg3<Hg2<Hg4

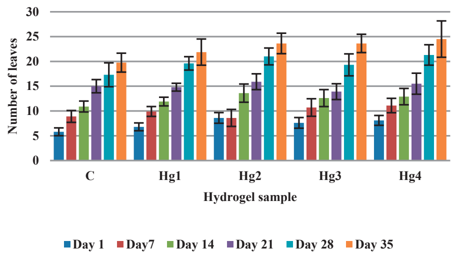


Figure 10. Total number of leaves of the lettuce samples with granular hydrogels

The average number of leaves of *Lactuca sativa* varies depending on each condition and treatment applied. In the study conducted by Chowdhury and Rahman (2021), the maximum leaf number was 27 using Approshika organic manure. Overall, the utilization of hydrogels has exhibited advantageous impacts on parameters associated with leaves in lettuce; however, the precise impact on the quantity of leaves may differ contingent upon the experimental circumstances such as applied treatments. Ponce-Lira et al. (2022) tested a new eco-fungicide on lettuce and the maximum number of leaves recorded was only 15.50.

Relative content of chlorophyll

The assessment of the nutritional status of plants can be facilitated by its utilization, as the availability of crucial nutrients has an impact on the production of chlorophyll. The presence of alterations in the relative concentration of chlorophyll may potentially serve as an indicator of stress or physiological issues within plants, including deficiencies in sustenance or environmental factors. The measurement of the relative chlorophyll concentration offers a non-invasive and easily accessible approach to assess the overall photosynthetic performance and well-being of lettuce plants (Camen et al., 2022).

The relative content of chlorophyll (µg/cm^2) of the samples proved to be higher for the lettuce plants in the presence of montmorillonite based hydrogels.

Regarding the bead form, after 35 days, the highest relative content of chlorophyll was registered for the lettuce plants in the presence of hydrogels with the lowest and highest concentration of montmorillonite - Hb2 (28.42±1.61µg/cm^2) and Hb4 (27.10± 6.14 µg/cm^2). The lowest relative content of chlorophyll was registered for the lettuce samples in the presence of hydrogels with no montmorillonite Hb1 (19.15±1.22 µg/cm^2).

The relative content of chlorophyll after 35 days for lettuce in the presence of hydrogel beads was recorded as:

Hb1<Hb3<C<Hb4<Hb2

For the granular form, the highest relative content of chlorophyll values were recorded for the lettuce plants in the presence of hydrogel containing medium and higher concentrations of montmorillonite, Hg4 (25.27±7.78 µg/cm^2) and Hg3 (23.21±5.24µg/cm^2). The lowest values were recorded for the samples Hg2 (19.93±4.34µg/cm^2) and Hg1 (19.96±3.29 µg/cm2) (Figure 11).

The relative content of chlorophyll after 35 days for lettuce in the presence of hydrogel granules was recorded as:

Hg2<Hg1<C<Hg3<Hg4

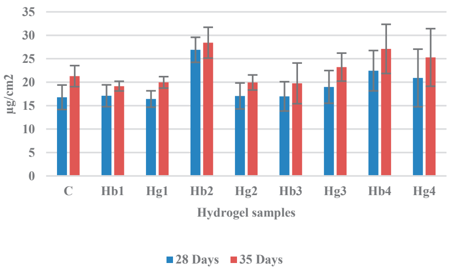


Figure 11. Relative content of chlorophyll of the lettuce samples

A study conducted by Lu et al. (2019) showed that the range of the chlorophyll content varied from 7.61 to 44.62µg/cm² for the Romaine lettuce.

CONCLUSIONS

The changes in lettuce plant over time were influenced by various factors including growth stage, environmental conditions, and nutrient availability (Ahmed et al., 2020).

The results of this study showed no noticeable differences regarding the height of lettuce in the presence of hydrogels compared to the control sample with a few exceptions, such as Hb3 (hydrogel with medium concentration of montmorillonite). The remaining samples registered close of slighly lower values than the control.

Meanwhile, the total number of leaves and relative content of chlorophyll proved to be significantly higher for lettuce cultivated in the presence of hydrogels (bead and granules) compared to the control sample. The hydrogels with a high (Hg4 and Hb4) and medium (Hg3 and Hb3) concentration of montmorillonite proved to enhance these two parameters for the lettuce plants in both forms of administration (bead and granules). Therefor, the montmorillonite based hydrogels proved to enchance some morphological parameters of lettuce.

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THE STUDY OF SOME AMINO ACIDS WITH A ROLE IN THE ADAPTATION OF THE BITTER CUCUMBER (*MOMORDICA CHARANTIA*) TO SALINITY STRESS

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Abstract

Bitter cucumber (Momordica charantia L.) is an annual tropical plant in the family Cucurbitaceae, cultivated worldwide for its bitter fruits that are used both for food and for its many medicinal properties. Salinity is a widespread problem globally and is constantly growing. This limits plant growth and biomass production, especially in arid, semi-arid and tropical areas. To adapt to new environmental conditions, plants can accumulate or consume different metabolites. In this work, proline and aromatic amino acids were determined by the spectrophotometric method. To carry out the determinations, two varieties of bitter cucumber and three experimental lines were used that were treated with saline solutions of different concentrations. Following the analyzes carried out, a tendency to increase the amount of proline was noticed in the variants treated with saline solutions compared to the control, and in the case of aromatic amino acids, a tendency to their decrease was observed proportional to the increase in the concentration of saline solutions. The determinations made highlight the degree of resistance of the studied genotypes to saline stress and the preferential accumulation of metabolites.

Key words: *Momordica charantia, saline stress, amino acids.*

INTRODUCTION

Momordica charantia L., or bitter cucumber, is an annual tropical plant in the Cucurbitaceae family that is cultivated in many places around the world for its particularly bitter fruits (Alisofi et al., 2020). Analysis have shown that this plant has the highest nutritional value of all Cucurbitaceae, as it is an excellent source of fiber, vitamins, minerals, carbohydrates and proteins. Vitamins C, A, P, thiamine, riboflavin, niacin and minerals are found in green leaves and fruits but also in young shoots (Bortolotti et al., 2019). The more than 60 medicinal properties of bitter cucumber fight almost 30 diseases such as diabetes, cancer and AIDS (Patel et al., 2020). Due to these properties, the plant is one of the most popular and valued fruit vegetables in the Cucurbitaceae family, cultivated both as food and for use in traditional medicine (Li et al., 2020).

The deterioration of food security is caused by the reduction of arable land due to abiotic stress. Food production must increase by 70% to meet the increase in global population, which is expected to reach 9.1 billion by 2050 (Iqbal et al., 2014). A major problem limiting plant growth and biomass production is salinity, found especially in arid, semi-arid and tropical regions where rainfall is scarce and irrigation systems are common (Mohsen et al., 1980; Gamalero et al., 2020).

Salinity manifests itself largely as osmotic stress and causes an ionic imbalance in the cell. The occurrence of this abiotic stress triggers cell signalling pathways and cellular responses, such as the accumulation of compatible solutes and the increase of antioxidants (Zhu, 2001).

Proline is a non-essential amino acid that plays a significant role in plant growth and development. There are two known pathways for proline synthesis: the glutamate pathway and the ornithine pathway. The most known and accepted pathway for the synthesis of this amino acid in plants subjected to abiotic stress is the glutamate pathway (Sharma & Verslues, 2010). Proline is synthesized from glutamate, as are γ-aminobutyric acid, glutamine and arginine. The main genes involved in the synthesis of this metabolite are: pyrroline-5-carboxylate synthetase (P5CS), pyrroline-5-carboxylate reductase (P5CR), and ornithine-δ-aminotransferase (OAT), and those responsible for proline catabolism are: proline dehydrogenase (PDH) and pyrroline-5-carboxylate dehydrogenase (P5CDH) (Kishor et al., 2015).

In conditions of abiotic stress, proline fulfills an important role as a cellular osmoprotector. It regulates water absorption under conditions of water and salt stress. In addition to its role as a compatible solvent, proline is known to improve the stability of subcellular structures and cell membranes. It serves as a redox buffer, facilitates the activity of enzymes and is a powerful chelator of metals in the cytoplasm. Proline is also known as a cell signaling molecule (Wang et al., 2014).

In plants, aromatic amino acids (AAA) are synthesized from chorismate, the end product of the shikimate pathway, and are precursors to a wide range of secondary metabolites (Trovato et al., 2021). The enzymes 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase (DHS) catabolize the first reaction of the shikimate pathway (Keith et al., 1991). Phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp) are aromatic amino acids characterized by the presence of an aromatic ring in their structures (Aydin et al., 2021). Aromatic amino acids are fundamental elements in plants, fulfilling multiple roles. These amino acids are important precursors in the biosynthesis of proteins, they play a role in the synthesis of some secondary compounds involved in the plants' fight against biotic and abiotic stress, and they also have the ability to function as antioxidants, ensuring the protection of plant cells against oxidative stress induced by environmental factors. adverse environment (Trovato et al., 2021).

MATERIALS AND METHODS

Materials

Spectrophotometer Specord 210 Plus, AnalitikJena, Vertex 70 FT-IR spectrometer, Bruker, Ninhydrin ($C_9H_6O_4$) - Merck, Sulfosalicylic Acid ($C_7H_6O_6S$) – Chemical Company, Glacial Acetic Acid ($C_2H_4O_2$) - Merck, Acid Phosphoric (H_3PO_4) - Merck, Toluene (C_7H_7) - Merck, L-Proline ($C_5H_9NO_2$) - Sigma Aldrich, Ammonium Hydroxide NH_4OH – Chemical Company, Nitric acid (HNO3) - Sigma Aldrich, *Momordica charantia* leaves.

Plant material

Five genotypes of *Momordica charantia* were used for the experiment, including two Romanian varieties (Rodeo variety and Brâncuși variety) and three experimental lines (Line 1, Line 3 and Line 4). The experience was located within the didactic resort "Ferma Vasile Adamachi" which belongs to the "Ion Ionescu de la Brad" University of Life Sciences in Iași.

Plants were treated with different concentrations of saline solutions: 100 mM and 200 mM. in total, three treatments were applied at intervals of 10 days between them. The leaves used for the determinations were harvested 7 days after the application of treatment 3. According to the BBCH scale, the plants were in phenophase 701 (corresponding to the appearance of the first fruit) at the time of application of the treatment and in phenophases 702-703 (corresponding to the appearance of the -second and third fruit) at the time of sampling. They were harvested from the main shoot of the plant and an average sample of 9 leaves was made. 3 leaves from 3 different plants belonging to the same variant (control, treatment V1 or treatment V2) were harvested and used.

Proline determination method

To perform the analysis, 1 g of plant material was used, which was mortared with 5 mL of sulfosalicylic acid 3%. The obtained mixture was centrifuged for 15 minutes at 5000 rpm. After centrifugation, the supernatant was used, from which 2 mL were extracted. 2 mL of acid ninhydrin and 2 mL of glacial acetic acid were added to these. Acid ninhydrin was obtained by dissolving 1.25 g of ninhydrin in 30 mL of glacial acetic acid and 20 mL of 6M phosphoric acid. For dissolution and homogenization, the substances were heated to 60 ºC.

The resulting mixture was placed on a water bath and subjected to heat treatment at 100 ºC for one hour. After the heat treatment, the resulting red solution was allowed to cool to room temperature. After cooling the mixture was extracted with 4 mL toluene.

The readings and calculation of the amount of proline were made using a calibration curve made in the range 1-18 mg/L. For proper fitting on the calibration curve, the samples obtained were diluted with another 2 mL of toluene.

Proline was read at 520 nm using a 1 cm quartz cuvette, after which the extract was dried for FTIR analysis.

Method for determining aromatic amino acids

AAAs were determined by means of the xanthoprotein reaction. An amount of 0.3 g of leaf was used, over which an amount of 10 mL of distilled water was added and which was then heated on a hot plate at a temperature of 60 ºC for 20 minutes. 2 mL of the obtained extract was used to which 0.5 mL of 65% HNO_3 was added and heated again at 60 ºC for 15 minutes. The solutions were cooled to room temperature, then 1 mL of 20% ammonia solution was added, after which the obtained solution acquired a yellow color. In order for the samples to be read by the spectrophotometer, they were centrifuged for 10 minutes at 2000 rpm.

An amount of 100 µL was used from the supernatant and diluted in 3 mL of distilled water. The resulting solution was read at 305 nm using a 1 cm quartz cuvette and then dried to perform FTIR analysis.

Statistical analysis

For the statistical analysis of the data, the Anova Two Factor test was used, following the influence of the concentrations of the saline solutions applied but also of the studied genotypes.

RESULTS AND DISCUSSIONS

Dramatic proline accumulation is a common physiological response in plants exposed to various types of abiotic stress. This increase may be due to de novo synthesis of proline, decreased degradation rate, or protein hydrolysis (Kaur & Asthir, 2015). Proline can preferentially accumulate in different plant organs. According to the literature, it can accumulate in roots, leaves, stems, flowers, fruits and seeds fulfilling different roles (Meenu Rani et al., 2022).

In leaves, proline accumulates with the aim of stabilizing cellular structures. Proline contributes to the stabilization of subcellular structures in chloroplasts. It helps maintain the integrity of membranes, proteins and enzymes. This stability is crucial for efficient photosynthesis even under stress conditions (Hayat et al., 2012)

Following the determinations made on the leaves of bitter cucumber after applying the last saline treatment (Figure 1), proline content values were recorded in the control plants between 1.5 mg/L in the case of the Rodeo variety and 2.18 mg/L registered in the Brâncuși variety. The differences between proline amounts in untreated plants were relatively low, with no statistically significant differences being recorded. Plants treated with 100 mM NaCl (V1) showed proline values between 1.94 mg/L in the case of the Rodeo variety and 5.59 mg/L in the case of Line 3. Following the V1 treatment, significant differences can be observed between the five various tested. The smallest differences compared to the control were also recorded in the Rodeo variety where the plants treated with V1 recorded 29.33% higher values, unlike Line 3 which showed a 225% increase compared to the control. In the case of plants treated with 200 mM NaCl (V2), very pronounced differences were observed between the amounts of proline in the five studied genotypes. Proline values fluctuated between 1.5 mg/L in the case of the Rodeo variety and 20.9 mg/L in the case of Line 3. Considerable amounts of proline were also recorded in the case of the Brâncusi variety (14 mg/L) and the Line 4 (13.9 mg/L). The smallest difference between the control and V2 was recorded in the Rodeo variety (150.66%) and the most pronounced difference was observed in the case of Line 3 (1115.11%). Significant and highly significant differences between controls and treatments are confirmed by two-way Anova test (Table 1). Among the genotypes studied, the Rodeo variety presented the lowest proline value both in the case of the control and the treated variants. This can be explained both by its poor resistance to salt stress and by the lack of the natural tendency of the genotype to accumulate proline. Compared to this, in the case of the Brâncusi variety and Line 4, the high amounts of proline may also be due to the general tendency of the genotype to accumulate proline under normal conditions. Following the analysis of the values, it can be concluded that the best adaptation to salt stress was recorded in the case of Line 3. Unlike the genotypes that present proline under normal conditions, Line 3 did not present this characteristic, which highlights this line as the most resistant to applied salt stress.

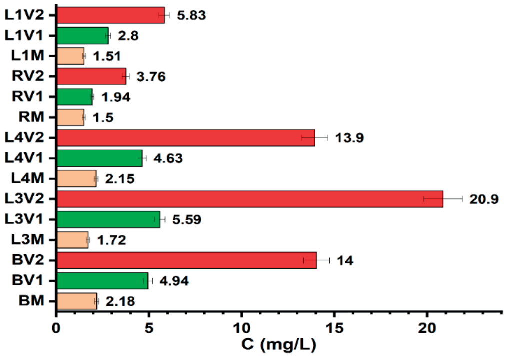

Figure 1. Free Proline content in bitter cucumber (*Momordica charantia*) leaves subjected to salt stress

After spectrophotometric analysis the toluene extract was dried. The residue formed was analyzed by infrared spectrometry in order to determine the type of functional groups present. This analysis was performed to highlight the presence of clusters characteristic of the analyzed compound. Analysis (403.11 m, 478.33 m, 621.05 m, 1018.37 w, 1220.89 vw, 1232.46 vw, 1244.03 vw, 1263.32 w, 1618.21 s (υC=O), 1637.50 m (υC=C), 1710.79 w (υC= O), 1753.22 vw, 1778.29 vw, 2925.89 vw (υC-H), 3236.42 w (υCH), 3413.86 vs, 3477.51 vs, 3552.73 s, revealed the formation of the compound of interest.

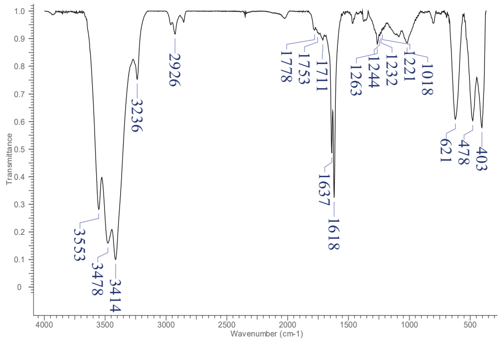

Figure 2. FTIR spectrum of the solid formed after drying the sample used for the determination of Proline

Table 1. Analysis of the variance of the amount of proline in bitter cucumber genotypes subjected to salt stress

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Significance*
Rows	99.392	4	24.848	1.933453	0.198253	3.837853	NS
Columns	268.829	2	134.4145	10.45895	0.005857	4.45897	**
Error	102.813	8	12.85162				
Total	471.0339	14					

Anova Two-Factor: NS non-significant statistical differences (p≥0.05); *significant statistical differences (p≤0.05); **distinctly significant statistical differences (p≤0.01); ***highly significant statistical differences (p≤0.001)

Following the performance of the Anova Two Factor test (Table 1), distinctly significant statistical differences (p<0.01) were revealed between the amounts of proline obtained following treatments with saline solutions, which demonstrates that the most important role is played by the concentration of NaCl in the amount of proline.

Aromatic amino acids

Studies on aromatic amino acids have shown that, under abiotic stress conditions, the expression levels of genes in the AAA biosynthesis pathway are up-regulated, leading to higher levels of them and their secondary metabolites in plants resistant to the abiotic stress encountered (Oliva et al., 2021).

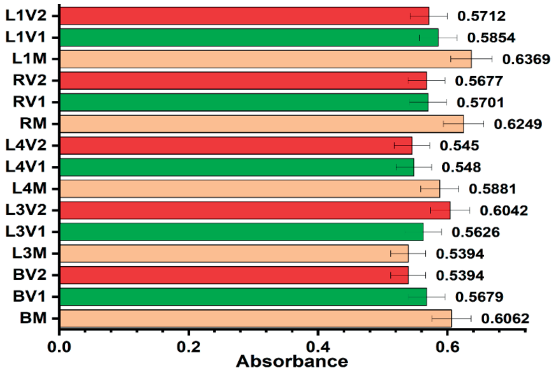

Figure 3. Aromatic amino acids from bitter cucumber leaves (*Momordica charantia*) subjected to salt stress

The analyzes of bitter cucumber leaves on the absorbance of aromatic amino acids, in the case of the five studied bitter cucumber genotypes, showed values between 0.5394 in Line 3 and 0.6369 in the case of Line 1, in the case of control plants. These values did not show statistically significant differences. In plants treated with V1, the absorbance values ranged from 0.548 in the case of Line 4 to 0.5854 in the case of Line 1. Following the comparison between the AAA absorbances of the control and V1-treated plants, a general downward trend was observed in the treated plants, except for Line 3 where a slight increase could be noted. The same phenomenon of decrease in absorbances was also noted in the case of the analyzes performed on the leaves that came from the plants treated with V2. The values fluctuated between 0.5394 in the Brâncusi variety and 0.6042 in the case of Line 3, which presented higher values than those of the untreated control and the V1 treatment. According to a study carried out on two varieties of chickpea (*Cicer arietinum* L.), by Kumar et al. (2021), it was highlighted that among the AAAs, only tryptophan was present in a greater amount compared to the control in the variety resistant to salt stress, while tyrosine decreased, compared to control plants, in both the salt stress-resistant and the sensitive variety (Kumar et al., 2021). Comparing the results obtained with this study, it can be concluded that Line 3 is a genotype resistant to abiotic stress conditions. The relatively low difference between the control and the treated variants in the case of this genotype can be explained by the increase in the plant only of the amino acid tryptophan.

According to specialized literature, AAAs not only play a role in protein synthesis, but also fulfill numerous other roles, including the adaptation of plants to abiotic stress (Trovato et al., 2021). Thus, the reduced amounts of AAA in the variants subjected to NaCl treatments could be explained by the plant's use of these amino acids in the synthesis of substances with a protective role.

After performing the spectrophotometric analysis, the yellow extract was dried in order to perform the infrared spectrometry analysis. This analysis was performed to determine the bands characteristic of the aromatic and aliphatic C-H vibrations, the C=O bands of carboxylic acids, and the NO vibration of nitroderivatives.

Analysis 387.68 vw, 586.34 vw, 626.84 w, 713.63 s, 827.43 vs, 1039.59 m, 1076.23 w, 1352.04 vs, 1400.26 vs, 1762.86 m, 2083.03 w, 2331.84 w, 2345. 34 w, 2354.99 w, 2395.49 m, 2505.43 vw, 2798.60 m, 3030.04 m, 3134.20 m revealed the presence of the compound of interest (Figure 4).

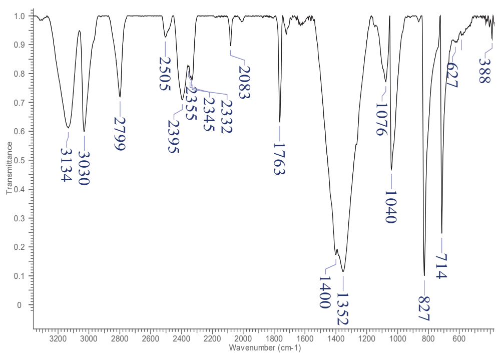

Figure 4. FTIR spectrum of the solid formed after drying the sample used to determine AAA

To carry out the statistical analysis of the AAA absorbance values in control and treated plants, the Anova Two Factor test was used (Table 2). After performing the Anova Two Factor test (Table 2), insignificant differences were found in the AAA absorbances (p≥0.05) obtained after the treatments with saline solutions and those of the control plants. In this situation the null hypothesis is accepted.

The result obtained from the Two Factor Anova test can be explained by the different behavior of Line 3, which showed an increase in AAA in stressed plants compared to the general trend of decreasing absorbances in the case of treated variants in the other studied genotypes.

Table 2 - Analysis of the variance of the absorbance of aromatic amino acids in the leaves of bitter cucumber genotypes subjected to salt stress

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*	*Significance*
Rows	0.002769	4	0.000692	0.86649	0.523528	3.837853	NS
Columns	0.003636	2	0.001818	2.275971	0.165012	4.45897	NS
Error	0.006391	8	0.000799				
Total	0.012796	14					

Anova Two-Factor: NS non-significant statistical differences (p≥0.05); *significant statistical differences (p≤0.05); **distinctly significant statistical differences (p≤0.01); ***highly significant statistical differences (p≤0.001)

CONCLUSIONS

Following the study of the proline content determined in the analyzed bitter cucumber (*Momordica charantia*) genotypes, an increase in the amount of this amino acid directly

proportional to the increase in the concentration of the applied saline solution was noted. The most pronounced difference between the control and the V1 and V2 treatments was recorded in the case of Line 3 where the amount of proline increased in V1 by 3.25 times compared to the control and in the V2 treatment by 12.53 times. This significant increase may demonstrate an adaptation of the genotype to the salinity conditions to which it was exposed.

Another evidence of the increased resistance to salt stress of this line is given by the lack of a large amount of proline in the control plants. Among the genotypes studied, Line 3 was highlighted by the lowest proline synthesis capacity under normal environmental conditions. This demonstrates that salt stress is the main factor that caused the increase in proline, a fact also observed by the result of the Two Factor Anova test.

The determinations regarding the analysis of aromatic amino acids in the leaves of the studied *Momordica charantia* genotypes revealed a tendency to decrease the absorbance of these substances proportional to the increase in NaCl concentration with which the plants were stressed. A distinct behavior was noted in Line 3 where AAA absorbance increased in saline-treated plants compared to the control. This phenomenon can be explained by the adaptation of this line to the abiotic stress conditions to which it was subjected, which allows us to recommend it to be cultivated on saline soils, in order to obtain a profitable harvest and some satisfactory nutritional properties.

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CONTENT OF POTASSIUM AND IRON IN TOMATO PRODUCTS

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Abstract

Tomato (Lycopersicon esculentum Mill.) is an annual vegetable crop whose fruits can be consumed fresh or processed. The aim of this thesis was to determine the amount of potassium and iron in samples of tomato products from different production batches. Sampling was carried out on six tomato products (ketchup, double concentrate, canned tomatoes, pureed tomatoes and chopped tomatoes from conventional and organic farming). Potassium was determined by flame photometry while iron was determined by AAS (Atomic Absorption Spectroscopy). The determined content of potassium in dry matter ranged from 1.36 to 5.37 % K DW. Potassium levels in fresh matter ranged from 185.19 to 1224, 46 mg K/100 g fresh weight. The determined content of iron in dry matter ranged from 24.30 to 155.07 mg Fe/kg DW. Iron levels in fresh matter ranged from 0.42 to 1.23 mg Fe/100 g fresh weight. The highest potassium content in dry and fresh matter was determined in tomato concentrate. The highest iron content in dry matter was determined in chopped tomatoes, while the highest iron content in fresh matter was determined in tomato concentrate.

***Key words**: Lycopersicon esculentum Mill., minerals, microelement, macroelement, processing.*

INTRODUCTION

The tomato (*Lycopersicon esculentum* Mill.) is a herbaceous plant and one of the most famous plants in the Solanaceae family. It originates from South America and was cultivated in Europe in the 16th century (Borošić, 2016; Lešić et al., 2016; Matotan, 2004).

The tomato is a vegetable that is cultivated worldwide due to its high yields, and every year 189,133,955 tons of fruit are harvested from around 5,167,388 hectares of cultivated land with an average yield of 36.6 t/ha (FAO, 2021). The world's largest producers of tomatoes are China and the USA (Lešić et al., 2016).

In developing countries, annual tomato consumption is less than 10 kg per inhabitant, while in developed countries it is three times as high (Lešić et al., 2016).

Tomatoes are most often consumed fresh, but due to their diverse nutritional properties, they are also highly valued in processed products. Tomatoes intended for processing are mostly grown in the open field and their cultivation is based on the use of mechanization. There are numerous products on the market obtained from the processing of tomatoes, such as concentrates, peels, ketchup, passata, chopped tomatoes, juice and dried tomatoes. The processing of tomatoes meets consumer demand for products with a longer shelf life (Thakur et al., 1996; Matotan, 1994).

Ripe tomato fruits can contain different amounts of dry matter: 3-6% (Parađiković, 2009), 5-7% (Lešić et al., 2006), 4-6% (Matotan, 2004), therefore their energy value is low and amounts to only 20-25 kcal per 100 g of fruit (Matotan, 2008).

Tomatoes are also an important source of vitamins and minerals for the human body. Of the minerals, potassium (92-376 mg/100 g fresh matter), phosphorus (7-53 mg/100 g fresh matter), magnesium (13-20 mg/100 g fresh matter), calcium (10-21 mg/100 g fresh matter) and iron (0.4-1.2 mg/100 g fresh matter) are the most abundant (Lešić et al., 2016).

Clay soils with a heavy structure and texture contain the most potassium, in contrast to sandy soils with a lighter mechanical composition, where its amounts are very low. Under humid climatic conditions, there are significant losses of potassium through leaching (Čoga and Slunjski, 2018; Vukadinović, 2011). The decomposition of primary minerals leads to the release of potassium, which is mostly bound to the adsorption complex of the soil (Vukadinović, 2011).

There are two main physiological roles of potassium: the activation of enzymes and the regulation of the permeability of living membranes. Plants do not incorporate it into organic compounds, but it acts as an activator for about 60 enzymes (by changing pH, ion concentration, temperature and the presence or absence of inhibitors). It also plays a very important role in the synthesis of protein, sugar, cellulose and fat (Vukadinović, 2011).

Potassium is required for the formation of adenosine triphosphate (ATP), influences the physiological activity of ribosomes and has a positive effect on the process of photosynthesis. It also improves the quality of the yield and influences the resistance of plants to diseases and drought (Butorac, 1999).

Potassium is one of the most important elements in the human body and is absorbed through plant food. It is contained in large quantities in tomatoes and has a positive effect on the work of the heart and blood vessels and lowers blood pressure and cholesterol levels (Vukadinović, 2011; Butorac, 1999).

Its deficiency in humans is usually the result of excessive secretion during sporting activities or heavy physical work. It also plays an important role in muscle function, and its deficiency can result in muscle cramps, weakening of the heart and weak intestinal function (Vukadinović, 2011).

The daily requirement of an adult for potassium is around 3.5 g, while safe amounts are up to 5 g per day (Vukadinović, 2011).

Iron is mainly present in the ferrous form (Fe^{2+}) in acidic soils and under reducing conditions, while the ferric form (Fe^{3+}) is more common in oxygen-rich soils with a higher pH value. The ferrous form of iron is more easily accessible to plants than the ferric form, which is usually insoluble. In the adsorption complex of the soil, the iron is bound in the exchangeable form as Fe^{2+}, while the Fe^{3+} form is very tightly bound (Čoga et al., 2010; Butorac, 1999).

Iron plays an important role in the work of various enzymes: peroxidase, catalase and cytochrome. Iron deficiency affects the reduction in the number of photosynthetic units (PS I), the reduction in the number of cytochrome molecules and the decrease in the concentration of carotenoids (Vukadinović, 2011; Butorac, 1999).

Its mobility is low, as 80-90 % of the iron in plants is firmly bound. The uptake of iron can lead to competition with other elements, such as Cu > Ni > Co > Zn > Cr > Mn (Kobayashi et al., 2019).

Iron plays an important role in the human body and is an essential component of hemoglobin. In plants, it regulates plant growth and metabolism and is involved in the process of photosynthesis, which influences plant productivity (Vukadinović, 2011; Butorac, 1999).

Iron deficiency in the human body is a common phenomenon. It occurs most frequently in athletes, women during the monthly cycle, pregnant women and children due to inadequate consumption of fruits and vegetables.

The daily iron requirement of an adult man is about 10-15 mg, for adult women and developping children about 20 mg and for pregnant women about 60 mg (Vukadinović, 2011).

The aim of this work is to determine the amount of potassium and iron in samples of tomato products from different production batches.

MATERIALS AND METHODS

For the purpose of this study, 6 different tomato products were sampled: (i) ketchup, (ii) peels, (iii) chopped tomatoes, (iv) tomato concentrate, (v) pureed tomatoes and (vi) chopped organic tomatoes. Sampling was carried out three times (in triplicate) between December 16, 2022 and February 16, 2023, taking care to sample the same product from the same producer from a different production series (lots) each time (Table 1).

Samples of tomato products were submitted to the Analytical Laboratory of the Department for Plant Nutrition, Faculty of Agriculture, University of Zagreb. The samples were homogenized, prepared for drying and dried at 105°C. After drying, the samples were ground. The dry matter was determined by the gravimetric method by drying to a constant mass. Dry and ground samples of tomato products were digested with concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a microwave oven, after which potassium was determined by flame photometry and iron by atomic absorption spectrometer.

Table 1. Datum uzorkovanja i prikaz šarži uzoraka proizvoda od rajčice

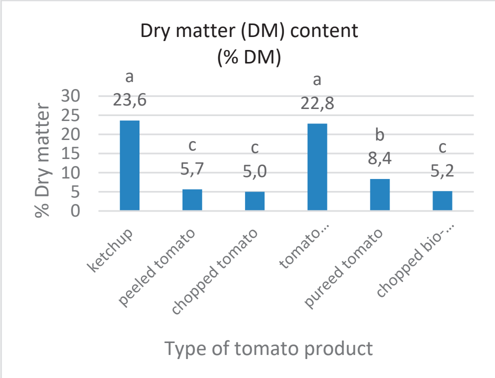
TYPE OF PRODUCTS	LOT NUMBER
SAMPLING 1, Date 16/12/2022	
ketchup	12.04.2024. 18:37
peeled tomato	VTL1 U226 BBE:31/12/2024
chopped tomato	VTL1 E211 31.12.2023.
tomato concentrate	19.06.2024. 19:56
pureed tomato	04.07.2025. L21:38 42078
chopped bio-tomato	L1521 31/05/2023 13:08
SAMPLING 2, Date 16/01/2023	
ketchup	03.07.2024. 18:23
peeled tomato	VTL1 F231 BBE:31/12/2025
chopped tomato	VTL1 U239 BBE:31/12/2024
tomato concentrate	17.07.2024. 16:38
pureed tomato	18.10.2025. L11:31 15514
chopped bio-tomato	VTL1 U210 BBE:30/06/2024
SAMOLING 3, Date 16/02/2023	
ketchup	08.11.2024. 20:09
peeled tomato	VTL1 E218 31/12/2023
chopped tomato	VTL1 E208 BBE:31/12/2025
tomato concentrate	15.12.2024.
pureed tomato	14.11.2025. L21:02 15068
chopped bio-tomato	VTL1 U210 PLP BBE:31/12/2024

Statistical data processing was carried out using the analysis of variance (ANOVA) model. The program used was SAS System for Win, Version 9.1 (SAS Institute Inc.), and the Tukey test of significant thresholds was used to test the results (SAS, 2002-2003).

RESULTS AND DISCUSSIONS

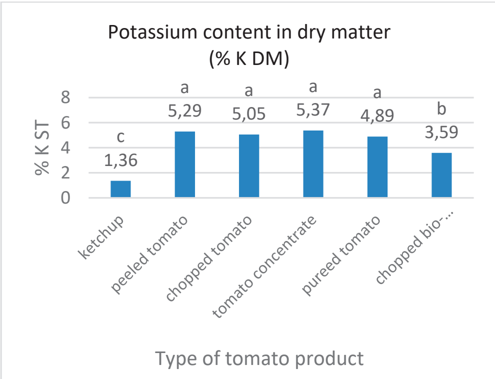
Figure 1 shows the dry matter content of tomato products (% ST). The dry matter in the tested products was determined in a range from 5.02 to 23.62% ST. The statistically significant highest percentages of dry matter were found in ketchup (23.62% ST) and tomato concentrate (22.80% ST). The statistically lowest percentages of dry matter were found in pellets, chopped tomatoes and organically chopped tomatoes.

Figure 2 shows the potassium content in tomato products in relation to dry matter (% K ST). The potassium content, based on dry matter, was determined to be in the range of 1.36 to 5.37% K ST in the products tested. The statistically significant highest proportion of potassium in the dry matter was found in tomato concentrate, pellets, chopped and pureed tomatoes. The statistically lowest proportion of potassium in the dry matter was found in ketchup.



Different letters represent significantly different values according to Tukey's test, $p \leq 0.05$. The non-letter values are not significantly different.

Figure 1. Dry matter content (% DM) of investigated tomato products

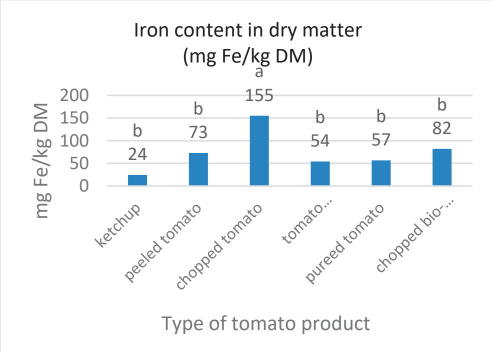


Different letters represent significantly different values according to Tukey's test, $p \leq 0.05$. The non-letter values are not significantly different.

Figure 2. Potassium content in dry matter (% K DM) of investigated tomato products

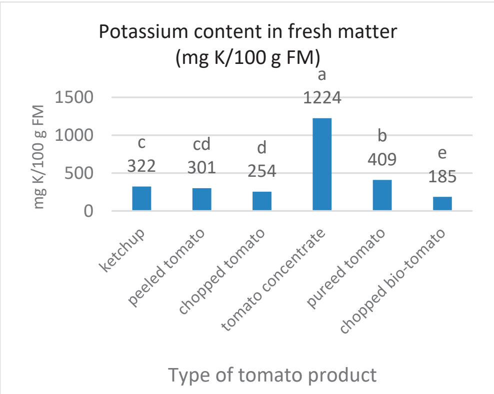
Figure 3 shows the amount of potassium in fresh mass in tomato products (mg K/100 g fresh mass). The potassium content in the fresh matter of the tested products was determined in a range from 185.19 to 1224.46 mg K/100 g fresh matter. The statistically significant highest potassium content in fresh matter was found in tomato concentrate. The statistically lowest potassium content in fresh matter was found in organically crushed tomatoes.

Figure 4 shows the iron content of tomato products in relation to the dry matter (mg Fe/kg ST). The iron content, based on dry matter, was between 24.30 and 155.07 mg Fe/kg ST in the products tested. The statistically significant highest iron content was found in chopped tomatoes. The statistically lowest iron contents in the dry matter were found in organically chopped tomatoes, pellets, tomato concentrate and pureed tomatoes.



Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

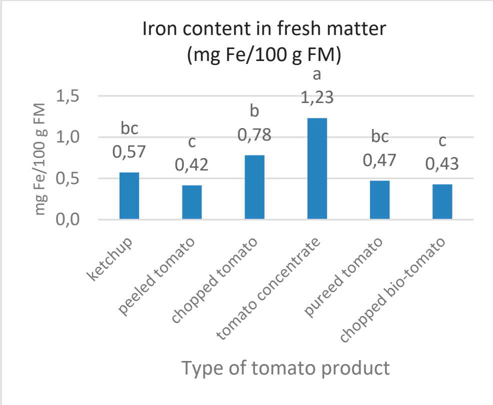
Figure 3. Iron content in dry matter (mg Fe/kg DM) of investigated tomato products



Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

Figure 4. Potassium content in fresh matter (mg K/100 g FM) of investigated tomato products

Figure 5 shows the iron content in fresh matter in tomato products (mg Fe/100 g fresh matter). The iron content of the products tested was between 0.42 and 1.23 mg Fe/100 g fresh substance. The statistically significant highest amount of iron in fresh matter was found in tomato concentrate. The statistically lowest iron contents in fresh matter were found in ketchup, seals, pureed tomatoes, chopped and organically chopped tomatoes.



Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

Figure 5. Iron content in fresh matter (mg Fe/100 g FM) of investigated tomato products

Tomatoes can be eaten fresh or processed into various products due to their perishable nature: Peels, tomato juice, concentrated tomato juice, tomato puree or pureed tomatoes (Bannwart et al., 2008).

Petek et al. (2021) state that the nutritional value of tomatoes and tomato products can be improved by fertilization.

Budak et al. (2016) state that there are highly significant differences in the composition of fresh tomatoes and tomato-based products in terms of sodium, phosphorus, calcium, copper, zinc and molybdenum, while no significant differences were found for magnesium, potassium, iron and manganese. Similarly, the content of microelements in food depends on soil properties, such as organic matter content, pH and clay mineralogy.

Budak et al. (2016) state that tomato juice and chopped tomatoes are most similar to fresh tomatoes in terms of mineral content and are the best choice for consumption in the human diet compared to other tomato products.

Porretta et al. (1993) state that despite the large market for tomato products in many countries, chopped tomatoes tend to be neglected in the technical and scientific literature. Lovrić and Piližota (1994) state that double tomato concentrate should generally contain 28-38% ST, while the dry matter in ketchup should be 25-35% ST. In the analyzed samples of tomato concentrate (22.80% ST) and ketchup (23.62% ST), lower values were found than those given by Lovrić and Piližota (1994). The lowest proportion of dry matter in the analyzed samples was found in chopped tomatoes (5.02%). In the production of ketchup, a concentrate is made according to the usual procedure or by diluting a concentrate containing larger amounts of dry matter, to which various spices are then added. In the production of chopped tomatoes, on the other hand, the end product consists of finely chopped tomatoes with a juice content of at least 30%. In most cases, the tomatoes are peeled, the stems removed and the core cleaned, which may be the reason for the lower dry matter content (Lovrić and Piližota, 1994).

The percentage of dry matter in the tomato varies depending on the variety, soil properties and especially the amount of irrigation and rainfall during the growth and harvest phase (Barringer, 2004). In this study, the dry matter in tomato products was determined in a range from 5.02 to 23.62% ST. The highest proportion of dry matter was found in ketchup, the lowest in chopped tomatoes.

In the literature, data on the dry matter content can only be found for three tomato products: peel, concentrate and ketchup. Peels contain an average of 5.64% dry matter (Lončarić et al., 2015, after Petek et al., 2021). Petek et al. (2021) state that 26.75% ST was found in tomato concentrate and 30.45% ST in ketchup. The following amount of dry matter was determined in the tomato products tested Peels (5.67% ST), concentrate (22.80% ST), ketchup (23.62% ST). Compared to the data cited by Lončarić (2015), a higher proportion of dry matter was found in the pellets examined here. On the other hand, the data reported by Petek et al. (2021) for the proportion of dry matter in concentrate and ketchup are higher than the data obtained in this study.

Maher (1976) states that 4.5% K ST was found in tomato fruits. The potassium content of the products examined was determined to range from 1.36 to 5.37% K ST. The highest potassium content in the dry matter in this study was found in tomato concentrate (5.37% K ST). The determined potassium content in the dry matter of the concentrate is higher than the values given by Maher (1976) for tomato fruits. It is assumed that the higher amounts of potassium in tomato concentrate compared to other processed products are due to the high proportion of dry matter in the end product. Concentrates are categorized according to the proportion of dry matter: rare juice (no prescribed proportion of dry matter), single concentrate (14-16 %), double concentrate (28-30 %), triple concentrate (36 %) and sixfold concentrate (55%) (Lovrić and Piližota, 1994). The tomato concentrate investigated in this study belongs to the double concentrate category. As a rule, it should have an ST content of 28 to 30%, and a dry matter content of 22.80% ST was determined.

The potassium content in the fresh mass of the tested products was determined in a range of 185.19 to 1224.46 mg K/100 g fresh mass. The highest potassium content in fresh mass was found in tomato concentrate, the lowest in organically chopped tomatoes. The amount of potassium in fresh tomatoes varies between 244 (Sainju et al., 2003), 279 (Breecher, 1998) and 376 mg K/100 g fresh mass (Lešić et al., 2016). In the tomato concentrate, a higher potassium content was determined in the fresh substance than in the data reported by various authors for fresh tomatoes.

Ayari et al. (2015) state that pureed tomatoes can contain 249-332 mg K/100 g fresh mass, tomato concentrate 890-1110 mg K/100 g fresh mass and ketchup 243-334 mg K/100 g fresh mass. In this study, it was found that pureed tomatoes contain 408.61 mg K/100 g fresh matter, tomato concentrate 1224.46 mg K/100 g fresh matter and ketchup 321 mg K/100 g fresh matter. The determined data on potassium content in fresh matter in pureed tomato and concentrate are higher than the data reported by Ayari et al. (2015), while the data on potassium content in ketchup do not differ.

Vukadinović (2011) states that potassium plays an important role in human nutrition. It is important for the work of muscles, and as a result of its deficiency, muscle cramps, weakening of the heart and weak intestinal activity can occur. An adult's daily requirement for potassium is around 3,500 mg, while safe levels are up to 5,000 mg per day. According to the results of this study, tomato concentrate contains the most potassium (1224.46 mg K/100 g fresh mass). About 35% of the daily potassium requirement can be met by consuming 100 g of tomato concentrate. It was found that pureed tomatoes contain the most potassium in the fresh mass after the concentrate (408.61 mg K/100 g fresh mass). Only 12% of the daily potassium requirement can be met by consuming 100 g of pureed tomatoes. Lovrić and Piližota (1994) state that tomato products are mostly used as an addition to certain dishes and are not consumed as a meal in their own right. Considering the fact that the consumption of tomato products analyzed in this study can cover a maximum of 35% of the daily potassium requirement, it is necessary to include other potassium-rich products in the daily meal, for example bananas, spinach, milk and dairy products, etc.

The iron content, based on dry matter, was determined to be in the range of 24.30 to 155.07 mg Fe/g ST in the products tested. The highest iron content, based on dry matter, was found in chopped tomatoes, the lowest in ketchup. Chopped tomatoes and organically grown chopped tomatoes are produced using a similar process to peelings, with the difference in the production process being that the tomatoes are cut into small pieces. The process is based on steaming the raw material, which, unlike other tomato products, does not require prolonged heat treatment (Lovrić and Piližota, 1994).

The iron content in the fresh mass of the tested products was between 0.42 and 1.23 mg Fe/100 g fresh mass. The highest iron content in the fresh substance was found in the tomato concentrate, the lowest in the pellets. It is assumed that the higher iron content in the concentrate compared to other processed products is a result of the high proportion of dry matter in the end product.

The potassium content in fresh tomatoes varies between 244 (Sainju et al., 2003), 279 (Breecher, 1998) and 376 mg K/100 g fresh mass (Lešić et al., 2016).

The iron content in fresh tomatoes varies between 0.33 (Matotan, 1994), 0.5 (Sainju et al., 2003) and 1.2 mg Fe/100 g fresh mass (Lešić et al., 2016). The values determined in this study for the iron content in fresh matter agree with the values of other authors. Barringer (2004) states that 100 g of tomato juice contains at least 1.0 mg of iron.

Kabore et al. (2022) state that iron and iodine are very important minerals for the normal functioning of human metabolism. Vukadinović (2011) states that iron in the human body is an integral part of red blood cells and hemoglobin, whose task is to transport oxygen from the lungs to the tissues. Iron deficiency leads to migraines, deconcentration, poor mobility and poor blood circulation in the skin. The daily iron requirement for an adult male is around 10-15 mg, for adult women and developing children around 20 mg and for pregnant women around 60 mg. The toxic effect occurs with an intake of more than 5 g of iron per day. According to the results of this study, the highest iron content in fresh material was found in tomato concentrate (1.23 mg iron/100 g fresh material). Only about 6% of the daily potassium requirement can be met by consuming 100 g of tomato concentrate. Since the consumption of the tomato products examined in this study can cover at most 6% of the daily iron requirement, it is necessary to include other iron-rich foods such as beans, red meat, walnuts, millet and lentils in the daily meal.

CONCLUSIONS

An analysis of tomato products showed that tomato concentrate contains the highest levels of potassium and iron in fresh mass. It is assumed that the highest content of potassium and iron in the fresh substance was determined due to the high proportion of dry matter (22.80% ST) in the tomato concentrate. A high proportion of dry matter (23.62% ST) was also found in the ketchup. The determined amount of potassium and iron in the fresh substance of ketchup is lower compared to the tomato concentrate. The production of ketchup is based on single, double or triple concentrate to which a considerable amount of sugar and various spices are added, resulting in a lower potassium and iron content in the fresh substance.

The daily requirement of potassium and iron in the human diet cannot be met by consuming tomato concentrate and other tomato products. It was found that 35% of the daily requirement of potassium and only 6% of the daily requirement of iron can be met by consuming tomato concentrate.

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USING BIOCHAR AND ORGANIC FERTILIZER IN THE CULTIVATION OF BOK CHOY (*BRASSICA RAPA* L. SSP. *CHINENSIS* L.)

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Abstract

In period during 2022 and 2023 years a pot experiment was conducted in a greenhouse using alluvial-meadow soil. Two seeding dates were tested. Five variants with different doses of biochar and humate fertilizer (Bioforce) was developed. The test crop was leafy vegetable - Bok Choy (Brassica rapa L. ssp. chinensis L.). Results indicate that increased moisture and temperature in the vegetative house caused premature budding and flowering of the plants. In the variant with Soil + Bioforce (humic fertilizer) *showed high photosynthetic activity, while variant Biochar 112.5 g per pot + Bioforce (20%) exhibited the best biometric indicators. Plants grown in March-April were taller but had less mass compared to those from December. The mass flowering makes the method suitable for seed production but not for consumption. Sugar levels followed a similar trend in both assessments. The least amount of accumulated sugars was in variant 3 with 50% Biochar. Variant 4 consistently had the highest sugar content, ranging from 7.6 to 7.7% Brix. The low nitrate content in leaf tissue was evidenced. The nectar`s sugar content and pollen quantity were assessed to determine the honeybee potential of Bok Choy by the tested variants.*

Key words*: Bok Choy, Brassica rapa L. ssp. chinensis L., biochar, humate fertilizer, honeybee potential.*

INTRODUCTION

In recent years, research in agriculture has been focused on developing good agricultural practices not only to achieve high yields but also to produce products that meet international quality standards and recommended levels of environmental impact. One of the primary environmental problems stemming from agricultural activities is the pollution of surface and groundwater with nitrates, as well as harmful emissions of greenhouse gases released from the soil. That is why countries developing modern agriculture are placing increasing importance on technologies for utilizing crop residues. In the context of climate change, the idea of applying biochar in agriculture has drawn considerable attention among scientific communities (Lehmann, 2007). Biochar has a wide range of applications and advantages due to its distinctive characteristics such as high cation exchange capacity, adsorption capacity, microporosity, high carbon content, and stable structure, and it is widely used in carbon sequestration, soil restoration, water and wastewater purification (Mohanty et al., 2013; Rizwan et al., 2016).

Leafy vegetables that require minimal processing are of great interest to consumers due to their easy usage. Bok choy (*Brassica rapa*) is a vegetable from the Brassicaceae family, which also includes cabbage, broccoli, cauliflower, and Brussels sprouts. It is also very closely related to turnips and rutabagas. In their results, Genesio et al. (2012) confirm the positive influence of applying biochar from rice husks on the growth and development of lettuce (*Lactuca sativa*) and Chinese cabbage (*Brassica chinensis*). The use of biochar in vegetable cultivation has been studied by many authors, and the conducted research unequivocally demonstrates that its application, in combination with balanced organic fertilization, leads to better results (Mikova et al., 2015).

Priadi and Nuro (2017) conducted experiments with Bok choy using organic and inorganic substances and found that their combination led to the highest parameters of seedling growth and leaf indices, as well as positive correlations between growth parameters.

Bok choy is still a relatively unknown vegetable crop in Bulgaria. There is scant literature data regarding its cultivation in our country, which is the subject of the present study.

The aim of the research is to evaluate the impact of combined application of carbonized plant residues (biochar) rate and humic fertilizer as soil amendments on the growth and development of Bok choy.

MATERIALS AND METHODS

During 2022 and 2023 years, a pot experiment was conducted on alluvial-meadow soil from the field of Vrazhdebna distruct - Sofia. The experiment was set up in the glazed greenhouse of the University of Forestry. The tested crop was leafy vegetable - Bok choy (*Brassica rapa L. ssp. chinensis L.*).

Five variants were developed, in three replications:

- Control - clean soil without amendments;
- Soil + Bioforce (humic fertilizer);
- Biochar 225 g per pot + Bioforce (50%) (BC 50%);
- Var. 4 Biochar 112.5 g per pot + Bioforce (20%) (BC 20%);
- Ver. 5 Biochar 67 g per pot + Bioforce (15%) (BC 15%).

The quantity of biochar (BC) applied was chosen based on literature studies (Haryanto et al., 2022), aiming to track the influence of fertilization with the humic fertilizer product name “Bioforce”, and the biochar's ability to absorb and release nutrients more slowly. The application of the biofertilizer occurred through irrigation water, with the same quantity used for all variants.

For the experiment setup, pots with 5 kg of soil were used, to which the respective presenting of biochar p pot were added. Two sowing dates were tested on December 2 (2022), and March 10 (2023), with 10 seeds of Bok choy planted per pots. At the stage of the second true leaf, three plants were left in each container.

The biochar used for the experiment was produced from wood chips.

The humic fertilizer was applied twice with the irrigation water at both sowing dates, in the initial phase of development and at the stage of 3-4 true leaves. The dosage was 100 mL per pots diluted 1:10 according to the manufacturer's recommendations.

Maintaining an optimal watering regime close to the field capacity was ensured by frequent irrigation with a small amount of water, with the quantity of water used determined using the gravimetric method.

Phenological observations and biometric measurements were conducted during the vegetation period. The photosynthetic activity, total sugar content, nitrates, and absolute dry matter content in the experimental plants were determined. After determining the fresh weight, the leaves were placed in a dryer at 60°C, where they were dried to obtain the absolute dry weight. The leaf water content was determined using the formula described by Jin et al. (2017). The chlorophyll content was measured according to Sarah C. et al., 2013, methodology. The percentage content of sugars in the plant cell (Brix, %) was measured with a refractometer model - Digital refractometer 32145, manufactured by B & C Germany.

The biochar used to conduct the experiment was produced from wood chips. Table 1 presents the chemical analysis of biochar the data being provided by the manufacturer.

Table 1. Chemical characteristic of biochar (BC)

	pH	EC mS/m	W%	C%	N%	P%	K%	Ca%	Mg%	$CaCO_3$
BC	10.8	45	10.3	61.8	0.39	0.22	0.85	2.18	0.23	5.4

The pH reaction of biochar is strongly alkaline. It contains a large amount of carbon, which confirms the ability of BC to deposit carbon in the soil, reducing its release into the atmosphere.

Pre-measured standards were introduced into each vessel in the form of a fine powder fraction.

The humate fertilizer used for pot experiment is “Bioforce” based on lombricompost with the following commercial characteristics:

Table 2. Chemical characteristic of Bioforce

pH		Total N g/kg	P_2O_5 mg/ 100 g	K_2O mg/100 g	Humus %
H_2O	KCl				
9.0	8.4	155.5	260.0	760.3	32.3

According to data given by producer the reaction of humic fertilizer is highly alkaline, it is well stocked with nutrients.

RESULTS AND DISCUSSIONS

Evaluation of the abiotic factors during the vegetation experiment

Temperature, light, and air humidity play an important role in greenhouse production and can have a significant impact on the growth and development of plants. Light and warmth are essential vital factors determining the growth and development of vegetable crops.

Deviation from these factors can halt life processes, leading to plant death. High temperature is one of the stressful factors that significantly reduce photosynthetic activity and plant productivity (Oukarrorum et al., 2002). Agrometeorological and phenological observations are valuable sources of information for understanding the relationship between climate and plant development during the vegetation period.

During the conducted experiment (December-May), the temperature in the greenhouse was monitored.

During the vegetative growth of Bok choy at both sowing dates, entirely positive temperatures were observed. Temperatures for the first vegetation period from December 2 (2022) to February 20 (2023) ranged between 8 and 15°C, with no sharp temperature fluctuations.

For the second sowing date from March 13 (2023), to May 26 (2023) temperatures increased up to 22°C at the end of April. The optimal temperature for the development of this crop is considered to be between 13°C and 24°C. The temperatures recorded by us are suitable for the normal growth and development of the crop. Despite the suitable temperature range at both sowing dates, the plants do not form well-rounded rosettes and transition to the budding and flowering phase.

Relative humidity (RH %) is the ratio between the amount of moisture present in the air and the maximum amount of moisture that the same air can hold at the same temperature and atmospheric pressure. Relative humidity is expressed in percentages. When the air is at 100% RH, it cannot hold more water vapor. Warm air can hold more water vapor than cold air. Therefore, as the air temperature increases, Figure 1. Air temperature during the period 2022-2023 Bok choy growing season RH decreases, and as the temperature drops, RH increases. If the air becomes cold enough, moisture condenses out of the air as dew.

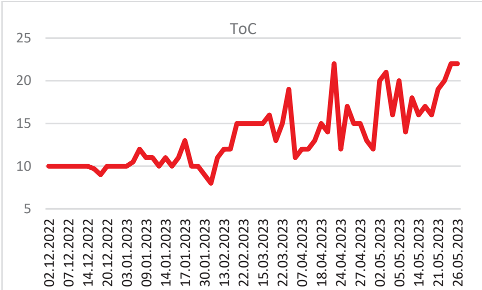

Figure 1 Temperature dynamics in greenhouse

The humidity during the crop's vegetation period is high (Figure 2), reaching up to 97%. The lowest values were recorded in early February, which resulted from the temperature rise. When values above 90% are recorded, the greenhouse is ventilated. High humidity is also associated with the plants' ability to absorb water, as the saturated air reduces transpiration in plants.

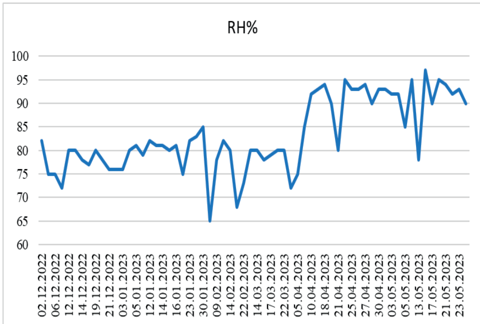

Figure 2. Relative humidity during the period 2022-2023 Bok choy growing season

The increased air humidity combined with high soil moisture likely exerts a suppressive effect on the development of the crop. Arve et al. (2011) reported that reduced ventilation, which consequently leads to increased relative humidity, affects plants in various ways and may result in plants that are less tolerant to water stress. This type of water stress leads to deformation of the stomata. To minimize the negative effects of this type of water stress,

plants respond by altering their growth pattern, producing stress proteins, enhancing regulation of antioxidants, accumulating compatible solutes, increasing the quantity of transporters involved in water and ion absorption and transportation, and by closing stomata.
The increased air humidity combined with higher temperatures is one of the probable reasons for the premature budding and flowering of Bok choy. Regardless of the temperature differences in the two development periods and at both sowing dates, mass flowering occurs between 50 and 55 days of vegetative growth.

Evaluation the impact of simultaneous application of organic fertilizers and biochar on the growth metrics and fresh yield of Bok Choy

About a week after sowing the Bok choy, the number of sprouted plants was counted in replicates.
The highest number of germinated plants were recorded in variant 3 with 50% biochar, with an average of 8.33 and 7 plants per variant, followed by variant 4 with 20% biochar. In both sowing dates, the lowest number of germinated plants was observed in the control variant, where a slower development of the plants was also observed.
Simultaneously with the recording of germinated plants, the phenological development of Bok choy was observed and described by variants.
It is noteworthy that the plants treated with biochar appear to be better developed compared to the control variant. The leaf rosette is better developed.

Table 3. Number of sprouted plants per pots during first showing date 02.12.2022

Variants	Replication			Aver. Number of germinated plants
	I	II	III	
Control	3	5	3	3.67
Bioforce	7	4	2	4.33
BC 50%	10	5	10	8.33
BC 20%	9	5	4	6.00
BC 15%	6	3	5	4.67

Table 4. Number of sprouted plants per pots during second showing date 10.03.2023

Variants	Replication			Aver. Number of germinated plants
	I	II	III	
Control	0	4	3	2.33
Bioforce	3	7	1	3.67
BC 50%	10	1	10	7.00
BC 20%	9	4	6	6.33
BC 15%	7	7	2	5.33

The data obtained upon completion of the experiment regarding the size of the leaves are presented in Tables 5 and 6.

Table 5. Size of the leaves at the first sowing date 02.12.2022

Variants	Leaves number	Leaves petiole cm	Leaves width cm	Leaves length cm
Control	6.44	2.87	2.29	3.57
Bioforce	7.44	3.27	3.48	4.94
BC 50%	9.30	2.10	3.85	4.95
BC 20%	9.70	1.96	4.17	5.01
BC 15%	9.80	2.60	3.59	4.81

The number of leaves varies between 6 and 14 in both sowing dates. Dinceva (2020) obtained the same data regarding the leaf count in a field experiment conducted at the Maritsa Vegetable Crops Research Institute. During the first sowing period, there are fewer leaves compared to the second growing period. In the first sowing date, the highest number of leaves were recorded in variant 5 with 15% biochar, and the lowest in the control variant. The plants from the first period have shorter petioles and smaller leaf rosettes compared to the second sowing date. The larger leaf mass is likely a result of higher air humidity, a statement supported by the results obtained by Arve et al. (2011). Another possible reason is the application of biochar, which during the second growth period has released a larger amount of NH_4^+. Biochar has a strong absorbing capacity and subsequently slow release of nutrients into the soil. Gomez-Eyles et al. (2013) report that the application of biochar increases the cation exchange capacity of the soil, with NH_4 ions being retained on the surface of the biochar.

Table 6. Size of the leaves at the second sowing date 10.03.2023

Variants	Leaves number	Leaves petiole cm	Leaves width cm	Leaves length cm
Control	10.25	4.44	6.16	8.95
Bioforce	10.88	5.15	5.81	9.04
BC 50%	7.29	4.80	6.04	8.39
BC 20%	9.25	5.25	5.84	9.01
BC 15%	9.71	5.63	6.37	8.50

The length of the whole plants together with the peduncles, which are shown in Figures 3 and 4, was recorded.

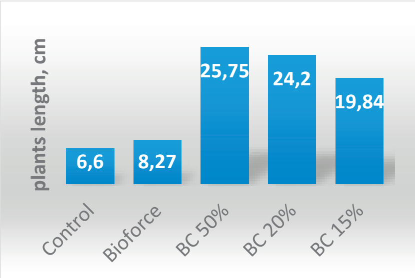

Figure 3. Plant height at harvest on the first sowing date

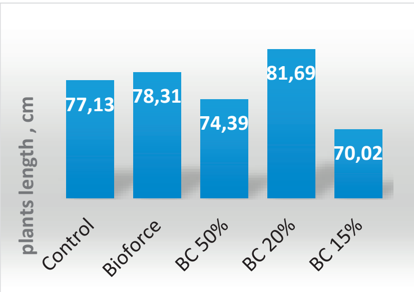

Figure 4. Plant height at harvest on the second sowing date

The height of the plants sown on December 2 (2022) ranges between 6.6 and 25.7 cm. The tallest plants were recorded in variant 3, followed by variant 2. Despite their relatively small height in the control variant, the plants from the first two variants approach the standard height of Bok choy, which ranges between 13 and 30 cm. The significant height of the plants is due to etiolation and elongation of the flowering stem.

The plants grown during the March-April period are on average about 50 cm taller than those sown in December. The strong elongation of the stems is likely due to insufficient lighting and the greater amount of infrared light. Infrared light has been proven to inhibit flowering in Bok choy. The tallest plants are in variant 4, while the shortest are in variant 5.

The number of flowering stems per variant is depicted in Figure 5.

The average number of flowering stems varies between 2.57 and 3.75. Variant 4 recorded the highest number of flowering stems, with individual plants reaching up to 6 stems, which is due to the combined effect of humic fertilizer and biochar. This variant demonstrates the highest biometric indicators, which determines the applied norm of 20% as suitable for Bok choy cultivation. The mass flowering during Bok choy cultivation in a glass greenhouse makes this method suitable for seed production but not for growing a crop suitable for consumption.

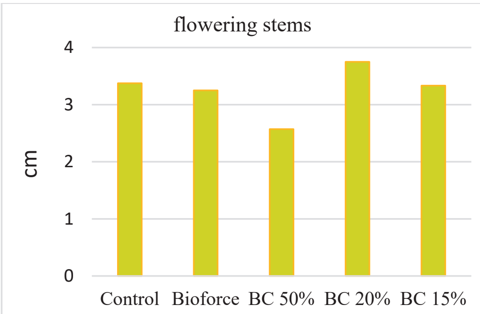

Figure 5. Average number of flowering stems

The yield of fresh weight for the first period, December to March, is lower than that recorded for the vegetation period from March to May. During the first period, it ranges between 15.2 and 17.7 g.

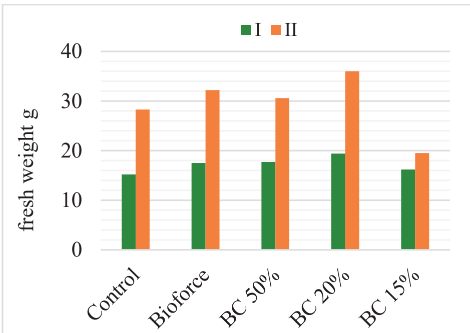

Figure 6. Fresh weight at harvest of Bok choy

It is noteworthy that the yield of fresh weight in the variant with the addition of biochar slightly exceeds that obtained from the control variant with pure soil. Although biochar itself is poor in nutrients, this is likely due to the improved water and air regime created as a result of its application, along with increased nutrient adsorption.

Chan et al. (2017) report that the application of biochar in combination with fertilization positively affects the growth and development of plants, but sometimes a negative effect is observed in variants with only biochar without added fertilizer due to reduced bioavailability, resulting from nitrogen adsorption. This is likely the reason for the lower fresh weight observed in variant 3 with the highest biochar application dose.

The fresh weight during the second vegetation period, March-May, is about 35% higher, with the highest average weight per plant observed in variant 4 with 20% biochar, corresponding to the soil volume, which is consistent with other biometric measurements obtained.

Crop yield is a result of the systemic interaction of processes occurring in plants. Assimilates produced by photosynthesis can be stored or distributed among different plant organs. Crop yield and plant development are not only a function of assimilate production and distribution but also an expression of the relationships between water and carbon, which interact with each other. This interaction is expressed through leaf temperature, stomatal conductance, relative water content in plant tissues, and leaf area.

The content of dry matter in Bok choy leaves is a direct indicator of the quality of the obtained product.

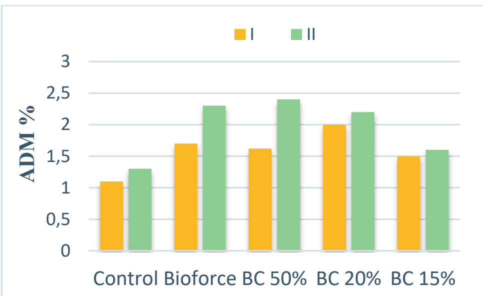

Figure 7. Absolute dry mass in 2022/2023 Bok choy plant samples

The percentage water content in plant samples is an indicator of maintaining an optimal water regime during vegetation.

Influence of combined organic fertilization and BC on some physiological indicators

Carbohydrates constitute the major portion (up to 85-90%) of the substances composing plant organisms. These are the structural, nutritional, and energetic materials of plant cells and tissues.

Measuring the content of total sugars in plant tissues (Brix %) is an important indicator because it provides us with a direct understanding of how the plant is functioning. A sugar molecule produced in the process of photosynthesis is the primary building block for everything we see growing above and below ground.

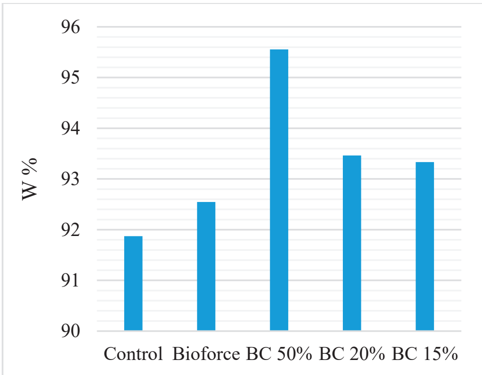

Figure 8. Moisture percentage in Bok choy plant samples 2022/2023

The percentage water content in plant samples is an indicator of maintaining an optimal water regime during vegetation.

Carbohydrates constitute the major portion (up to 85-90%) of the substances composing plant organisms. These are the structural, nutritional, and energetic materials of plant cells and tissues.

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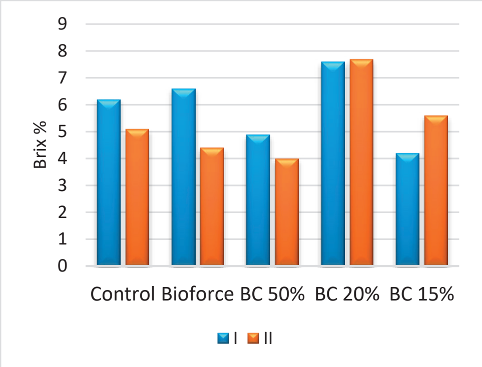

Figure 9. Percentage of total sugars in Bok choy leaf (2022/2023)

The amount of sugars follows the same trend in both observations. The least rate of accumulated sugars is found in variant 3 with 50% biochar. This is due to the higher water content in the leaf tissues observed in this variant. The highest amount of sugars is found in variant 4 for both sowing dates, ranging from 7.6 to 7.7%, which corresponds to the water content in the leaves of this variant.

Accumulation of nitrates in plants results from the uptake of nitrate ions and subsequent assimilation. Of all nitrogen sources in the soil, plants primarily uptake ammonium.

The regulation does not mention the permissible content for Bok choy, but the defined levels for spinach and lettuce are 3500 mg NO_3^-/kg.

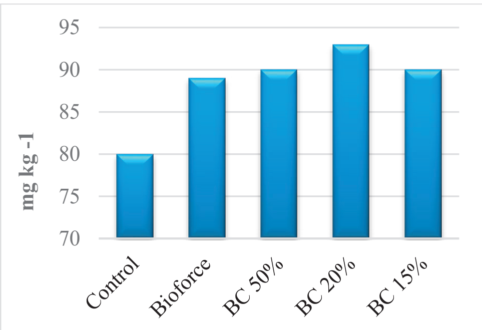

Figure 10. Nitrate content of Bok choy leaf (2022/2023)

The gradual increase in nitrates in the variants with added biochar is likely due to biochar's ability to absorb nutrients and release them over a longer period. The nitrate values range from 80 to 93 mg/kg, with the highest value recorded in variant 4, which also has the best-developed leaf mass.

Despite the presence of nitrates in fresh Bok choy leaves, the concentration does not exceed the regulated limit in Regulation 1881/2006, which classifies the product as suitable for consumption.

Photosynthesis is a unique physiological process inherent to photoautotrophic green plants.

Chlorophylls, the primary colour pigments in green vegetables, have two main types: chlorophyll "a" and chlorophyll "b". Chlorophyll "a" typically occurs in concentrations 2-3 times higher than chlorophyll "b" in agricultural products (Kirca et al., 2006). Carotenoids in plant tissues have two main functions: they participate in the absorption and transfer of radiant energy to chlorophyll "a" and protect chlorophyll molecules.

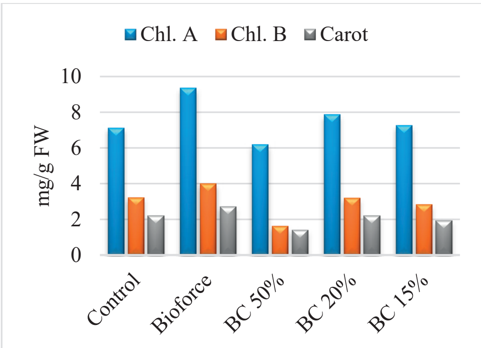

Figure 11. Content of plastid pigments in leaves of Bok choy

With the highest photosynthetic activity, variant 2 with the addition of humic fertilizer Bioforce. The absence of biochar accelerates the absorption of nutrients. Among the variants with added biochar, variant 4 with 20% of the norm again shows the best indicators. The ratio of 1:2 between chlorophyll a and chlorophyll b is preserved in all variants. The content of carotenoids ranges between 1.42 and 2.74 mg/g FW. The absence of elevated levels of carotenoids indicates the absence of stress in the cultivated plants.

CONCLUSIONS

The soil and climatic conditions inside the greenhouse are favourable for growing Bok choy, and the growth period progresses under favourable conditions.

The increased humidity combined with higher temperatures is one of the probable reasons for premature budding and flowering of Bok choy. Regardless of the temperature differences in the two development periods and at both dates, mass flowering occurs between the 50^{th} and 55^{th} day of vegetative growth.

The highest number of germinated plants is recorded in variant 3 with 50% biochar, averaging 8.33 and 7 plants per variant, followed by variant 4 with 20% biochar. In both seeding dates, the least germinated plants are observed in the control variant, where a slower development of the plants is also observed.

Plants grown during the March-April period are on average about 50 cm taller than those seeded in December. The strong elongation of the stems is likely due to insufficient light and a greater amount of infrared light. Infrared light has been proven to inhibit flowering in Bok choy. The tallest plants are in variant 4, while the shortest are in variant 5.

The mass flowering during the cultivation of Bok choy in a greenhouse makes this method suitable for seed production but not for growing a crop suitable for consumption.

The yield of fresh biomass for the first period from December to March is lower than that reported for the vegetation period from March to May. The fresh biomass during the second vegetation period from March to May is about 35% higher, with the highest average mass per plant observed in variant 4 with 20% biochar relative to soil volume, which corresponds to other biometric measurements obtained.

There is a gradual increase in nitrates in the variants with added biochar (BV), which is likely due to the ability of biochar to absorb nutrients and release them over a longer period. The nitrate values range between 80 to 93 mg/kg-1, with the highest value reported in variant 4, which also exhibits the best-developed leaf mass. Despite the presence of nitrates in fresh bok choy leaves, the concentration does not exceed the levels regulated by Regulation No. 1881/2006, which designates the production as suitable for consumption.

Variant 2, with the addition of Bioforce humic fertilizer, exhibits the highest photosynthetic activity. The absence of biochar results in faster nutrient absorption. Among the variants with added biochar, variant 4 with 20% of the recommended amount consistently shows the best performance.

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THE EFFECT OF CULTURE SUBSTRATE AND WATER STRESS ON TUBER DEVELOPMENT IN THREE SWEET POTATO CULTIVARS ACCLIMATIZED IN THE GREENHOUSE

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Abstract

Starting from the micropropagation and selection of sweet potato varieties that responded best to in vitro water stress conditions, this study aims to identify the most effective methods of plantlets acclimatization in protected space. The three-factor experiment was carried out by combining the following factors, analyzed in several gradations, as follows: experimental factor A-variety, experimental factor B-substrate, experimental factor C-irrigation. Regarding the influence of the culture substrate and the irrigation variant on the tuberization of the three varieties of sweet potato (CD/1, CD/3, CD/4), the CD/1 genotype is noted on the normal irrigation variant and the use of the culture substrate containing: red peat, black and perlite (2: 1: 1), with a very significant positive difference (5.00) at the average number of tubers/pot. On the same culture substrate, but in a deficient level of irrigation, the CD/3 variety recorded at the average mass and the number of tubers a distinctly negative difference (-131.63 g) and a negative significant difference (-3.25), compared to the control variety (CD/4).

Key words: *acclimatization, culture substrate, protectedspace, sweet potato, water stress.*

INTRODUCTION

The sweet potato crop grows well in marginal soils having a good yield with little demand for fertilizer or water. Although sweet potato is relatively drought tolerant, can provide good ground cover and can be grown without pesticides (Ewell, 1990), its productivity is limited by a large number of biotic and abiotic stresses (Alam et al., 2010). The sweet potato is the target of a large number of pathogenic organisms. This category includes viral, fungal and bacterial diseases and those caused by nematodes. Among the 20 viruses that have been identified, the most widespread sweet potato virus found worldwide is Sweet Potato Feathery Mottle Virus (SPFMV, genus *Potyvirus*, family *Potyviridae*) is widespread in all areas of sweet potato cultivation while the others are located in one or more geographical areas (Moyer & Salazar, 1989; Kreuze et al.,2000). In mixed infections with Sweet Potato Chlorotic Stunt Virus (SPCSV), SPFMV is associated with severe sweet potato virus disease (SPVD), which causes significant damagein many regions of the globe and can lead to a 98% yield loss in production. Viral epidemics of sweet potato were, in many cases, followed by the disappearance of an elite variety (Gibson et al., 1997; Gibson et al., 1998). Other viruses are: Sweet Potato Mild Mottle Virus (SPMMV), Sweet Potato Chlorotic Flecks Virus (SPCFV), Sweet Potato Virus G (SPVG), Sweet Potato Leaf Curl Virus (SPLCV). A regular supply of clean planting material is therefore necessary for sustainable production (Wang & Valkonen, 2008). Biotechnological approaches using different tissue culture techniques can circumvent these problems by producing a large number of plants of different species, through already proven studies. Plant tissue culture is an efficient and reliable method for large-scale production of high-quality, disease-free plants in a short period of time in a small space and providing elite planting material to farmers worldwide (Butt et al., 2015; Mukhopadhyay et al., 2016; Mukhopadhyay et al., 2019; Behera et al. 2019; Behera, 2022). Rapid propagation is based on the use of the *in vitro* propagation technique in aseptic conditions, in special culture vessels, the product obtained is named with the prefix "micro" (microplants, microtubers, microtuberization). The nutritional substrate consists of microelements, macroelements and vitamins considered as basic culture medium (Murashige & Skoog, 1962) with the addition of growth hormones depending on the culture phase (initiation, multiplication, tuberization) (Chiru & Antofie, 1997). Addition of cysteine to the culture medium in the pre-acclimation phase can further support the success of *de novo* shoot development for acclimation (Sand & Antofie, 2022). Microplants require special growing methods and cannot be directly planted in the field (Wiersema et al., 1987). Plantlets are very small plants obtained in completely sterile conditions and transplants are produced by *in vivo* planting of seedlings in non-sterile conditions (Struik & Wiersema, 1999), through a protected space (greenhouse, tunnel) preferably insect-proof, to avoid contamination with aphids where the seedlings are acclimatized. The success of feeding depends on a series of factors, among which we mention: the state of health and the size at transplantation, the choice of the culture substrate, the prevention of diseases and the control of some physical parameters. The choice of the substrate, which must be of good quality, with good permeability, well aerated with a suitable pH (Ourèye, 2013).

MATERIALS AND METHODS

Plant material

The origin of the plant material used consists of glass plates regenerated from shoots from three sweet potato (*Ipomoea batatas*) genotypes: CD/1, CD/3 and CD/4. The three varieties were obtained from the Research-Development Station for Agricultural Plants on Sands Dabuleni, and the country of origin of the varieties is Korea. Some of the morphological and production characteristics of these varieties grown in the experimental fields from Dabuleni are presented in Table 1.

Table 1. Characterization of the varieties used in the study

Genotype	Flesh color	Tuber number/ plant	Tuber weight/ plant	Tuber average weight	Production t/ha
DK 19/1	White	8	1673	210	32
DCh 19/3	Purple	5	767	105	25
DK 19/4	Yellow	5.33	1233	232	36

In vitro culture condition

For culture initiation and *in vitro* multiplication, the culture medium Murashige & Skoog (1962) is used, to which growth regulators (auxins), agar, sucrose and the antimicrobial agent PPM (Plant Preservation Mixture) are added. Sterilization of the culture medium is carried out at 121°C and a pressure of 1.1-1.2 atmospheres. The explants are inoculated inside the hood with laminar air flow. Incubation is carried out in the growth chamber, at a temperature of 25°C, with a light intensity of 3000 lux and a photoperiod of 16 hours of light, alternating with 8 hours of darkness.

The *in vitro* evolution of these varieties had superior results in terms of shoot proliferation and elongation, rooting and a very good tolerance of induced water stress. After eight weeks, the glass plants were prepared for their transfer to a protected space.

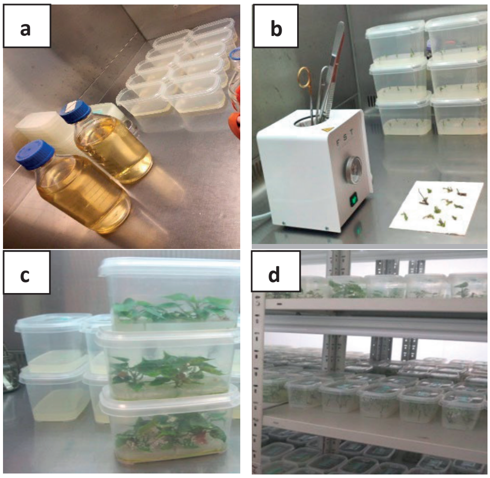

Figure 1. Distribution of the nutrient medium in the culture containers (a); Initiation of sweet potato *in vitro* culture (b); Multiplication by successive subcultures of varieties (c); Incubation of the culture containers in the growth chamber (d)

Experimental variants

The experiment was carried out in the greenhouse of the research laboratory for tissue culture from INCDCSZ Brasov. On the work tables in the greenhouse, the pots containing perlite (Pe) and substrate (T) containing: red peat, black peat and perlite (2:1:1), in which the three-threes were planted sweet potato varieties. The substrates used must be sterilized, permeable in order not to retain excess water, have a suitable pH. Irrigation of plants developed on the T substrate as well as on the substrate on according to their necessity, but there was also conditional irrigation on the same substrats (T stress, Pe stress). The temperature in the greenhouse oscillates from 15-20°C (in the morning) and can reach up to 40-43°C (in the afternoon) and the humidity is between values of 70-80% and 92.9%, depending on the registered temperature. Maintaining plants in an optimal phytosanitary state required the application of prophylactic treatments with fungicides and insecticides. For the growth and development of plants, both incorporable fertilizers were used in the culture substrate (Osmocote Exact) and foliage (Cropmax, Razormin, Agroleaf Power). All culture conditions have been controlled (temperature, humidity, bacterial, fungal, nematodes aphids). After about 120 days after planting the tubers were harvested and an assessment of the number of tubers and their mass, on each variety, type of culture and irrigation variant were made.

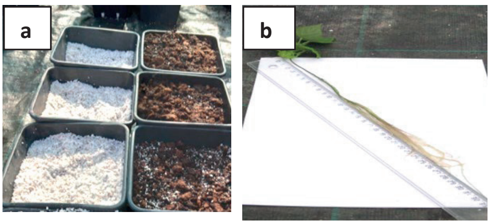

Figure 2. Studied substance (a); The length of the root of a sweet potato plant (b)

Data analysis

The analyzed factors were the variety (A) with three gradations, the substrate (B) with two gradations and the irrigation (C) with two gradations. The statistical analysis was carried out by the Duncan test and by the analysis of variation.

RESULTS AND DISCUSSIONS

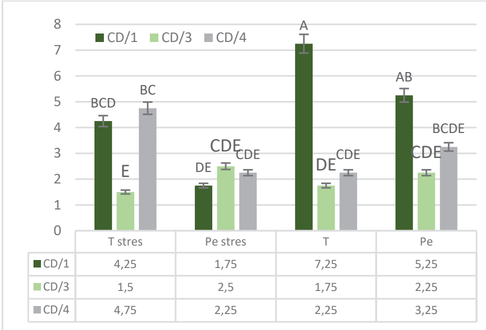

Figure 3. Average number of tubers/plant obtained in the experiment

*The means in the column inside the table followed by different letters are significant according to Duncan's MR test ($p < 0.05$).
** SX = 0.78 DS value: 2.27-2.67.

From the analysis of the average number of tubers obtained, the best results were recorded for the CD/1 variety (7.25), on the T culture substrate with better results than the control, and the CD/3 genotype shows a low productivity (1.5) on the T stress culture substrate, compared to the pots in this control variety CD/1.

From the data entered in Table 2 where the results of the influence between the variety and the culture substrate are presented, it follows that the variety CD/3 planted on the culture substrate T stress and Pe stress significantly negative differences (-3.25) compared to the control variety CD/4.The variety CD/1 surpassed the control variety, presenting very significant positive differences (5.00) in the culture substrate T, on the normal irrigation variant. Insignificant values were recorded for the other types of variants.

Table 2. The influence of the sweet potato variety in obtaining tubers for each culture substrate

Variety	Culture substrate	Avg. nr of tubers	%	Diff.	Sigf.
CD/4 (Ct)	T stress	4.75	100.0	0.00	Ct
CD/1	T stress	4.25	89.5	-0.50	ns
CD/3	T stress	1.50	31.6	-3.25	0
CD/4 (Ct)	Pe stres	2.25	100.0	0.00	Ct
CD/1	Pe stress	1.75	77.8	-0.50	ns
CD/3	Pe stress	2.50	111.1	0.25	ns
CD/4 (Ct)	T	2.25	100.0	0.00	Ct
CD/1	T	7.25	322.2	5.00	***
CD/3	T	1.75	77.8	-0.50	ns
CD/4 (Ct)	Pe	3.25	100.0	0.00	Ct
CD/1	Pe	5.25	161.5	2.00	ns
CD/3	Pe	2.25	69.2	-1.00	ns

*LSD 5%=2.45; 1%=3.43; 0.1%=4.90

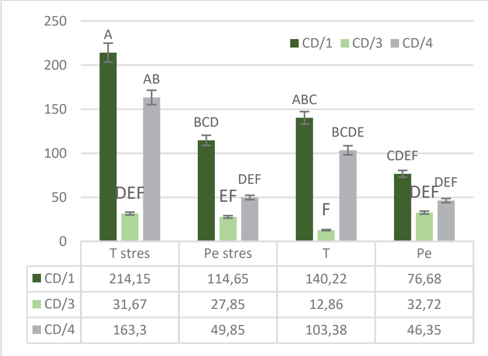

Figure 4. Mass (g) of harvested tubers/plant

* The means in the column inside the table followed by different letters are significant according to Duncan's MR test ($p < 0.05$).
** SX = 25.99 DS value: 75.51-88.77.

In the case of the analysis of the weight of the tubers obtained per plant, it can be seen in Figure 4 that the three varieties responded better to the T stress variant the total weight oscillating between 214.15-163.3-31.67 g compared to the T variant where the total weight of the tubers is lower, respectively 140.22-103.38-12.86 g, in the varieties analyzed (CD/1-CD/4-CD/3). In the Pe stress variant, only two varieties CD/1 and CD/4 recorded a higher weight of the harvested tubers, 114.65-49.85g, compared to the Pe variant 76.68-46.35g

Table 3. The average mass of tubers influenced by the sweet potato variety and the culture substrate

Variety	Culture substrate	Avg. nr of tubers	%	Diff.	Signf.
CD/4	T stress	163.30	100.0	0.00	Ct
CD/1	T stress	214.15	131.1	50.85	ns
CD/3	T stress	31.68	19.4	-131.63	00
CD/4	Pe stresss	49.85	100.0	0.00	Ct
CD/1	Pe stress	114.65	230.0	64.80	ns
CD/3	Pe stress	27.85	55.9	-22.00	ns
CD/4	T	103.38	100.0	0.00	Ct
CD/1	T	140.23	135.6	36.85	ns
CD/3	T	12.86	12.4	-90.52	0
CD/4	Pe	46.35	100.0	0.00	Ct
CD/1	Pe	76.68	165.4	30.33	ns
CD/3	Pe	32.72	70.6	-13.63	ns

*LSD 5%=85.42; 1%=120.69; 0.1%=174.18.

From the data presented in Table 3, where the results of the influence on each substrate of the same type are showedit results that the variety CD/3 planted on the substrate (T stress) has a distinctly significant negative difference (-131.63 g), and on the culture substrate variant T, the same variety registers a negative difference (-90.52 g) compared to the control variety CD/4.

CONCLUSIONS

Starting from the micropropagation method of some sweet potato genotypes to the acclimatization in protected spaces under conditions of water stress, this study constitutes a beginning in order to produce a planting material with high biological value and the identification of varieties with resistance to drought.

At the same time, the use of two culture substrates was experimented: a. T which contains in the composition: red peat, black peat and perlite (2:1:1); b. Pe on which it contains only perlite (industrial substrate). The acclimatization method was largely conditioned by the choice of the substrate for the transfer of the sweet potato vitroplants into pots, but also by the control of the factors specific to the *ex vitro* environment, the survival percentage of the seedlings was 98%.

The data regarding the evaluation of the weight of tubers obtained per plant show that the three varieties studied, CD/1, CD/3 and CD/4, responded better to the T stress variant compared to the T variant where the total weight of the tubers/plant was more small, there were distinctly significant negative differences in the variety CD/3 (-131.63 g) compared to the control variety CD/4. In the Pe stress variant, only two varieties CD/1 and CD/4 recorded a higher weight of the harvested tubers, compared to the Pe variant, but with insignificant differences.

Acclimatization of sweet potato seedlings in protected areas has been successful, developing and producing tubers that can be used as efficiently as acclimatized material in field culture.

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AGROBIOLOGICAL AND TECHNOLOGICAL CHARACTERIZATION OF *PHASEOLUS COCCINEUS* L.

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Abstract

Phaseolus coccineus L. (runner bean) belongs to the Fabaceae family and is the second most important species of the Phaseolus genus, after the common bean (Phaseolus vulgaris L.), which is often confused. The species is cultivated especially for seeds (mature or immature) and some cultivars are grown for pods. The runner bean is grown as an annual plant in pure crop or intercropping, being appreciated, to a lesser extent, also for its ornamental value. In Romania it is cultivated in small areas, especially in family gardens, using local populations without using a standard technology, but rather one that is adaptable to environmental conditions. The study focuses on aspects such as morpho-physiological, ecological, and phenological features, and different cultivation technologies, but also on genetic features and potential breeding methods.

Key words: *runner bean, crop systems, Fabaceae.*

INTRODUCTION

P. coccineus is a perennial species originating from the mountainous, humid tropical areas of Mesoamerica. In its native area, it can live up to ten years, but outside this area, it behaves as an annual plant because its aerial parts cannot tolerate frost (Rodiño et al., 2007).

ORIGIN AND DISTRIBUTION AREA

The best-known species of the genus *Phaseolus*, both in terms of area and geographical distribution, is the common bean (*Phaseolus vulgaris* L.). Next to this, but cultivated in smaller areas is the broad bean (*Phaseolus coccineus* L.). Both species are native to America, more precisely from northern South America, Central America (Guatemala and Honduras), and southern North America (Mexico) (Salinas, 1988).

The two species were brought to Europe after the discovery of the American continent by Columbus (1402). Here they underwent a process of adaptation to lower temperatures (Spataro et al., 2011; Rodriguez et al., 2013).

It seems that these two species were cultivated in the Neolithic, about 10-12 thousand years ago, when in fact the first great agrarian revolution was recorded. Evidence in support of what has been presented has been brought by various scientific works dedicated to this subject, such as those of authors such as Salinas (1988) and Philips (1993), Kaplan and MacNeish (1960), Kaplan (1965), Flannery (1973) or Smart (1976). Traces of the great bean, cultivated in Mexico, have been found in archaeological sites at Telmacan, Puebla, Rio Zaze, and Durango, dated to between 2200 and 1300 BC (Popa, 2010; Vargas et al., 2012).

However, bio-geographic research, which uses the cpSSR and nonSSR techniques, suggests multiple evolutionary processes of the species in Mesoamerica as well (Angioi et al., 2009; Spataro et al., 2011; Rodriguez et al., 2013).

It is important to remember, for the explanation of some ecological peculiarities of the broad bean species, as it results from the works of the authors mentioned above, that the broad bean was formed in the mountainous regions of Mexico, delimited by the states of Puebla, Oaxaca, and Chiapas.

The species *P. coccineus* is represented in culture, especially in regions close to the area of origin, by two subspecies, *coccineus* and *darwinianus* (Salinas, 1988) opines that the subspecies *darwinianus* would be an ancestral hybrid between *P. vulgaris* and *P. coccineus*, i.e. it would be a kind of "bridge species" between them.
Salinas opinion that *P. coccineus darwinianus* is the said hybrid may be true given the extremely varied environmental conditions during the periods of thousands or hundreds of thousands of years of coevolution of the two species.
In Romania, this species once arrived, probably, with the common bean, with which it was and still is confused (Popa, 2010). The first information about this species appears in Alexandru Borza's "Ethnobotanical Dictionary" (1968) and the monographic book "Fasolea" by Olaru (1982). A distinct report with many details about the biology, ecology, mode of cultivation, and use of the crop was that made by Munteanu (1985) and entitled "*Phaseolus coccineus* L. - a vegetable species that deserves more attention", using the appropriate literature appearing in outside of Romania.

THE IMPORTANCE OF CULTURE

Food importance

Worldwide, different consumer preferences are known. Thus, in the USA and Western Europe, the broad bean is known especially for its large, wide pods (similar to the pods known for the common bean), of a raw green colour (Santalla et al, 2004). In Latin America, but also some European countries, such as those in South-Eastern Europe, the broad bean is known mainly for its mature or immature seeds, which are large, oblong, slightly flattened, white or coloured (Munteanu, 1985; Stan et al., 2003; Tay et al., 2008).
In general, this type of bean is known as a luxury food product and is valued at much higher prices than common beans (Kay, 1979)
From specialized research, it can be concluded that 60% of a pod can be consumed due to its nutritional value as well as its nutritional, catalytic, and mineralizing complex (Table 1).

Table 1. Content of seeds and pods in broad bean (Munteanu, 2005)

The composition	Pod (edible part)	Immature seeds (green	Mature seeds (dry)
Water	58,3	34,2	12,0
Protein (%)	7,4	2,6	20,0
Carbohydrates (%)	29,8	47,9	63,0
Fats (%)	1,0	0,3	1,5
Fibre (%)	1,91	12,2	5,0
Ash (%)	1,63	2,8	3,5
Calcium mg/100 g	50,0	60,6	120,0
Phosphorus mg/ 100 g	160,0	276,6	-
Iron mg/100 g	2,6	4,1	10,0
Thiamine mg/100 g	0,34	0,54	0,3
Riboflavin mg/100 g	0,19	0,14	0,1
Niacin mg/100 g	-	2,3	2,0
Ascorbic acid mg/100 g	27,0	0,2	-
Carotene mg/100 g	0,057	0,324	-

In Central America, the tuberized (fleshy) roots, rich in starch, are also used in food, after they have been boiled and the boiling liquid removed (Munteanu, 1985). Another method of preparation consists of drying the roots for a week, then portioning them and boiling them for 6-8 hours. Sometimes, the boiled roots are eaten as such (Salinas, 1988). In Oaxaca and Chiapas, the raw roots are used to regulate digestion, and in Guatemala, the water in which the roots have been boiled is used as a remedy against malaria (Salinas, 1988).
In Mexico, the young leaves and shoots, as well as the inflorescences, are boiled and then fried, eaten as such, after seasoning them with garlic and onion, or as side dishes.

Ornamental importance

In the countries of Germany, the USA, etc., the use of broad beans for ornamental purposes can be observed, but the plants being exposed to high temperatures, although they bloom profusely, do not bind pods because the atmospheric moisture requirement is not covered.
This species is decorative due to the diversified colouring of the flowers (from intense red to white or even bicolor) which are arranged on a raceme up to 60 cm long that stands out from the abundant foliage, especially in the climbing forms (Schwember et al., 2017).

Due to the diversified colour of the flowers, the broad bean attracts various insects such as butterflies, bees but also birds (hummingbirds) (Teliban, 2015).
The broad bean is cultivated as an ornamental plant in Romania, and is known as a "flower bean" (Popa, 2010).

Agrotechnical importance
The large bean, due to its positive characteristics, is of very high agrotechnical interest. This lends itself very well to growing between rows of plants (corn) but also cabbage, carrot, lettuce, etc. (Munteanu et al., 2007).
As a precursor plant, the bean is essential because it contributes high amounts of biological nitrogen in crop rotations due to its ability to symbiosis with nitrogen-fixing bacteria belonging to the genus *Rhizobium*, which use nitrogen from the atmospheric air (78%), and which transforms into ammoniacal nitrogen (NH_4) (Ciofu et al., 2003; Stan et al., 2003).
Due to the lower temperature requirements for germination and plant growth, *P. coccineus* L., can grow in cooler environmental conditions than those required by *P. vulgaris* L. making it important for the Great Britain (Rodiño et al, 2007). Due to its tolerance to low temperatures, it has become an important crop in the northern part of Europe and in the mountainous areas of southern Europe where it is grown from spring to summer. It is also cultivated in the upper regions of Africa and Asia.
The broad bean can be grown in various places and cropping systems: as an annual plant (monoculture or intercropping), as a perennial plant, interspersed with trees in the orchard, or cultivated on fences, lines, and hedgerows (Hernandez et al., 1979), of mention is that in greenhouses and solariums, it is cultivated only to obtain green pods (Teliban et al., 2014).

Economic importance
From the point of view of the economy, broad beans are preferred by hypermarkets, markets and other areas of trade due to the fibre and protein intake as well as the special taste. Although it has a higher price compared to the common bean, it is marketed very easily (Teliban, 2015). For this reason, broad beans have attracted the attention of both researchers and producers.
To ensure the profitability of broad bean crops, it is very important to apply the appropriate cultivation technology of the species and ensure favourable growing conditions (Teliban et al., 2015).

Risk factors
The main risks associated with the cultivation of this plant are mostly related to environmental conditions (Popa, 2010). Below are some of these factors: temperatures below 5 ºC stop plant development (Rodiño et al., 2007), and temperatures below 0ºC destroy plants; temperatures above 25ºC can prevent formation; placing the culture in areas with periods with temperatures between 0 and 5ºC during the 120-130 days of vegetation; drought can delay seed germination, lead to uneven germination and/or the appearance of a large number of voids in the crop; prolonged drought during flowering can lead to abundant flower drop or lack of fertilization, leading to the formation of small pods and seeds of poor quality; heavy rainfall during harvest can be a real calamity because it can cause disease in the pods, leading to quality deterioration and quantitative reduction of the harvest (Popa et al., 2020).

BOTANICAL AND BIOLOGICAL PARTICULARITIES

Phaseolus coccineus has a tuberous taproot, rich in starch, with nodules and vegetative buds in the neck area. The emergence of broad beans is hypogeic (Giurcă, 2009). The 2-7 m stem is tall, vigorous, and slightly twisted. The buds from which the stem branches grow and develop are formed in the axils of the leaves at the base, and the flower buds are formed in the upper part (Popa, 2010). The leaves are trifoliate, with oval leaflets, acuminate to the tip and round at the base. The inflorescence is zygomorphic, hermaphrodite, multifloral raceme longer than the leaves, 25-60 cm, with red, white, pink, bicolour flowers and allogamous pollination, rarely autogamous. Cross-pollination is specific to climbing beans and occurs before the flower opens. Considering that flowering extends over a longer period, allogamy is favoured (Hamburdă and Munteanu, 2016). The fruit is linear oblong to oblong. The seeds are very large, 20-25 mm long, 13-14 mm wide and 8 mm thick. They have different colours: white, black, beige, and purple (Teliban, 2015). The weight of 1000 grains is between 1000 and 3000 g (Labuda, 2010).

RELATIONS WITH ENVIRONMENTAL FACTORS

Relations with temperature

The broad bean is a species adapted to the humid tropical conditions found in the high plateaus. Compared to common beans, it is more tolerant of low temperatures. Germination occurs starting from temperatures of 8-12ºC, and the optimum temperature for growth and development is 16-18ºC (Popa, 2010). Usually, temperatures above 25ºC inhibit the formation of pods, and temperatures below 5ºC adversely affect the plants, possibly even leading to their destruction by frost. Thus, to ensure optimal growing conditions, the vegetation period without the risk of frost should be 130-150 days (Salinas, 1988; Popa, 2010).

Relations with water

Water is a crucial factor for growing broad beans, and for this reason, it is mainly grown in temperate areas of Europe where irrigation is possible. The critical periods when water availability becomes crucial are during germination, flowering and pod formation. Even a short period of drought at these stages can result in the dropping of buds, flowers, and newly formed pods (Popa, 2010). On the other hand, excessive moisture at these times can be harmful, causing excessive vegetative growth, affecting the grain, and increasing susceptibility to diseases, especially anthracnose.

During the flowering period, lack of water can shorten this phase and reduce the number of flowers and pods per plant. During grain formation, insufficient water can lead to grain size reduction (Kay, 1979).

Broad beans are not very resistant to soil drought and atmospheric drought during the flowering and fruiting stages. These conditions can cause a significant decrease in flower formation and production. To ensure an optimal bean crop during the flowering and pod formation stages, it is critical to have well-irrigated soil and a moist and warm environment (Kay, 1979).

Relations with the soil

In growing broad beans, the soil plays an essential role. Among legumes, broad beans are known for their high soil requirements. To obtain quality production, it is necessary to cultivate on deep, well-drained soils with a light or medium texture and a pH between 6.5 and 7.4 (Popa, 2010).

Beans get some of their nitrogen needs from nitrogen-fixing bacteria found in the nodules on their roots. However, to ensure the healthy growth of the plant, it is important to supplement the nutrients in the soil, especially in the first stages of vegetation. Thus, less fertile soils must be enriched with chemical and organic fertilizers (in quantities of 40-80 tons/ha) (Stan et al., 2003).

Relations with nutrients

The growth and development of bean plants is strongly influenced by the availability of nutrients. Their lack can have significant effects on the plant. For example, the lack of nitrogen in the soil can cause the yellowing of leaves in young plants, a phenomenon accentuated in cold weather conditions (Olaru, 1982).

In the case of a lack of phosphorus in the soil, the growth and development of young plants can be significantly delayed, and the leaves can acquire a pale colour, later turning yellow and even red (Olaru, 1982).

A lack of potassium can cause chlorosis, characterized by yellowing of the leaves. The absence of calcium in the soil can lead to problems such as the curling of young bean leaves and the deformation of their edges.

Micronutrients such as zinc, boron, manganese, and molybdenum have a positive impact on the growth and development of beans. For example, a lack of boron in the soil can slow growth and reduce production, while a lack of manganese can cause severe chlorosis and leaf necrosis (Olaru, 1982).

Molybdenum in the soil has multiple beneficial effects, including the accumulation of calcium and phosphorus in leaves and roots, as well as active nitrogen fixation, an activity carried out by nitrogen-fixing bacteria. Lack of

molybdenum can lead to small plant sizes and reduced production (Olaru, 1982).
Studies in the specialized literature highlight the ability of rhizobacteria to solubilize organic and inorganic phosphates in the soil and to produce indoleacetic acid. Seed inoculation with bacteria from the rhizosphere of bean plants, such as strains S4 and S7, can increase photosynthesis, transpiration, water use efficiency, leaf chlorophyll content, and yield (Ștefan et al., 2013a; 2013b).

GROWING TECHNOLOGY

The choice and preparation of the land

The choice and preparation of the land are carried out similarly to the common bean crop. Romanian specialized literature does not provide a standard methodology in this way.
The culture is located on flat or sloping land, with a uniform slope, with deep, well-worked, well-drained, loamy soil, from light to medium in texture. In Europe, the crop is practiced only under irrigation conditions (Kay, 1979).
Heavy, wet, and cold soils, as well as those that form a crust, are unsuitable, since, in such conditions, the beans sprout hard and unevenly (Olaru, 1982).
In principle, any of the vegetable species are accepted as precursor plants, except those from the same botanical family (Stan and Stan, 2010). Sometimes, monoculture can be practiced, if the disease attack was not strong or if too large a reserve of pathogens did not form in the soil (Stan, 2005).
Basic fertilization is differentiated, depending on the fertilization system of the preceding plant, as well as the nature of the type of soil on which the crop is placed. A quantity of 60-80 t/ha of well-decomposed manure, 400-600 kg/ha of superphosphate, and 200-300 kg/ha of potassium salt is administered. On soils with high fertility, no manure is applied, and the doses of chemical fertilizers are reduced by half (Davidescu and Davidescu, 1992).
When manure is applied, it must be well decomposed, and in this case, facial fertilizers are quantitatively reduced (Kay, 1979).
Basic ploughing is performed immediately after fertilizing, at a depth of 28-30 cm, with PP-3x30 M; the land is left in the furrow until spring (Stan et al., 2003).
In early spring, the land is worked through 1-2 passes with the harrow with adjustable tines, to keep the soil loose and destroy any weeds that appear, as well as for levelling the basic ploughing (Dumitrescu et al., 1998).
Starter fertilization follows harrowing and is done with 100-150 kg/ha of ammonium nitrogen (Dumitrescu et al., 1998). An insecticide can also be applied to prevent the attack of the *Delia platura* pest, but also herbicide to control weeds (Stan et al., 2003).

Crop establishing

The establishment of the crop must be carried out 10-14 days after weeding. Culture is usually done by direct sowing, but also by seedling.
The establishment period begins with the achievement of a temperature above the threshold of 10-12ºC, which corresponds, in the southern areas of the country, to the period of April 15-20, and in the more northern areas, to the period of May 10-20. The establishment of the spring crop can continue, staggered, until the end of May (Popa and Munteanu, 2009).
The establishment scheme is variable, the technical element that differentiates the crop establishment scheme is the palisade method. If the palisade is made on canes, the culture is established in nests of 4-6 plants, arranged in parallel rows 80-100 cm apart; the distance between the nests in a row is 50-60 cm. In this way, a density of 17-25 thousand nests/ha or 70-150 thousand plants/ha is achieved (Stan et al., 2003).
If the plant stringing is carried out on the trellis, the same density and, partially, the establishment scheme are respected. Usually, the trellis consists of a row of 200 cm high posts, arranged at a distance of 8-10 m between them; a 2-3 mm thick galvanized wire is attached to the top end of the poles (Popa et al., 2020). A row of nests of 3-5 seeds each, at a distance of 40-80 cm, is placed on one side and the other of the trellis; the distance between the nests is 40-60 cm. In this way, the densities that can be achieved vary between 18 and 36 thousand nests/ha (Stan et al., 2003; Hamburda et al., 2014).

The sowing rate is 70-80 kg/ha, depending on the density and the mass of a thousand grains. The sowing depth is 3-4 cm; emergence occurs more easily when sowing is done in nests (Popa et al., 2020).

Care work

Care work does not differ much from climbing garden beans, but a standard technology is not known. In the households of the population, as a rule, the same works are applied to the corn culture, with which it is most often associated. Here, as a distinct work, we can distinguish the palisade, which is made on long arches (over 2 m), which ensures good exposure to light (the palisades can also be made on different types of trellises). In some countries, bamboo stems are used, which are resistant and very cheap. After digging into the soil, the arachnids are caught in pairs at the level of the trellis wire with raffia or twine (Munteanu et al., 2007).

For the success of the culture, pinching and sausage are practiced. Pinching is mandatory work, which ensures a good branching of the stems and a remarkable increase in the harvest. The pinching is done by breaking the tops of the stems, when they reach 60-80 cm, then at 100-120 cm, and after they have reached the level of the trellis wire. Sausage is recommended to stop the growth of the stem and to speed up the ripening of the formed pods (Ciofu et al., 2003).

During the growing period, a good distribution of rainfall is required, because broad bean is very sensitive to drought and requires a relatively high humidity at seed setting. In England, the crop is often irrigated from the green bud stage, normally from June to August (Kay, 1979).

Combating diseases has some peculiarities, due to the phenomenon of "disease escape", determined by the fickle behaviour of plants (Ruşti and Munteanu, 2008). Pest control is based on their biology and ecology.

Due to its allogamy, the runner bean requires the presence of pollinating insects such as bees (*Apis mellifera* L.) and bumblebees (*Bombus* spp.), which significantly influence both early pod formation and overall yield, which is of paramount importance for varieties grown for both green pods and dry seeds (Łabuda, 2010).

Harvesting

The experiments at the Bacau Vegetable Research Station, in the period 1980-1986, allowed an evaluation of the production that varied between 1000-2500 kg/ha of dry beans. The culture was carried out in its field, as a monoculture, on a specially arranged land with a palisade system, used for the culture of climbing garden beans (Munteanu et al., 1985).

The pods will be harvested every five days or even more often. The pods should be harvested when they are almost full length, usually 20-30 cm, and before they form threads. It is very important to continue harvesting all the pods as soon as they reach the technical time of ripening; otherwise, the plants will slow down their flowering. In the vast majority of gardens, the plants will then continue to produce flowers and pods until the vegetation is stopped by the first frost (Brink and Belay, 2006).

Cultivation systems in the field and tunnels

Worldwide, broad beans are grown almost exclusively in the field, in row or palisade cropping systems, as well as in succession or double cropping. In Mexico, for example, four cultivation systems are used, namely: as an annual plant, as a perennial plant, interspersed with orchard trees, and cultivated on fences, hedgerows, etc. In Grand Britain, systems with climbing plants or bush plants are very popular; the latter system is mainly used in areas with strong winds. In other Western European countries (the Netherlands, Belgium, France, Spain), the culture system with climbing varieties in monoculture, similar to the common climbing bean, is mostly used.

In Romania, most often, broad beans are grown in association or interspersed with corn, suppor-ted on canes, but also in their cultivation system next to fences (Haburda and Munteanu, 2016).

Greenhouses practices is mainly used to obtain pods, especially in countries such as the Netherlands and Belgium. A variety that lends itself to this cultivation system is the Desiree variety (Teliban, 2015).

Intensive (conventional) and sustainable (unconventional) cultivation systems

Sustainable farming systems are already known worldwide, as well as in our country.

These systems have been imposed by the fact that they represent a viable alternative to the current intensive or industrial agriculture systems (Stan et al., 2003).

Broad bean lends itself very well to both cultivation systems. Lately, with the promotion of non-conventional (organic/biological) cultivation, the broad bean, as well as the climbing common bean, has become a preferred species for organic cultivation, because, for this cultivation system, plants with a high ecological plasticity are recommended, with a certain rusticity towards environmental factors, with genetic resistance (especially horizontal) to pathogenic and harmful agents, etc. The specialized literature confirms what was presented above (Munteanu et al., 2007).

Ways of growing broad beans

Ways of growing broad beans vary according to region, traditions and available technical resources.

In UK, both seed cultivation and tuberous root planting are practiced. The preferred seeds are those obtained from disease-free early or mid-season crops (Kay, 1979).

Systems with climbing plants or bush plants are also encountered. In areas with strong winds, bush plants are preferred, but they are more exposed to diseases and require more attention. Another form is to support plants with canes from bamboo stems or wire, allowing them to climb naturally (Wood, 1979).

In other European countries such as the Netherlands, Belgium, France and Spain, the cultivation system with climbing varieties in monoculture, similar to that of the common bean, is used more. In Central America, broad beans are associated with corn or grown in a household system, in pots, where 10-15 plants can grow.

In Romania, broad beans are grown in all agricultural areas, in small areas, and family households. It is often grown in association with other crops or alone, supported on trellises or next to fences. The culture is established by direct sowing and germination is hypogeic (Munteanu et al., 2007; Łabuda, 2010).

Optimizing planting schemes and densities is essential for achieving efficient production, taking into account the specific environmental conditions in each area (Munteanu, 2005; Hamburda et al., 2016).

BREEDING AND SEED PRODUCTION

The species *P. coccineus* L. belongs to the group of allogamous species, a fact determined, first of all, by the genetic mechanisms that govern fertilization and which are specified as the genetic mechanisms specific to gametophytic incompatibility (Munteanu, 2005).

The runner bean, within the context of genetic improvement for the common bean, presents a significant range of variability across various traits, as noted in studies by Salinas (1988), and Singh (2001). This species offers valuable agronomic features and resistance to diseases, such as resistance to lodging due to sturdy stem bases, tolerance to cold conditions, extended epicotyls and racemes, the presence of a tuberous root system enabling perennial growth cycles (Santalla et al., 2004), potentially high pod counts per inflorescence, and resilience against pathogens like *Sclerotinia sclerotiorum* (Gilmore et al., 2002), *Ascochyta blight* (Schmit and Baudoin, 1992), and root rots caused by *Pythium* or *Fusarium* (Dickson and Boettger, 1977), among others.

As theoretical and practical support for the establishment of a conservation scheme for runner bean, can be considered knowledge of the biology and genetics of the species, the biological and genetic basis for breeding and conservation selection, and, respectively, the model of the selection scheme for annual autogamous plants, including that of the climbing common bean (Popa et al., 2020).

CONCLUSIONS

Following the research carried out both in Romania and abroad and summarized in the review, regarding the botanical and biological peculiarities, the high ecological plasticity, the important biochemical and agro-productivity characteristics, the adaptability to the different schemes and cultivation methods. At the same time, it's also sensitivity to atmospheric drought, as well as the species' allogamy, we consider it opportune to carry out for the first time in Romania a runner bean breeding program, to create genotypes with specificity for pods or grains, superior from quality and quantity point of view, adapted to current climate change and with high economic efficiency.

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ABIOTIC STRESS RESISTANCE OF SEVERAL TOMATO LANDRACES FROM SALINE ZONES OF BIHOR COUNTY

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Abstract

A major constraint to global food production is the selection of crops that are better adapted to resource limited environments and soil conditions. In the past decades, the research in this field has focused on the study of the effect of abiotic stress on plants and elucidating signalling pathways that govern the appropriate and coordinated response to abiotic stress. This study identified some tolerant tomato landraces to salt and drought stress from saline zones of Bihor County, in North-Western Romania. Phenotypic characterization of collected landraces provided information regarding drought and salt stress resistance compared with the 'Marmande' cultivar. Root density, leaf area and the reaction under different concentrations of salt and different water content were compared to conclude which landraces are more suitable for future genotypic studies in order to use them in breeding programs. The aim is to obtain genotypes with increased drought and salt stress resistance to improve crop yield and quality in saline environments. This study highlights the potential of local tomato landraces from Bihor County as valuable genetic resources for developing resilient varieties that can thrive in challenging environmental conditions.

Key words: *Solanum lycopersicum L., Marmande cultivar, drought stress, salt stress, leaf area, root density.*

INTRODUCTION

According to recent estimates by the United Nations Food and Agriculture Organization, cereal production must double before 2050 to meet the world's expanding population's demand for food and the growing competition for crops, considering their use as sources of fibre, bioenergy, and other industrial uses (www.fao.org).

A majority of the Earth's arable land is affected by salinity, due to the expansion of drought and excessive use of agricultural land. All these are due, mostly, to the increase in global temperature and climate variability (Dolferus et al., 2014). The development of new crop plant varieties with enhanced resistance to abiotic stresses (i.e., heat, drought, salinity, and UVB rays) is a major concern on a global scale (Nowicka et al., 2018; Martinez et al., 2018; Machado R.M.A. et al, 2017, Ors et al, 2017)). These varieties or local landraces make it possible to grow them on soils already affected by increased salinity or drought (Broccanello et al., 2023), ultimately increasing food security and sustainability in regions facing these challenges. By incorporating traits that improve resilience to these stressors, farmers or breeders can continue to develop crops in areas that were previously unsuitable for cultivation.

It is believed that phenotypic plasticity rather than genetic diversity may prove to be crucial for plants to thrive in specific surroundings amid swift changes in the environment (Vitasse et al., 2010; Gratani, 2014). Under harsh circumstances like cold, drought, and salt, the genotypes cultivated under different environmental conditions may demonstrate phenotypic plasticity (Tester and Langridge, 2010). Therefore, there is a need for quantitative analyses of plant traits to accelerate the selection of crops that are highly adapted to resource-limited environments and soil conditions, which is also a significant barrier to the production of food worldwide. (Fiorani and Schurr, 2013).

Bihor County, located in the north-west of Romania, has an important area of soil affected by salinization. From the total of polluted soils of the county, charted during the period 2013-2017, 9,11% of the surface is affected by different degrees of salinity: weak (38122 ha), moderate (900 ha), strong (400 ha) (http://anpmbh.ro). Tomatoes (*Solanum lycopersicum* L.) are one of the most valuable and economically significant vegetables grown worldwide for their fruits, and their productivity is steadily declining due to their vulnerability to abiotic stresses (Raja et al., 2020). Developing strategies to mitigate the impact of drought and salt stress on tomato plants is crucial for maintaining high yields and ensuring food security. Implementing innovative agricultural practices, such as precision irrigation and genetic engineering, can help improve tomato resilience to these challenging environmental conditions (Polenta et al, 2020). The purpose of this study is to identify some tolerant tomato landraces to salt and drought stress, analyse the synergistic effect of salinity and drought stress on plant growth and development and their use for initiation of a breeding program in order to obtain resistant hybrids, with tolerance to salinity and stable production (Sahin et al., 2018), Cui et al. (2020), Arshad et al. (2023).

Phenotyping platforms using non-invasive technologies have achieved strong development in the last decades Pieruschka et al. (2019). For simulation and measuring the effect of environmental conditions on tomato plants in a greenhouse a high throughput installation, Phenotyping Platform HAS-R(S) SDS was used.

In the present study, early plant growth was subjected to several forms of abiotic stress, including drought stress, salt stress, and a combination of the two (Ors et al., 2017; Ors et al., 2021). In terms of comparison, a conventional cultivar was subjected to the same treatment. Under two salt concentrations (0.2%, and 0.3%) and soil water content (60%, 20%), leaf area and water consumption were studied.

MATERIALS AND METHODS

Experimental design and stress treatments

For the experiment 4 different tomato varieties were used, collected from local farmers in Bihor County. The landraces were designated after the location: 'Ateaș 136', 'Ateaș 37', and 'Cefa 7'. 'Marmande' is the cultivar used for control. The seeds were sown in pots containing a mixture of 50% sandy (Maros) soil and 50% Terra peat soil. Fertiliser was added to each pot in equal quantity (SUBSTRLAL®, Osmocote Plus®, Wals-Siezenheim, Austria). After sprouting, the plants were grown in a 16/8 hour light/dark programme until they reached the first layer of true leaves when the water capacity of the soil was assessed. Within the group of plants for drought stress experiment, water was limited to 20% of the total soil water capacity and the control group of plants was normally watered to 60% of the total soil water capacity. All the pots of the experiment were watered automatically by a phenotyping platform moving system that had a balance connected to a computer-controlled peristaltic pump. A radio-frequency identifier was added to each pot. The plants were grown in 8 replicates and the plantlets were exposed to 4 different experimental conditions.

The plants were watered according to the preset watering protocols, and the growth of their above ground, green leaf and shoot area was monitored by digital photography. The measurements were made at an interval of seven days.

Plant Phenotyping

Leaf area, water consumption and root density measurements were performed on the Phenotyping Platform HAS-R(S)SDS (Shoots/Roots Stress Diagnostic System) at the Biological Research Centre of Hungarian Academy of Science, Szeged, Hungary, as described by Cseri et al. (2013), Kondić-Špika et al. (2022) and Dénes et al. (2023). The platform monitored the plant growth by digital photography using an Olympus C-7070WZ (Olympus Ltd., Southend-on Sea, UK) digital camera. The photographs of the plants were made from different sideways positions due to multistep rotations of the plant pots and the semi-automated system provided data for each individual plant in terms of leaf area and water consumption. The leaf area was automatically determined using the images from where the background was subtracted and the remaining green pixels were counted. The water consumption parameter was calculated from the weight measurement of the pots before watering. The data regarding the development of the roots was obtained from minirhizotrons by photographing the transparent pots (cylinders) from different side views and bottom. The growth of the plants was monitored for 3 weeks. The Matlab software tools with the Image Processing Toolbox Ver. r2013b (The MathWorks Inc., Natick, MA, USA) was used to analyse the data from the phenotyping platform (Figure 1).

Figure 1. Tomato plants with the first layer of true leaves. Day 1 of the experiment

Statistical analysis

The measurements were done on 8 plantlets from each landrace and standard cultivar. The parameters from the performed treatments were assessed using a one-way ANOVA. For data analysis, the OriginPro 8 data processing program was used.

RESULTS AND DISCUSSIONS

The studied tomato landraces were treated with 0.2% and 0.3% salt for 21 days and the leaf area, the water consumption and the root density were measured by the phenotyping platform.

In the experiment with 0.2% salt treatment the leaf area measurements for 21 days (Figure 2) under normal watering (a) and under water limitation (b) were plotted. In the normal watering conditions (panel a) there is a minor difference between control plants (with no salt treatment, solid symbol and line) and 0.2 % salt watered plants (open symbol, dotted line). It can be observed that 'Cefa7' landrace (solid and open orange diamond) and commercial cultivar 'Marmande' (solid and open pink down triangle) reacted to the salt treatment evidenced by the reduced leaf area (open orange diamond and open pink down triangle, respectively), whereas 'Ateas136' (open up blue triangle) and 'Ateas37' (open green circle) presented increased leaf area as compared to the control plants that were not subjected to salt stress (solid blue triangle and solid green circle, respectively) by day 14. In the case of 'Ateaș 37' (green circle) on day 7 of the treatment the treated plants (empty green circle) presented the same leaf area as the control plants (solid green circle), whereas starting from day 14, the leaf area of the treated plants increased. In the case of 'Ateaș 136' (blue up triangle), on day 7 and day 14, the leaf area of the treated plants (blue open up triangle) slightly decrease; however, from day 14 onward, the measured leaf area of the treated plants started to increase as compared to plants that were not subjected to salt stress (blue solid up triangle); in the day 21 the leaf area of treated plants is bigger than control. Previous studies have also reported that plants subjected to multiple abiotic stresses adversely affect their development, including leaf area (Ors & Suarez, 2017). Physiological responses of cabbage to different levels of salinity and drought proved to have a negative impact on the development of tomato seedlings. When applied independently, drought and salt stress have had a detrimental impact on plant growth, including fresh weights of the roots, shoots, and roots. However, the negative effects of each stressor were amplified when drought and salinity were combined (Ors et al., 2021). This outcome has also been observed in multiple crops, including barley, onion (Hanci & Cebebci, 2015), tomato (Al-Omran, A. M., Al-Ghobari, H., & Alazba, A., 2004) and squash (Abd El-Mageed, T. A., & Semida, W. M., 2015).

Regarding the water restricted conditions (panel b) it can be observed that there is a slight difference between the control plants (solid symbol) as compared to the salt treated plants (open symbol). The plants subjected to salt stress presented reduced leaf area by up to two-fold between days 7 and 14 as compared to the control plants that are grouped together (i.e., in the upper part of the figure, panel b, Figure 2).

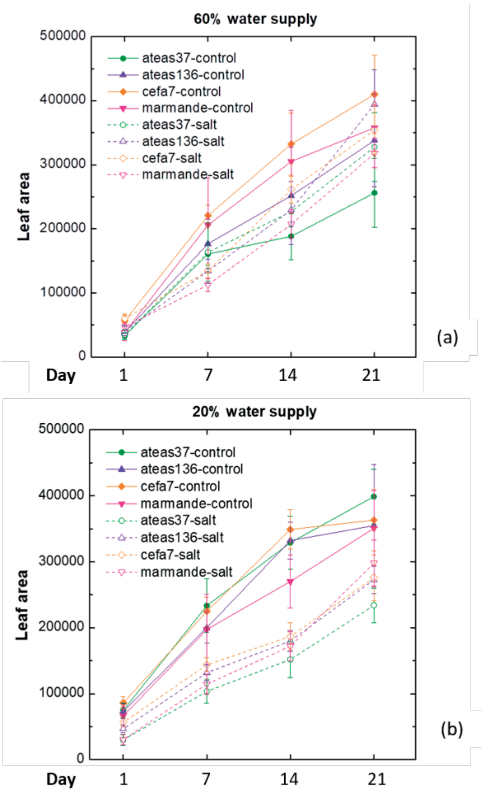

Figure 2. Measurements of leaf area under control and 0,2% saline stress in normal watered (panel a) and drought stress (panel b); for each landrace 'Ateaș37' (circle), 'Ateaș136' (up triangle), 'Cefa7' (diamond) and (solid line and symbol) and salt added substrate standard cultivar Marmande (down triangle), leaf area measurements of plants grown in normal (dotted line, open symbol)

In Figure 3 the leaf area measurements at the last day of treatment are presented and each salt-stressed landrace compared with its control can be visualised. It can be observed that under water restricted conditions 'Ateaș 37' presented significantly reduced leaf area compared with the other landraces and the control cultivar 'Marmande'. Conversely, under normal watered conditions, the most elevated leaf area has been observed in 'Ateaș 136'.

In the tomato experiment, we highlighted that the presence of 0.2% salt in the soil did not induce significant differences in the growth of plants under well watered conditions (60% soil water content). On the other hand, the presence of salt induced a 20-40% decrease in green shoot/leaf area (Figure 2b) under water limited conditions (20% soil water content). Assimakopoulou et al. (2015) evaluated the responses of several cultivars and hybrids of cherry tomatoes to different salt concentrations and revealed that the growth inhibition proved to be due to the toxicity of Na^+ and Cl^- ions and imbalances at nutritional levels impacted by salt stress. In a different study, at a degree of salinity of 5.5 dS m^{-1}, the cultivar Raf's leaf area was recorded to be around 2700 cm^2, however at 11 dS m^{-1}, the foliage was reduced in dimensions and their area drastically dropped to 1800 cm^2 (Sánchez et al., 2010). Salinity-induced morphological alterations, including plant height, dry matter (%), leaf area, and fruit count/plant in tomato cultivar PKM 1 were evaluated. Tomato plants suffered adverse effects from treatment with water containing NaCl at multiple concentrations of 25, 50, 100, 150, and 200 mM for 90 days after seeding. For instance, it was discovered that the 200 mM NaCl treatment decreased the number of fruits per plant to 4 from 15 in the control and the plant leaf area by 43.91%. Furthermore, the plants were 76.17 cm smaller than the control at this dosage (Babu et al., 2012).

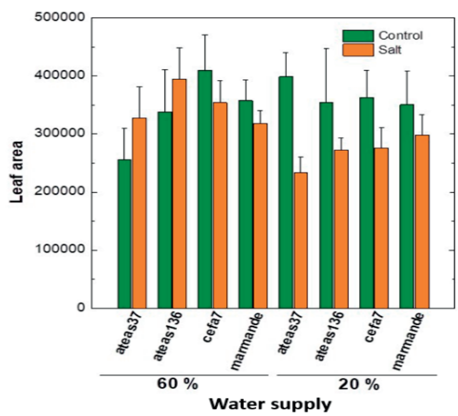

Figure 3. Leaf area measurements of control and 0.2% salt stress samples on the last day of treatment under different watering regimes. Data shown are expressed as means of 8 independent measurements ± SD

Numerous other researchers have demonstrated that morphological alterations were all affected either negatively or favourably by the salt variation in the growth medium. The findings of certain studies that evaluated the morphological alterations in tomato cultivars under salt stress have been reported in this regard (Maeda et al., 2020; Tanveer et al., 2020; Parvin et al., 2016). One adaptive morphological method to prevent water loss through transpiration may be to reduce plant height, leaf area, number, and length when exposed to salt stress. The toxicity of Na^+ and Cl^-ions that build up in cells, which inhibit the development of new leaves, could possibly constitute the root of the problem.

The water consumption measurement (Figure 4) in the case of normal watering (panel a) shows an evolution for 'Marmande' (solid and open pink down triangle) and 'Cefa7' (solid and open orange diamond), with bigger water consumption for the salt treated plants (open pink down triangle and open orange diamond) compared with control plants (solid pink down triangle and solid orange diamond) for the first 14 days and in day 21 the water consumption equalises for both salt treated and control plants.

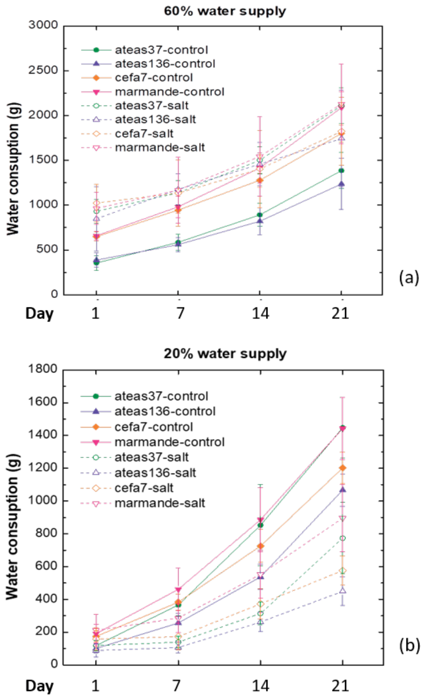

Figure 4. Measurements of water consumption under control and 0,2% salt stress in normal watered (a) and drought stress (b); for each landrace plants grown in normal (solid line and symbol) and salt added substrate (dotted line, open symbol)

For 'Ateaș37' (solid and open green circle) and 'Ateaș136' (solid and open blue up triangle) the salt treated plants (open green circle and open blue up triangle, respectively) show a bigger water consumption than control plants (solid green circle and solid blue up triangle, respectively). When water limitation is imposed (Figure 4, panel b), it is observed a lower water consumption for salt treated plants (open symbols) than the untreated control plants (solid symbols) for all the tomato landraces studied. This difference between control (solid lines) and treated plants (dotted lines) evolves proportionally with time.

End point water consumption measurements on the last day of salt treatment (Figure 5) under normal water showed little to no difference between 'Marmande' and 'Cefa7' control (green columns) and treated plants (orange columns) and a significant level of water consumption in treated plants particularly for 'Ateas37' and 'Ateaș136' under the same regime. When water consumption was measured under drought stress, all the studied landraces showed an elevated consumption for control plants (green columns), almost 2 times bigger as compared to the 0.2% salt treated plants (orange columns); however, lower water consumption was noticed for control drought stress plants as compared to normal water regime control plants.

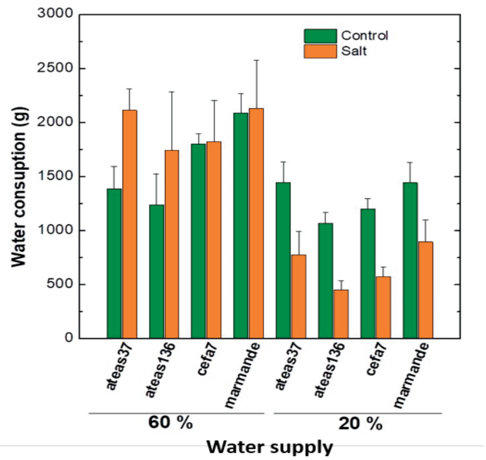

Figure 5. Comparison of leaf area measurements for control and 0,2% salt stress samples on the last day of treatment under different watering regimes. Data shown are expressed as means of 8 independent measurements ± SD

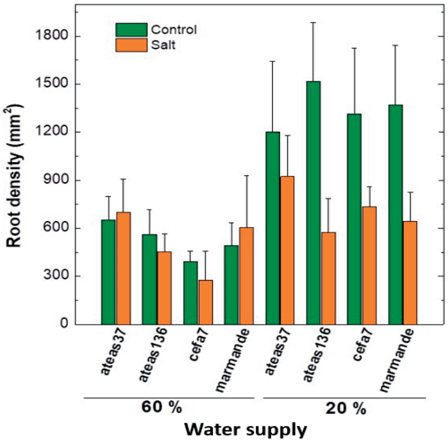

Figure 6. Comparison of root density measurements for control and 0,2% salt stress samples at the last day of treatment under different watering regimes. Data shown are expressed as means of 8 independent measurements ± SD

Root density measurements under 0,2% salt treatment show a decrease compared with the non-treated plants. Under salt and drought stress combined, the root density of treated plants is decreased compared with no salt treated plants, but there is an increase in root density compared with normally watered plants (control and salt treated). 'Ateaș37', under a normal watering regime and with 0.2% salt treatment, was the only landrace that presented an increase in root density (Figure 6). It was demonstrated for certain crops (rice, tomato) that under mild salinity the plants tend to modify their toot architecture system developing deeper roots with fewer lateral roots in this way maximising the opportunity to access deeper soil with more water and less salinity (Ijaz et al., 2019; Gandullo et al., 2021; Shelden and Munns, 2023)

In the 0.3% salt experiment, the leaf area (Figures 7 and 8) and the water consumption (Figures 9 and 10) were measured under normal watering (panel a Figures 7 and 9) and drought stress (panel b, Figures 7 and 9). The leaf area measurements under normal water supply (Figure 7 panel a) showed a greater value with regard to the control plants (solid symbol) as compared to the salt treated plants (open symbol), with the smallest variation during the 12 days measurements for 'Marmande' (pink down triangle). Thus, the 'Marmande' cultivar in day 21 presented leaf area measurements approximately equivalent to those identified in the control plants, with the most significant variation in 'Cefa7' (orange diamond).

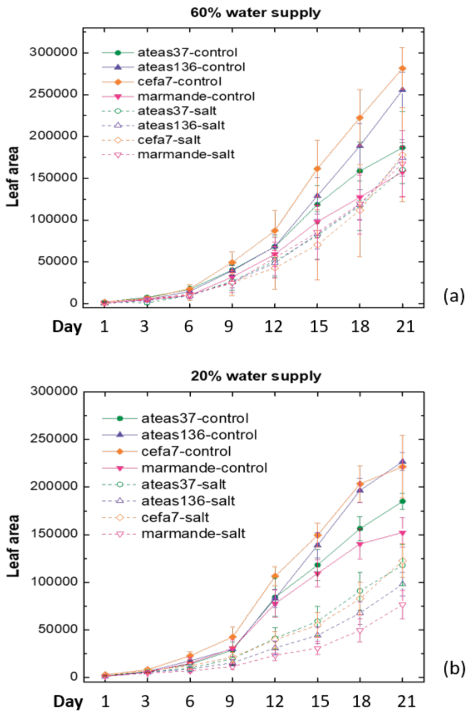

Figure 7. Leaf area measurements of control and 0,3% salt stress in normal watered (a) and drought stress (b); for each landrace plants grown in normal (solid line and symbol) and salt added substrate (dotted line, open symbol) are shown

When drought stress is applied jointly with salt stress (Figure 7, panel b), the leaf area slightly decreased till day 9 and continuing its downward till day 18, when the difference between the control (solid line and symbol) and treated plants (dotted line and open symbol) is revealed to be three times greater.

At the end of the experiment, leaf area measurements (Figure 8) under salt stress only, the leaf area of 'Marmande' proved to be similar in both control (green columns) and stressed plants (orange columns), whereas under both stresses (i.e., salt and drought), the leaf area of the treated plants (orange column) significantly decreased approximately at half the value.

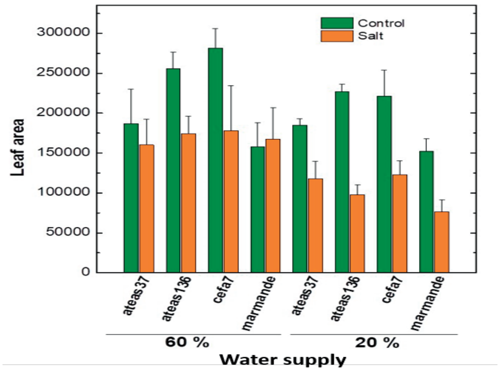

Figure 8. Leaf area comparison of control and 0.3% salt stress samples at the end of the experiment under different watering regimes. Data shown are expressed as means of 8 independent measurements ± SD

In the case of 'Ateaş37', under salt stress only, the leaf area values of treated plants were 15% smaller compared to the control plants. When both stresses were applied, salt and hydric, the values of stressed plants decreased up to 40%. For 'Ateaş136' and 'Cefa7' the salt stressed plants and the salt and drought stressed plants presented lower values for stressed plants, whereas the values in the combined stressed plants were significantly lower as compared to both the controls and their counterparts with only salt stress.

Regarding the water consumption (Figure 9), when 0.3% salt stress was applied, the 'Marmande' commercial cultivar showed a major difference between control (solid pink down triangle, solid pink line) and salt treated plants (open down triangle, dotted pink line), whereas the other studied landraces evolved together with minimum differences between control (solid symbol and solid line) and treated plants (open symbol and dotted line).

When the water consumption is compared at the end of the experiment (Figure 10), the control plants utilise water better than salt stressed plants and, in the case of double stressed plants, (salt and drought together) the water intake of control plants is considerably lower than control salt stressed only plants whereas the measurements for double stressed plants are extremely low.

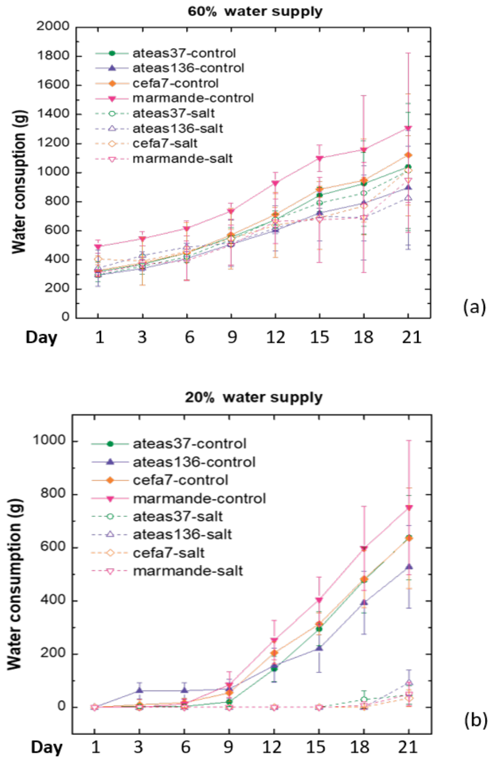

Figure 9. Influence of water supply on water consumption under control and 0,3% salt stress in normal watered (a) and drought stress (b); for each tomato landrace

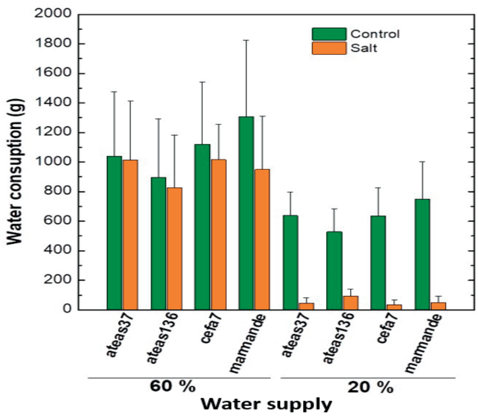

Figure 10. Comparison of control and 0,3% salt stress samples at the end of the experiment under different watering regimes. Data shown are expressed as means of 8 independent measurements ± SD

In the study conducted by Reina-Sánchez et al., 2005, tomato plants cultivated in particularly salinized environments used 40% less water than control plants. The salinity of the nutrient solution accounted for nearly all of the variances in plant water absorption, and the connection among salinity and water uptake was linear. This indicates that plants can modify their water uptake to saline conditions, which could potentially conserve water resources in arid situations (Wan et al., 2007). In order to maximise water, use in agriculture, more studies may also examine how various plant species react to differing salt levels, as correlations between water uptakes can also be cultivar-specific.

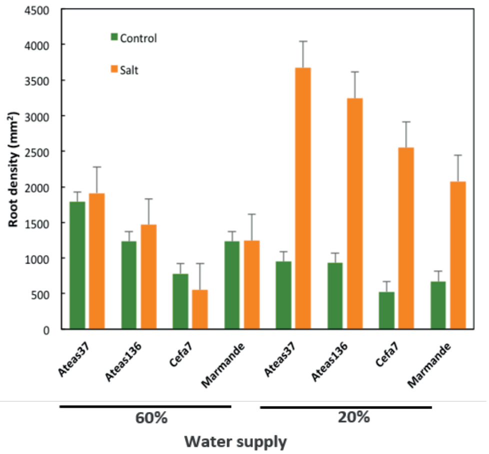

Figure 11. Comparison of root density measurements for control and 0,3% salt stress samples at the last day of treatment under different watering regimes. Data shown are expressed as means of 8 independent measurements ± SD

Root density measurement under a normal watering regime showed a slight increase for 'Ateaş37' and 'Ateaş136' compared with no salt treated controls and a small decrease for 'Cefa7'. Control cultivar 'Marmande' had the same root density in control plants and 0,3% salt treated plants. When both stresses (salt and drought) were applied, the root density of the treated plants exhibited a 4-5 fold increase as compared to their controls that presented smaller root density than the normal watered control plants. This extended root system of the tomato plants grown in saline soil is in line with the research of Anqi et al. (2020) that showed a broad root system contributes to decrease in soil salinity and crops with bigger root systems coop better with increased soil salinity.

CONCLUSIONS

It was noteworthy to observe that the presence of 0,3% salt in the soil seriously limited the water uptake. On day 9, the leaf area measurements of the 'Marmande' cultivar were roughly similar as those observed in the untreated plants, with 'Cefa7' showing the greatest variation. When salt stress and drought stress were jointly applied, the leaf area gradually diminished until day six and continued to decline until day eighteen, when the difference between the treated plants (dotted line and open symbol) and the control (solid line and symbol) turned out to be three times larger. Therefore, preeminent tolerance against the effect of salt was observed in the case of 'Cefa7' landrace and 'Marmande' cultivar. The findings suggest that these landraces have the ability to adapt and thrive under combined drought and salt stress, making them promising candidates for breeding programs aimed to improve crop resilience in harsh environments. Ultimately, utilising these local tomato landraces could contribute to sustainable agriculture practices and food security in regions prone to environmental stressors.

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RESEARCH ON THE EVOLUTION OF TOMATO VARIETIES AND HYBRIDS CULTIVATED IN ROMANIA

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Abstract

The study was carried out at USAMV Bucharest and aimed to identify the assortment of tomato varieties and hybrids grown in protected spaces and open fields in Romania. Tomatoes are traditional vegetables, rich in carbohydrates, vitamins, lycopene and carotene. Currently, in our country, imported F1 hybrids, extra-early and early, are cultivated in a smaller proportion and in a larger proportion Romanian or imported varieties. F1 hybrids are preferred, because show the phenomenon of heterosis which gives crops resistance/tolerance to diseases, pests, drought, a higher production yield and a very good quality of fruit production. F1 tomato hybrid seeds are procured from vegetable growers from authorized stores that import them to production companies from the Netherlands, Italy, France, Israel. The Romanian tomato seeds come from research stations in Buzău, Bacău, Vidra, etc.
This study aims to highlight the large number of varieties adapted and used in the different cultivation areas in Romania, varieties that are recommended for both conventional and organic crops in the different cropping systems.

Key words*: Solanum lycopersicon, genotypes, productions.*

INTRODUCTION

Tomatoes are considered to be the specie with the largest number of genotypes that are grown on the largest areas, both in protected spaces, and in the field. Ensuring quality tomatoes throughout the year is the essential priority of the Romanian market. Their fruits have a high vitamin, mineral salts and oligoelements content, as a good human health support. Can be consumed as fresh products or processed as juice, sauce or ketchup. Due to these properties, the way their harvests are obtained have to be managed properly (Popescu and Dinu., 2019).

Modern methods have been introduced into tomato culture with the aim of increasing tomato production and quality. The use of high-quality hybrids, grafting methods which reduce the number of chemical treatments and other technological methods represent viable alternatives for sustainable agriculture. Tomato production is influenced by many factors, including temperature, humidity, fertilisation, pests and diseases, etc (Soare, Dinu and Băbeanu, 2018). Since they must be provided throughout the year, their culture is mainly done in classic greenhouses, hydroponic greenhouses and tunnels.

The cropping systems are varied depending on the possibilities of the farmers, the crops being able to be practiced in a conventional system on the ground but also in a non-conventional system on different culture substrates.

The advantage of the non-conventional system is that, the crop cycle can be throughout the whole year, compared to the conventional system, unheated greenhouses, where 2 crop cycles can be planted, one from spring to summer for early crops and from summer to autumn for successive crops with production assurance until November.

The disadvantage of the non-conventional culture, in the II cycle but also in the I cycle, is that the temperatures in the autumn during the ripening period of the fruits are ensured with great difficulty, and for the spring crops in the first part of the growth and development of the plants, the temperatures can also be very low, thus influencing the period of early tomato harvest (Garofalo and Rinaldi, 2015).

Another aspect to take into account is that of ensuring pollination both during the autumn period in the second cycle and the spring period in the first cycle.

Choosing the tomato variety is a priority for any farmer. The great diversity of the assortment leads to a more careful analysis of it with the aim of obtaining the highest possible production performance but also for the constant assurance of the market.

Schouten et al. (2019) in the study mentioned that, until 1960 genetic diversity was very low, and concerns regarding the improvement of fruit quality as well as resistance to diseases and pests began to take shape after 1970.

Lake (1966) and Mut Català (1972) mention the production is influenced by the place of culture as well as Heinonen0 et al. (1979) that high temperatures can influence the rate of physiological maturity.

The performance of tomato crops is influenced by the management of the plantation, which should promote the efficient development of the plants, as well as both the quantitative and qualitative production of fruit (Jerca et al., 2021; Arshad et al., 2023; Dinu et al., 2023).

The main concerns regarding the selection of genotypes concerned resistance to diseases and pests but also the productivity and quality of tomato fruits (Erba et al., 2013; Lin et al., 2014; Mayer et al., 2008; Garcia et al., 2013).

According to the Food and Agriculture Organization of the United Nations (FAO), in 2019, the global tomato production was about 1808 million tons. The countries with the highest production in the same year were China (628 million tons), India (190 million tons) and Turkey (128 million tons) (FAO, 2020). In addition, in 2019, the organic farming in general increased by 2.9% compared to the previous year, and for the following years a considerable increase in trade with organic products is expected (IFOAM-Organics International, 2020), tomatoes being the most relevant species of this type of unconventional agriculture (Dinu et al., 2023).

Data presented in this study on tomato production were collected from the Ministry of Agriculture and Rural Development and from the FAO, were analyzed, processed and interpreted statistically in order to highlight the large number of varieties adapted and used in the different cultivation areas in Romania.

MATERIALS AND METHODS

Tomato production in Romania from 1965 to the present was analyzed based on the data recorded in the FAO Statistical Annals.

Also, the data on the varieties cultivated in Romania were taken from the official list of varieties and hybrids from 1965 to the present.

Tomato production data were collected from the Ministry of Agriculture and Rural Development and analyzed, processed, and interpreted.

The present study refers to tomato production in Romania from 1965 until now, and the processed data are collected from the FAO Statistical Yearbooks and from the Ministry of Agriculture and Rural Development and analyzed, processed and interpreted. Also, the data on the varieties grown in Romania were taken from the official list of varieties and hybrids from 1965 to the present.

RESULTS AND DISCUSSIONS

Analyzing the data on the production made in the period 1965-1989, we could find that the lowest productions were obtained in 1969 and 1970 respectively, which were 119,619.0 tons and 120,636.0 tons respectively, and the highest productions were obtained in 1984 of 280,872 tons followed by year 1985 with 272,682 tons. On average over the period 1965 - 1989, the total tomato production recorded was 186,778.08 tons, production covering both domestic consumption and export of tomatoes.

It should be noted that before 1989 tomato export was a priority, and tomato imports were almost non-existent. Practically, domestic production covered the entire consumption of tomatoes in Romania. At the same time, it should be noted that 99% of the assortment of varieties was represented by non-assortment created in Romania (Figure 1).

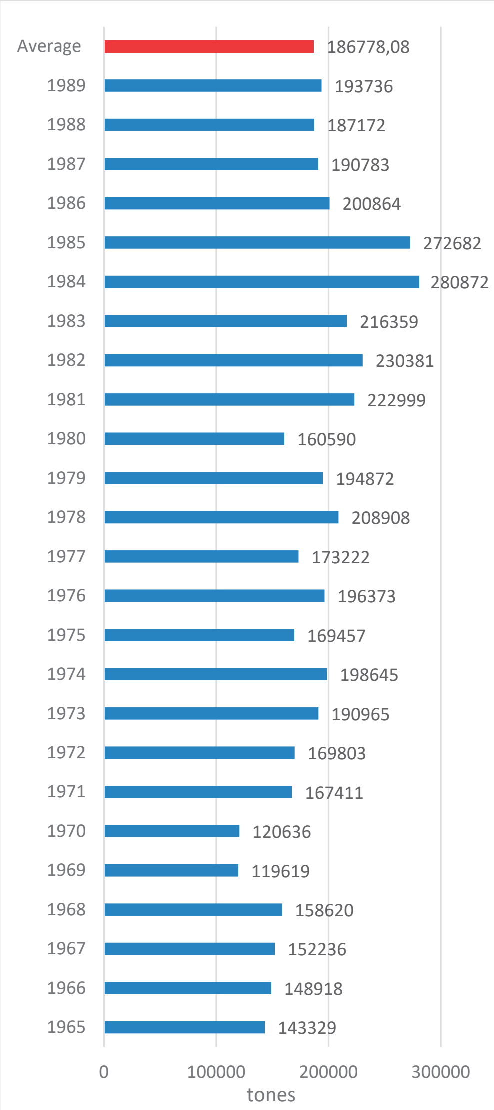

Figure 1. Tomato production during the period 1965-1989 in Romania

Analyzing the data from the period 1990-2020, it was found that the lowest tomato production recorded was 107,918 tons, in 1997, while the highest production was recorded in 2020, reaching 282,610 tons. The average production recorded during this period was 164,606.4 tons of tomatoes (see Figure 2), which is lower than the assessment for the period 1965-1989.

The research stations created the varieties according to the climate zone as follows: The Research and Development Station for Vegetable Culture from Bacău recently created the Tombac variety, a variety with a very high productivity of up to 150 tons per hectare, under protected conditions. It has an indeterminate growth and is a semi-early variety with a vegetation period of 98-102 days until harvest. The fruits show a very good firmness with an average of 200 g per fruit.

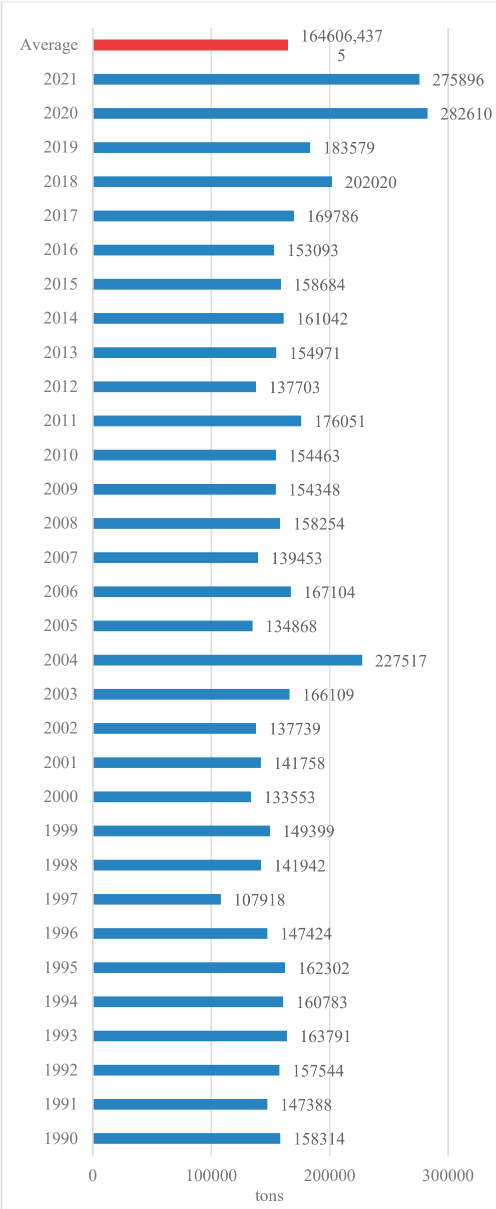

Figure 2. Tomato production realized in the period 1991-2021 in Romania

Another variety created at SCDL Bacău was the Unibac variety with fruits weighing 70-90 g and a production of up to 110 t/kg.

At the Vegetable Research Station in Buzău, more than 20 varieties of tomato were approved, including the varieties Buzău 1600, Siriana and Inimă de Bou. The appearance of the varieties Buzău 47, Buzău 1600 and Inimă de Bou are shown in the Figure 3.

a. Buzău 47

b. Buzău 1600

c. Inimă de Bou

Figure 3. The appearance of the varieties Buzău 47 (a); Buzău 1600 (b) and Inimă de Bou (c)

Among the cherry varieties with indeterminate growth, the Flaviola variety, created at SCDL Buzău, is cultivated with very good results. This variety lends itself to cultivation both in non-conventional soil culture and in non-conventional culture on nutrient substrates (Figure 4).

Figure 4. The appearance of the tomato culture, variety Flaviola, on the substrate, in an unconventional system

Analyzing the variety of cultivars cultivated between 1961 and 1989 as well as from 1990 to the present, it was found that before 1989 most cultivars were created in the country, but after 1990, cultivars, and performing hybrids were introduced to Romania. Knowing the properties and characters of different varieties is also necessary in the process of producing seeds for valuable varieties in order to establish their authenticity.

The adaptability of tomato plants to certain environmental conditions sometimes lead to compromising the harvest. Both varieties and hybrids created for a certain way of use give the maximum yield if they are grown in the conditions and for the purpose for which they were created. Studies have shown that if they are given another use, if they are grown in the most indicated areas or if they are applied with an inappropriate technology, they do not give the expected results in terms of production.

Recently, cultivars have been created with a high ecological plasticity regarding the influence of the environmental conditions in which they develop. Varieties created for a certain area retain their valuable properties and therefore it is recommended to cultivate them for the recommended conditions.

In 1971 hybrids were cultivated in Romania: N1 10xBison of Bulgarian origin, the fruits being

about 50-70 g and being recommended for fresh consumption and especially for export.
Another hybrid cultivated during this period was Delicates F1 obtained by crossing the Bulgarian variety XXTV/13 and Stupike polni rani. Also, Argeș 408 was one of the earliest hybrids, the first fruits being obtained after 90-95 days from emergence and its destination being culture in protected spaces.
The Temnocrasnîi cultivar from the USSR (Russia) has a smaller distribution in Romania but is appreciated for its quality fruits weighing 70-90 g.
The Export II hybrid, created at the Nicolae Bălcescu Agronomic Institute - Bucharest (UȘAMV Bucharest), Horticulture faculty, resulted from this variety as part of a breeding work and by crossing with line XXIV/13. This hybrid has been maintained for a long time in cultivation, being highly appreciated in export for its taste qualities but also the size of the fruit of about 70-80 g of the fruits obtained per plant, about 80% were classified as I and extra quality.
Another valuable variety was Aurora 100, created at the Ciolpani vegetable experience resort, this one being extra-early, the fruits having between 100 and 120 g, which can be grown both in protected space and in early crops in the field.
Also, for the early crops, another variety with indeterminate growth, but of Dutch origin, was Money Maker, with fruits of 65-70 g, being in the category of first quality and extra in percentage of over 85%. The Money D'or variety, with the same origin as the previous variety, has been successfully cultivated, especially under greenhouse conditions.
In Romania, in 1991, the following cultivars were cultivated in protected spaces, for early crops, according to the official list of varieties and hybrids in Romania from 1991: Export II (S1), H 601 (S1), Ioana, Işalniţa 50, Lucia, Solara. All this assortment being intended for cultivation in greenhouses and solariums for early crops, these being Romanian creations. The Export II hybrid was created and entered into the official list of varieties since 1970.
Analyzing the official catalog of varieties and hybrids in Romania, for the year 2016 as well as for the year 2017, it could be observed that no new tomato hybrids were registered on the list of new tomato varieties for the greenhouse. The official catalog includes the following variety of tomatoes: Augustina, Bacovia, Buzău 1600, Carisma, Coralina, Doljbrid, Eclipsette, Efrat 70, Ema de Buzău, Ghittia, Ișalnita 29, Ișalnita 50, Kalina, Kazanova, Llissete. Medeno SRCE, Nectaria, Omer, Parris, Ralisaisa, Rita, Rinoko, Rubystar, Siriana, Smarald, Sony, De Buzău, VP Viva and Yarden.
However, it should be emphasized that in 2017 the following cultivars were also included: Caitlin, Carisma, Corina, Danamari, Edina, MCSU07, which have indeterminate growth and can be recommended for crops in protected spaces.
In 2018, the tomato cultivars Lamoneda, Panda, Silvia, Toprak, Andrada, Flaviola and Fulya were introduced.
In the official catalog of varieties and hybrids for 2019, a new assortment was introduced, mostly of Romanian origin: Dorca, Florelia, Hera, Miruna, Moldoveanu, Oltena, Roliana, Sandybelle, Sorada, USAB29 and Zaira.
We were able to find that in the Official Catalog of Varieties and Hybrids grown in Romania recommended for 2020, a new variety created in 2019 was introduced, which is: Banato, Crina, Emiliana, Florelia and Tomtim. It was found that in 2021 only one Chandona cultivar created in 2020 was entered into the official catalog of varieties and hybrids in Romania.
The cultivar Edanur was entered into the official list of varieties and hybrids in Romania in 2022, and in 2023 two more cultivars Akemi and Oshin were entered, according to the Official Catalog - ISTIS.

CONCLUSIONS

Research on the evolution of tomato varieties and hybrids grown in Romania is a particularly interesting and relevant subject for agriculture and the food industry in the country. These studies are essential to understand and address the specific needs of the domestic market, as well as to adapt to global demands and trends by utilizing varieties developed in Romania.
When considering the evolution of tomato varieties and hybrids, several factors are considered: adaptability to climate and soil conditions in various regions of Romania, resistance to region-specific diseases and pests, yield, and quality of production concerning

taste, texture, size, and appearance of tomatoes, as well as early maturity and storage and transport potential.

Special emphasis should be placed on promoting varieties and hybrids adapted to sustainable and ecological agricultural practices, which are increasingly crucial for ensuring food safety and environmental protection. Ultimately, research on the evolution of tomato varieties and hybrids in Romania plays a crucial role in ensuring competitive, sustainable agricultural production and meeting the needs of a continuously growing population.

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BIOLOGICAL AND CONVENTIONAL CONTROL OF *SPHAEROTHECA FULIGINEA* PATHOGEN ON ZUCCHINI CROPS

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Abstract

Maintaining a good phytosanitary level of vegetable crops in greenhouses, it is conditioned by application of a complex of measures and means to prevent and control the attack of the pathogen and the pests present in the crops. The main objective of this experience was the control of the pathogen Sphaerotheca fuliginea on zucchini crops in organic and conventional systems, and a comparison was to be made between the efficacy of applied chemical products and the efficacy of biological products. The chemical products (Topas 100 EC 0.5/ L/ha, Amistar 1 L/ha, Cidely Top 1 L/ha, Dagonis 0.6 L/ha) had an efficacy between 73.44 and 96.33%, and the biological products (Fytosave 2 L/ha, Funres 3 L/ha, Mimoten 3 L/ha, Canelys 3 L/ha) between 20.74 and 56.90% for Diana, Perfect and Eskenderany varieties. In the zucchini crops in greenhouses, the chemical products applied in the conventional system had a much higher efficacy than the biological products.

Key words*: disease, zucchini, Sphaerotheca fuliginea, pathogen, greenhouse.*

INTRODUCTION

Powdery mildew, caused by *Sphaerotheca fuliginea* (Schlecht) Pollacci), is a major foliar disease worldwide, reducing crop quality and yield.

Powdery mildew (*Podosphaera xanthii*) is considered the most serious disease-causing yield losses, as it affects the leaves, stems and fruits of squash grown under different conditions (Hafez et al., 2018, Elsisi A., 2019).

The disease is generally controlled in commercial cucurbit crops via frequent applications of fungicides; however, heavy fungicide application has led to the development of resistant *Podosphaera xanthii* populations that can no longer be controlled by fungicides (McGrath et al., 1999). The need for new control strategies for the management of powdery mildews has led researchers and growers to explore suitable environmentally friendly alternatives or complementary to chemicals, biological control being the most investigated of these approaches (Bélanger and Labbé, 2002).

The organic management of crop diseases, such as powdery mildew in squash, is extremely important in order to ensure global food security, to reduce pesticide applications and GHG emissions, to maintain sustainable production practices, and to minimize the vulnerability of farmers to the negative impacts of climate change (El Chami D., 2020; El Chami et al., 2020; Frem et al., 2022).

Plant resistance inducers, also referred to as elicitors, are agents that confer improved protection to pathogen or pest attacks by inducing host defence mechanisms (Siah A. et al. (2018). COS-OGA, a novel oligosaccharidic elicitor, induced a large reduction in the severity of powdery mildew on cucumber crops (Van Aubel et al., 2014).

Sodium bicarbonate can be a safe and effective organic bio-agent for the control of powdery mildew in squash plants (Frem et al., 2022). The inhibitory effectiveness of phosphate and potassium salts makes them useful biocompatible fungicides and possibly ideal foliar fertilizers for disease control in the greenhouse (Reuveni et al., 1996). Foliar applications of potassium silicate reduce severity of powdery mildew on cucumber, muskmelon, and zucchini squash (Menzies et al., 1992).

MATERIALS AND METHODS

The main purpose of this experience was the control of pests in the zucchini squash crop in conventional and organic system, to make a comparison between the effectiveness of chemical and biological products.

The biological material is represented by three cultivars: Diana, Perfect and Eskenderany, which were planted on August 8, 2023. To the crop in the conventional system, 3 treatments were applied, at intervals of 10 days, as follows: August 25 (T1), September 4 (T2) and September 14 (T3). To the crop in the biological system, during the vegetation period, 5 treatments were applied, at intervals of 5 days, as follows: August 25 (T1), August 30 (T2), September 5 (T3), September 11 (T4)) and September 15 (T5).

In the conventional system, a bifactorial experience was organized that includes 5 experimental variants, laid out according to the method of randomized blocks, as follows:

1. Topas 100 EC (penconazole 100 g/L, local systemic, translaminary) 0.5 L/ha;
2. Amistar (azoxystrobin 250 g/L, local systemic, translaminary) 1 L/ha;
3. Cidely Top (difenoconazole 125 g/L + cyflufenamid 15 g/L, local systemic, translaminary) 1 L/ha;
4. Dagonis (fluxapyroxad 75 g/L + difenoconazole 50 g/L, local systemic, translaminary) 0.6 L/ha;
5. Untreated control.

Products with a very short break time until harvesting (3 days) were chosen, because in protected areas harvesting is done much more often than in the field.

In the ecological system, the experience also includes 5 experimental variants, arranged according to the method of randomized blocks, as follows:

1. Fytosave (COZ-OGA) 2 L/ha;
2. Funres (Mimosa tenuifolia and citrus extract) 3 L/ha;
3. Mimoten (Mimosa tenuifolia 80% extract + 1.95% Zn + 0.05% Mo) 3 L/ha;
4. Canelys (cinnamon extract) 3 L/ha;
5. Untreated control.

Observations and determinations were made on the leaves (3 plants/variant) regarding the frequency and intensity of the *Sphaerotheca fuliginea* pathogen, based on which the degree of attack and the effectiveness of the products were calculated. Final observations were made on September 22, 2023.

Climate data monitoring in greenhouse was done with the help of thermohygrometer, which recorded air temperature and humidity at hourly intervals.

The high temperatures from July to September, associated with low atmospheric humidity (50.9-56.9%), have created very favorable conditions for the appearance and evolution of the powdery mildew attack on zucchini squash crops in greenhouse (Figure 1).

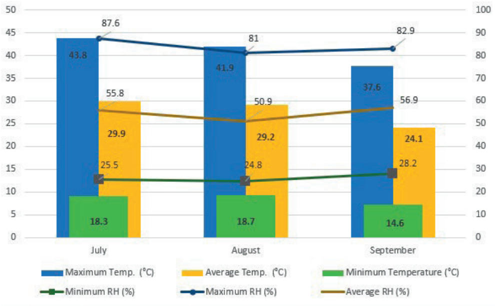

Figure 1. High plastic tunnel climate data for the period July - September 2023

RESULTS AND DISCUSSIONS

The Diana zucchini variety, the best results were obtained when applying the Dagonis product with an effectiveness of 96.33% on the upper part of the leaves and 83.67% on the lower part (Table 1, Figure 2). The untreated control showed a very strong powdery mildew attack with a frequency of 75%, intensity of 87.96% and an attack degree of 65.97% (Figure 3).

Figure 2. Squash plant, Diana variety treated with Dagonis product

Figure 3. Untreated control, Diana variety

Table 1. Efficacy of products to control the pathogen *Sphaerotheca fuliginea*, in the squash crop in the conventional system, Diana variety

Var.	The product	Dose / ha (L/ha)	Attack frequency (%)	The intensity of the attack (%)	The degree of attack (%)	Efficacy (%)
			The upper part of the leaf			
1	Topas 100EC	0.5	55.00c	27.90b	15.34b	76.74
2	Amistar	1.0	51.53cd	28.43b	14.64b	77.80
3	Cidely Top	1.0	53.63d	18.57c	9.95c	84.88
4	Dagonis	0.6	60.00b	4.04d	2.42d	96.33
5	Untreated control	-	75.00a	87.96a	65.97a	-
			The underside of the leaf			
1	Topas 100EC	0.5	47.59bc	18.69c	8.89b	83.37
2	Amistar	1.0	47.69bc	25.00b	11.92b	77.71
3	Cidely Top	1.0	50.60b	19.25c	9.74b	81.78
4	Dagonis	0.6	41.14c	21.25c	8.73b	83.67
5	Untreated control	-	69.44a	77.00a	53.47a	-

And in the varieties Perfect and Eskenderany, the best results were obtained when applying the Dagonis product with an effectiveness of 91.77% on the upper part of the leaves (Table 2, Figure 4) in the Perfect variety, respectively 91.84% in the Eskenderany variety (Table 3, Figure 6). The untreated control showed a very strong attack of powdery mildew, with a degree of attack of 58.74% (Figure 5) in the Perfect variety and 66.6% in the Eskenderany variety (Figure 7).

In the case of the crop in the conventional system, analyses were made on the squash fruits to determine the content of pesticide residues. In this sense, 10 samples were collected, one for each treated variant, which were sent to the National Phytosanitary Authority for analysis.

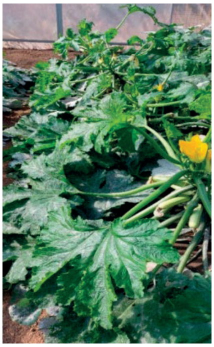

Figure 4. Squash plant, Perfect variety treated with Dagonis product

Figure 5. Untreated control, Perfect variety

Table 2. Efficacy of products to combat the pathogen *Sphaerotheca fuliginea*, in the squash crop in the conventional system, Perfect variety

Var.	The product	Dose / ha (L/ha)	Attack frequency (%)	The intensity of the attack (%)	The degree of attack (%)	Efficacy (%)
			The upper part of the leaf			
1	Topas 100EC	0.5	45.38c	24.41bc	11.07bc	81.15
2	Amistar	1.0	50.00b	31.25b	15.60b	73.44
3	Cidely Top	1.0	45.45c	18.30c	8.31bc	85.85
4	Dagonis	0.6	46.70c	10.35d	4.83c	91.77
5	Untreated control	-	65.00a	90.38a	58.74a	-
			The underside of the leaf			
1	Topas 100EC	0.5	31.53b	21.31b	6.71	79.34
2	Amistar	1.0	35.00b	18.72	6.55	79.50
3	Cidely Top	1.0	30.25b	15.95	4.82	85.16
4	Dagonis	0.6	31.90b	15.30	4.88	84.97
5	Untreated control	-	60.00a	54.16a	32.49	-

Table 3. Efficacy of products to combat the pathogen *Sphaerotheca fuliginea*, in the squash crop in the conventional system, Eskenderany variety

Var.	The product	Dose / ha (L/ha)	Attack frequency (%)	The intensity of the attack (%)	The degree of attack (%)	Efficacy (%)
The upper part of the leaf						
1	Topas 100EC	0.5	41.10c	35.35b	14.52bc	78.20
2	Amistar	1.0	48.27b	36.07b	17.41b	73.85
3	Cidely Top	1.0	36.66c	31.78b	11.65c	82.51
4	Dagonis	0.6	35.29c	15.41c	5.43d	91.84
5	Untreated control	-	70.37a	94.73a	66.60a	-
The underside of the leaf						
1	Topas 100EC	0.5	38.23bc	14.61b	5.58b	80.80
2	Amistar	1.0	44.82b	14.92b	6.67b	77.05
3	Cidely Top	1.0	61.90a	11.23b	6.95b	76.09
4	Dagonis	0.6	32.35c	12.73b	4.11b	85.86
5	Untreated control	-	66.67a	43.61a	29.07a	-

Figure 6. Squash plant, Eskenderany variety treated with Dagonis product

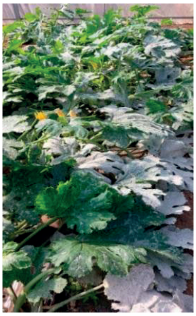

Figure 7. Untreated control, Eskenderany variety

The results of the analyzes showed that the samples comply with the provisions of Regulation (EC) 396/2005 with subsequent amendments and additions, regarding the analyzed pesticide residues.

In the squash crop, the 4 biological products: Fytosave, Funres, Mimoten and Canelys, showed lower biological effectiveness compared to the chemical products.

In the Diana variety, on the upper part of the leaves, the effectiveness of the products varied between 39.64% (Canelys) and 50.1% (Funres), in the conditions of a very strong degree of attack in the control 68.22% (Table 4). With the Perfect variety, the best results were obtained, with more than 56% effectiveness in the variants treated with Fytosave and Funres (Table 5).

In the Eskenderany variety, the lower results were obtained, with an effectiveness of over 30% in the case of the variants where the biological products Fytosave, Funres and Mimoten were applied (Table 6).

In the squash crops in greenhouses, the chemical products applied in the conventional system had a much higher effectiveness than the biological products (Figures 8, 9 and 10).

Table 4. Biological control of the *Sphaerotheca fuliginea* pathogen, in Diana variety squash crop

	Variant	Attack frequency (%)	The intensity of the attack (%)	The degree of attack (%)	Efficacy (%)
The upper part of the leaf					
1	Fytosave	68.00b	57.37c	38.94b	42.91
2	Funres	53.16c	64.09b	34.04b	50.10
3	Mimoten	56.00c	68.65b	38.33b	43.81
4	Canelys	66.66b	61.78bc	41.18b	39.64
5	Untreated control	81.55a	79.74a	68.22a	-
The underside of the leaf					
1	Fytosave	66.00b	26.51b	17.42b	37.89
2	Funres	50.63c	26.37b	13.35b	52.59
3	Mimoten	54.67c	28.90b	15.79b	43.92
4	Canelys	64.28b	18.61c	11.96b	57.52
5	Untreated control	85.55a	32.92a	28.16a	-

Table 5. Biological control of the *Sphaerotheca fuliginea* pathogen, in Perfect variety squash crop

	Variant	Attack frequency (%)	The intensity of the attack (%)	The degree of attack (%)	Efficacy (%)
		The upper part of the leaf			
1	Fytosave	63.33b	40.05c	25.36c	56.82
2	Funres	62.06b	40.83c	25.27c	56.90
3	Mimoten	70.37a	42.30c	29.76b	49.33
4	Canelys	64.51b	46.75b	30.15b	48.87
5	Untreated control	72.22a	81.34a	58.74a	-
		The underside of the leaf			
1	Fytosave	33.33bc	11.00c	3.66b	50.13
2	Funres	37.93b	14.54b	5.51b	24.93
3	Mimoten	31.85c	13.64b	4.34b	40.87
4	Canelys	42.38a	13.00b	5.51b	24.93
5	Untreated control	35.6b	20.62a	7.34a	-

Table 6. Biological control of the *Sphaerotheca fuliginea* pathogen, in Eskenderany variety squash crop

	Variant	Attack frequency (%)	The intensity of the attack (%)	The degree of attack (%)	Efficacy (%)
		The upper part of the leaf			
1	Fytosave	56.52b	73.07bc	41.29c	32.69
2	Funres	55.55b	68.66c	38.14c	37.82
3	Mimoten	57.89b	82.95b	48.01b	20.74
4	Canelys	48.38c	82.50b	39.91c	34.94
5	Untreated control	63.63a	96.42a	61.35a	-
		The underside of the leaf			
1	Fitosave	43.47b	14.50b	6.30b	49.27
2	Funres	37.03c	13.00b	4.84c	61.12
3	Mimoten	47.36a	15.27b	7.23b	41.78
4	Canelys	30.64d	14.73b	4.53c	63.52
5	Untreated control	42.42b	29.28a	12.42a	-

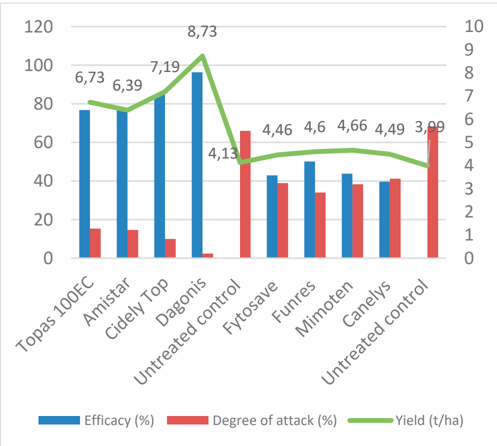

Figure 8. Efficacy of conventional and biological products and yield, Diana

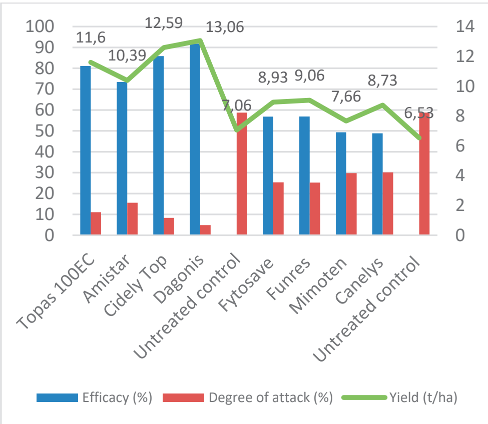

Figure 9. Efficacy of conventional and biological products and yield, Perfect

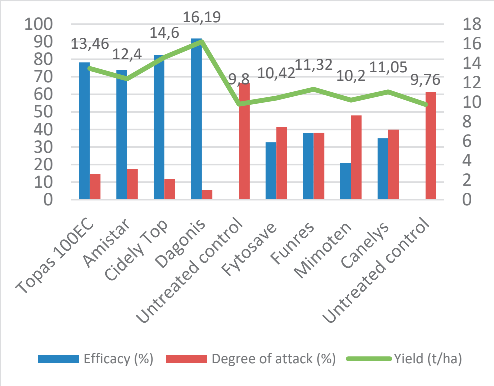

Figure 10. Efficacy of conventional and biological products on yield, Eskenderany

Between the degree of attack and yield there is a negative correlation, distinctly significant in the conventional system (-0.964, Perfect variety) and biological (-0.965, Diana variety). Significant negative correlation in conventional system (-0.932, Diana variety, -0.902, Eskenderany variety) and biological (-0.904 Perfect variety, -0.886, Eskenderany variety).

CONCLUSIONS

1. The biological products (Fytosave 2/ha, Funres 3 l/ha, Mimoten 3 l/ha, Canelys 3 l/ha), used to control the pathogen *S. fuliginea* had a much lower effectiveness compared to the chemical control products (Topas 100 EC 0.5/ l/ha, Amistar 1 l/ha, Cidely Top 1 l/ha, Dagonis 0.6, l/ha), used in conventional system;
2. In the conventional system, the best results were obtained when applying the Dagonis product (fluxapiroxad 75 g/l + difenoconazole 50 g/l) 0.6 l/ha, with an effectiveness of over 90% in Diana, Perfect varieties and Eskenderany;
3. The biological products had an average effectiveness between 48.27% in the case of the application of the Funres product (*Mimosa tenuifolia* extract 60% and citrus 20%) and 37.96% for Mimoten (*Mimosa tenuifolia* extract 80% + 1.95% Zn + 0.05% Mo).

ACKNOWLEDGEMENTS

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POPULATION MANAGEMENT OF *MYZUS PERSICAE* (SULZER) IN *SOLANUM TUBEROSUM* AGROECOSYSTEM USING CHEMICAL AND BIOLOGICAL PRODUCTS

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Abstract

Myzus persicae produces significant economic losses in the agroecosystem of Solanum tuberosum, through direct and indirect damage. The aim of this study was to control the population of Myzus persicae, in the potato crop, by applying the substance acetamiprid and biological products based on potassium salt (Kabon and Konia) and Bacillus thuringiensis (Bitoxybacilin). The study included seven variants, acetamiprid was applied in three doses (0.13 l/ha; 0.09 l/ha; 0.08 l/ha), 0.900 l/ha was applied in the variants treated with Kabon and Konia K Plus and 1.0 l/ha Bitoxybacilin. The efficiency of the biological and chemical products was compared with the control variant (untreated). Phytosanitary products were applied on 27.05.2022. The efficacy of the treatments was assessed 3, 7, 14, 21, 28 days after application, by analyzing 25 plants/variant. On the day of the treatments application, the number of individuals of Myzus persicae was 274-284/25 plants. Acetamiprid 0.13 l/ha significantly reduced the aphid population. Among the biological products, Konia K Plus stood out. 28 days after application, the effectiveness of the products to control the Myzus pericae species decreased.

***Key words**: Myzus persicae, efficacy, Solanum tuberosum, treatments, biological products.*

INTRODUCTION

Myzus persicae (Sulzer) is a hemipteran with a wide distribution, according to the information provided by CABI (https://www.cabidigitallibrary.org), being present in Africa, Asia, Europe, North America, South America and Oceania (Figure 1).

Myzus persicae (Sulzer) is present in all European countries (CABI, 2024) (Figure 1), the first report was in Sweden, in 1885 (Seebens et al., 2017 quoted by https://www.cabidigitallibrary.org).

This species is characterized by high polyphagism, attacking more than 400 plant species from 50 families, including *Brassicaceae, Fabaceae, Compositae, Solanaceae*, causing serious economic losses (Chiriloaie-Palade et al., 2020; Georgescu et al., 2020; Jiang et al., 2022). The green peach aphid attacks more than 24 species of plants in the Solanaceae family, including *Solanum tuberosum* (CABI, 2024). The first report of this species on potato plants dates back to 1986 (Jansson et al., 1986). The green peach aphid is considered an important pest in potato agroecosystems, being capable of reducing production by 10 to 80% in crops where it is present (Ali et al., 2023; Oprisiu et al., 2023).

Figure 1. Distribution of the *Myzus persicae* species on the continents and in Europe (map overtaken from https://www.cabidigitallibrary.org)

The damages produced by *Myzus persicae* (Sulzer) are classified as direct (sucking sap from the phloem) and indirect (through "honeydew" it favors the development of pathogens and thus the processes of photosynthesis and respiration are disturbed) (Capinera et al., 2020). In addition, the peach green aphid plays an important vector in the transmission of potyviruses, leading to significant yield losses (Naga et al., 2020; Qi et al., 2020; Bera S. et al., 2022). Studies by various researchers have highlighted the fact that this species causes both qualitative and quantitative losses in agricultural production, causing fruit abortion, wilting, chlorosis, necrosis and defoliation (Ali et al., 2023). *Myzus persicae* (Sulzer) is a challenge for entomological researchers and farmers because of the damage and factors that ensure the ecological success of the species (Figure 2).

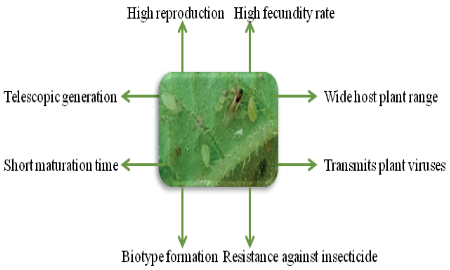

Figure 2. The factors that determine the ecological success of the species M*yzus persicae* (Sulzer) as a polyphagous and cosmopolitan pest (diagramm made by Ștef R., after the information presented by Ali et al., 2023)

From the up to date published studies, it appears that the management of the population of *Myzus persicae* (Sulzer) has been done using chemical, biological and cultural methods (Ali et al., 2023). The most common control methods, applied to the species *Myzus persicae* (Sulzer), were chemical, due to their availability, effectiveness and ease of use (London et al., 2020; Deguine et al., 2021). The most used pesticides to reduce the population of *Myzus persicae*, from antropic ecosystems, have as active ingredients: pyrethroids (sodium channel modulators), organophosphates (acetylcholinesterase inhibitors), carbamates (acetylcholinesterase inhibitors) and neonicotinoids (binds nicotinic acetylcholine receptors). Neonicotinoids are very effective against hemiptera species (Ștef et al., 2023; Lin et al., 2021). In 1990, the first neonicotide (imidacloprid) was introduced to the market, since then other types have been synthesized: thiamethoxam, acetamiprid, thiacloprid, clothianidin, dinotefuran, nitenpyram, sulfoxaflor, flurofuranone and triflumezopyrim (Matsuda et al., 2020). Even if *Myzus persicae* exerts a certain level of resistance to some neonicotinoids, some chemical compounds still have a high potential to be used in the control of the "target" species, so the application in doses lower than those approved and the study of the efficacies must continue. According to the studies published by Sabra et al. (2023), the population of *Myzus persicae* was significantly reduced by the use of new insecticides: flonicamid (chordotonal organ nicotinamidase inhibitors), spirotetramat (acetyl COA carboxylase inhibitors) and Afidopyropen (chordotonal organ TRPV channel modulators). Reducing pest populations by biological means is an alternative to chemical control (Grozea et al., 2016; Barratt et al., 2018, Marcu et al., 2020; Costea et al., 2023; Georgescu et al., 2023), involving the use of living organisms belonging to various kingdoms and phyla, including fungi, bacteria and viruses, nematodes and insects (parasitoids and predators). As biological control of the green peach aphid 150 predators, 50 parasitoids and 40 entomopathogens (Ali et al., 2023) including potassium salts of fatty acids (insecticidal soaps) (Oprisiu et al., 2023) have been reported.

Insecticides based on potassium salts of fatty acids are used against aphids, thrips (Wafula et al., 2017), flies, mites, etc. (https://blogs.k-state.edu).Their effectiveness is higher when applied to young life stages (nymphs, larvae).

The aim of the present study was to control the population of *Myzus persicae* (Sulzer), in the potato agroecosystem, using chemical insecticides (acetamiprid - applied in doses lower than those approved) and of biological products (based on *Bacillus thuringiensis* and based on potassium salts). We believe that the present study is important, in the context in which, at the level of the European Union, the reduction of the doses of pesticides applied and the withdrawal of some neonicotinoid substances are being discussed more and more frequently.

MATERIALS AND METHODS

Site location

The experimental plots in which the chemical and biological products were applied, in order to control the population of *Myzus persicae*, were located in the western part of Romania (Timiș County), in the localities of Beregsău Mic - Săcălaz Commune (45.748180, 20.986419) and Vizejdia - Tomnatic commune (45.960329, 20.645269) (Figure 3).

Figure 3. Trial location (https://www.google.com/maps)

Experimental design and Efficacy Assessments

The experimental plots (Figure 4), in which chemical and biological insecticides were applied, were arranged according to the Randomized Complete Block (RCB) with 4 replications for each treatment. The size of the variants was 30 m^2/plot. The potato variety used in both experimental lots was Actrice. The experimental field, located in Beregsău Mic, included five treatments: untreated (T1), acetamiprid 0.13 l/ha (T2); acetamiprid 0.0975 l/ha (T3); acetamiprid 0.078 l/ha (T4); thiacloprid (Calypso – T5) 0.015 l/ha. Thiacloprid was chosen as the reference product for acetamiprid. The first assessment of the population of *Myzus persicae* was performed on the day of application of the chemical treatments (19.05.2022).

The effectiveness of chemical treatments was assessed counting the number of larvae and adults/25 leaves (Figure 5).

The assessments were made on the diagonal of the plot, on the same leaf floor. Evaluations were made at 3 DAA, 6 DAA, 14 DAA, 21 DAA, 28 DAA.

The trial regarding the biological control of the *Myzus persicae* species was located in Vizejdia – Tomnatic and included four treatments: untreated (T6), Kabon (50% potassium salt from vegetable oil extract) 0.900 l/ha (T7); Bitoxybacillin (*Bacillus thuringiensis* $1.0x10^{9}$ CFU/cm^3) 1,0 l/ha (T8); Konia K Plus 0.900 l/ha (fat acids potassium salt) (T9).

Figure 4. Aspects regarding the experimental fields (photo Cărăbeț, 2022)

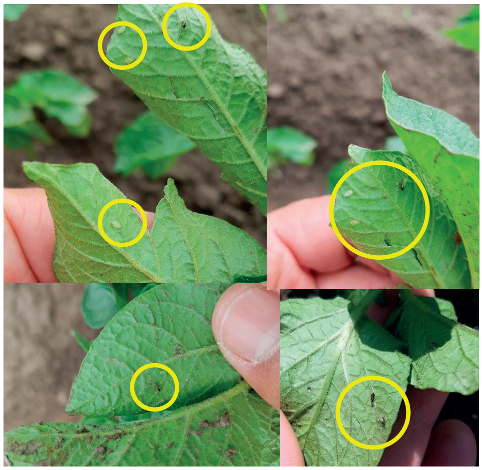

Figure 5. Assessing the effectiveness of treatments by counting the number of larvae and adults of *Myzus persicae* (Photo Cărăbeț, 2022)

Biological treatments were applied in BBCH 22-23 (potato plants had 2 lateral shoots - 5 cm) and BBCH 32-33 (20% plants closes the row).

The effectiveness of biological products was determined at: 5 and 10 DAA (from the second application), by analysing 10 plants diagonally. The value 0, in the statistical analysis of the results, represents the population level from the second application day (BBCH 32-33), which overlapped 10 days after the first application.

Statistical analysis

The statistical analysis was performed with the help of the statistical software IBM-SPSS 18. Elements of descriptive statistics were calculated (mean, standard deviation, minimum value and maximum value). The data were analysed by applying the ANOVA test and LSD post-hoc tests (least significant difference) at 5% significance level. The data were graphically represented by Box-plot diagrams and by representing the averages and the confidence intervals for the averages (Chiș, 2011).

RESULTS AND DISCUSSIONS

The results of variance analysis (Table 1) show us that both treatments timing had a real distinctly and significant influence on the number of adults and larvae during the study under homogenic condition across replicates. In respect of adults number, a significant higher variations from one assessment to another, compared to the variation of different treatments. The dose of the treatment exerted a highest contribution to the variability of the number of larvae of *Myzus persicae*, significantly superior to the effect of the period. Likewise, the interaction between the two factors showed significant influences on the variation in the number of larvae, but considerably less than the separate effects of the factors.

Table 1. ANOVA for adults number and larva number on surface of *Myzus persicae* plants

Source of variation	DF	Adults number		Larva number	
		MS	F value	MS	F value
Period	5	25.411	211.59***	9.256	38.563***
Treatment	4	3.744	31.17***	11.145	46.434***
Period x Treatment	20	0.329	2.73***	1.518	6.326***
Error	90	0.120		0.240	

*Significant at p<0.05; **Significant at p<0.01; ***Significant at p<0.001

In order to analyse the effectiveness of the treatments, at al six assessments the average values of the number of adults and the standard deviations were calculated, based on the experimental data (Table 2).

Table 2. Variation of adults number/25 plant leaves under the effect of different treatment during different period of evaluation

Treatment	Days after treatments: 0	3	6	14	21	28	Treatment mean
Control I (T1)	0.62±0.91a	1.21±1.22a	1.18±1.78a	2.76±2.27a	3.84±2.44a	4.00±3.34a	2.26±2.52X
0.13 L/ha Acetamiprid (T2)	0.60±1.21a	0.58±0.84b	0.37±0.79bc	1.55±1.53b	2.00±2.01c	2.05±2.73b	1.19±2.79Z
0.0975 L/ha Acetamiprid (T3)	0.61±1.14a	0.54±0.78b	0.50±0.83bc	2.19±2.17ab	2.67±2.77bc	2.77±3.08b	1.54±2.25Y
0.078 L/ha Acetamiprid (T4)	0.63±1.32a	0.52±0.85b	0.79±1.30ab	2.42±2.25a	2.95±2.42ab	2.99±2.81ab	1.71±2.23Y
0.015 L/ha Calypso (T5)	0.63±1.13a	0.53±0.87b	0.34±0.62c	2.20±1.99ab	2.65±2.34bc	2.78±2.89b	1.52±2.10Y
Period mean	0.62±1.15A	0.68±0.96A	0.64±1.19A	*2.22±2.09B*	2.82±2.47C	2.92±3.03C	1.64±1.15

Values represents mean ± SD. Values with different letters (a, b, c) in the column indicate a significant variation at p<0.05. For comparisons of period's means (A, B, C) and treatment's means (X, Y, Z) capital letters were used.

At spraying of the chemical treatments, there were no significant differences between the number of adults, among variants studied, the average values laying between 0.60 adults/25 leaves and 0.63 adults/25 leaves.

In the control untreated variant, the minimum average number of adults was 0.62 adults/25 leaves, at the beginning, and the maximum was 2.29 adults/25 leaves, at 28 DAA. Table 2 reveals that the minimum level of *Myzus persicae* population was recorded in the plot treated with acetamiprid 0.13 l/ha (T2) (the average number being 1.19 adults/25 leaves), and the maximum in the untreated (with an average number of 2.26 adults/25 leaves). By calculating the average number of adults, recorded in the 28 days (DAT – days after treatment), we observed that the chemical treatments significantly reduced the population of *Myzus persicae* compared to the untreated variant (T1). The number of adults (1.71 adults/25 leaves) recorded in the variant treated with acetamiprid 0.078 l/ha (T4) exerted significant differences compared to the population in the variants in which acetamiprid 0.13 l/ha was applied (T2 – 1.19 adults/25 leaves); acetamiprid 0.0975 l/ha (T3 – 1.54 adults/25 leaves) and thiacloprid 0.015 l/ha (Calypso - T5 - 1.52 adults/25 leaves). The first assessment, performed at 3 DAT, features the fact that all chemical treatments significantly reduced the adult population of *Myzus persicae* compared to the untreated control, the minimum average of 0.52 adults/25 leaves being recorded in the plot treated with acetamiprid 0.13 l/ha (T2), and the maximum average of 1.21 adults/25 leaves in the untreated plot.

The insecticides efficacy, expressed by the number of adults, at 6 DAA, from the variants treated with acetamiprid 0.13 l/ha (T2 – 0.37 adults/25 leaves) and thiacloprid (T5 – 0.34 adults/leaves) was superior to the other treatments (acetamiprid 0.0975 l/ha and acetmiprid 0.078 l/ha), the differences being significant. Chemical treatments, at 6 DAT, significantly reduced the population of *Myzus persicae* compared to the untreated. No differences were obtained between the variant in which acetamiprid was applied at a dose of 0.078 l/ha and the control (untreated). The III assessment, carried out at 14 DAA, highlights an increase in the population of *Myzus persicae*, possibly due to the overlap of generations. The average number of adults ranged between 1.55 (T2 – acetamiprid 0.13 l/ha) – 2.76 (T1 – untreated control), the differences between these two variants being significant (Table 2). In variants T3, T4 and T5, the number of adults was between 2.19 and 2.42.

The population growth trend was also observed at 21 DAA, in all experimental variants. However, the variance analysis shows that the applied insecticides significantly reduced the *Myzus persicae* species, from the potato agroecosystem, the results being significant compared to the control. Acetamiprid applied at a dose of 0.078 l/ha failed to significantly reduce the pest population compared to the untreated control.

At the last assessment, performed at 28 DAA, the minimum average of 2.05 adults/25 leaves was reached at T2 (acetamiprid 0.13 l/ha) and the maximum of 4 adults/25 leaves in the control group. The results from the variants treated with: acetamiprid 0.13 l/ha; acetamiprid 0.0975 l/ha; thiacloprid 0.015 l/ha exerts significant differences compared to the control. Acetamiprid 0.078 l/ha did not provide significant protection to potato plants against *Myzus persicae*, the results showing no significant differences compared to the untreated variant (Table 2). During the study, in the case of the control variant, the average number of adults varied from 0.62, at the beginning, to 4 adults, at 28 days, the significant increase being recorded from 6 to 14 days, with insignificant variations at 21 and 28 DAA (Figure 6).

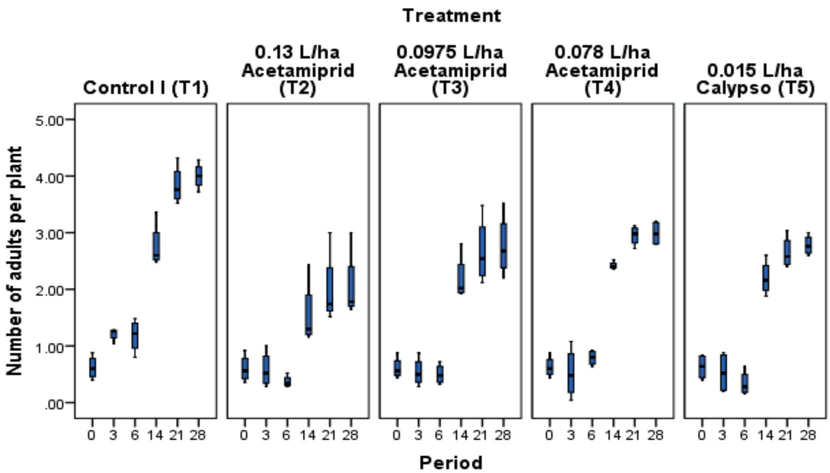

Figure 6. Boxplots of *Myzus persicae* number/25 leaves for different treatments

In the variants treated with acetamiprid 0.13 l/ha, the best efficacy in reducing the number of *Myzus persicae* adults was obtained at 6 DAA (0.37 adults). The result showing significant differences compared to the values recorded at 14, 21 and 28 DAA (Figure 6).

In the case of the variant treated with 0.0975 L/ha acetamiprid (T3), the average number of adults showed significant differences from 6 DAA to 14 DAA, registering an increase from 0.54 adults/25 leaves to 2.19 adults/25 leaves, the more specimens of *Myzus persicae* (2.77 adults) were observed at 28 DAA (Figure 6).

Acetamiprid at a dose of 0.078 L/ha (T4) protected the potato crop for 6 DAA after this interval the population of *Myzus persicae* started to increase, tripling (0.79 adults – 2.42 adults) from 6 days at 14 days (Figure 6).

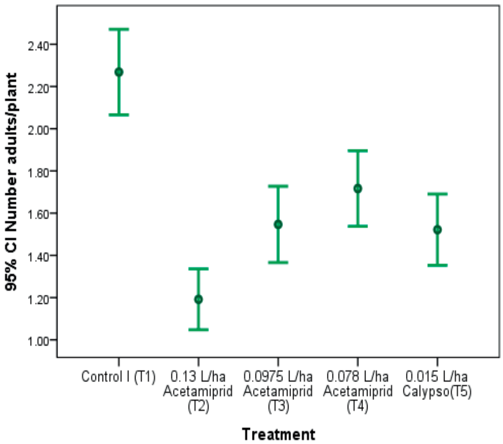

Figure 7. Mean and 95% CI for number of adults/25 leaves for different treatments

In the case of the 5 variants, significant differences were obtained between the number of adults as follows: between the control variant T1 and all the other variants, respectively between the variants treated with acetamiprid 0.13 l/ha and the variants treated with acetamiprid 0.0975 l/ha, acetamiprid 0.078 l/ha, thiacloprid 0.015 l/ha (Calypso) ($F=2.981$, $p=0.022$) (Table 2, Figure 7).

Analysing the number of larvae recorded in the experimental variants, the averages and standard deviations were calculated for each experimental variant, for each evaluation (3 DAA, 6 DAA, 14 DAA, 21 DAA, 28 DAA).

At the time of chemical treatments, the number of larvae in the experimental variants did not register significant differences, the minimum average being 2.11 larvae/25 leaves and the maximum 2.24 larvae/25 leaves. At 3 DAA, significant differences were observed between the average number of larvae in the case of the control group (3.67 larvae/25 leaves) and all other groups. Calypso insecticide 0.015 l/ha reduced the number of larvae to 0.28/25 leaves (Table 3).

From Table 3 it can be observed that at 6 DAA the significant differences are maintained as in the case of the previous assessment, the average maximum number of larvae, 3.26 larvae/25 leaves, being observed in the case of the control variant and the minimum in the variant treated with Calypso, of 0.15 larvae /25 leaves.

At the third evaluation, at 14 DAA, no significant differences were observed between the 5 experimental variants in terms of the average number of larvae.

Tabel 3. Variation for number of larva/25 leaves under the effect of different treatment during different period of evaluation

	Days after treatments						Treatment mean
Treatment	**0**	**3**	**6**	**14**	**21**	**28**	
Control I(T1)	2.14±0.47a	3.67±1.30a	3.26±1.31a	2.46±0.50a	3.47±0.42a	3.15±0.46a	3.03±0.93Z
0.13 L/ha Acetamiprid (T2)	2.24±0.59a	0.42±0.24b	0.3±0.05b	1.8±0.25a	1.78±0.38b	1.75±0.36b	1.38±0.82X
0.0975 L/ha Acetamiprid (T3)	2.15±0.39a	0.34±0.14b	0.39±0.15b	1.97±0.21a	2.26±0.31bc	2.16±0.31bc	1.55±0.89XY
0.078 L/ha Acetamiprid (T4)	2.19±0.37a	0.36±0.32b	0.6±0.18b	2.1±0.11a	2.56±0.21c	2.49±0.26c	1.72±0.93Y
0.015 L/ha Calypso (T5)	2.11±0.36a	0.28±0.14b	0.15±0.06b	1.57±0.77a	2.27±0.61bc	2.4±0.52c	1.46±1.03XY
Period mean	2.16±0.40B	1.01±1.46A	0.94±1.31A	1.98±0.50B	2.46±0.68C	2.39±0.58BC	1.83±1.10

Values represents mean ± SD. Values with different letters (a, b, c) in the column indicate a significant variation at $p<0.05$. For comparisons of period's means (A, B, C) and treatment's means (X, Y) capital letters were used.

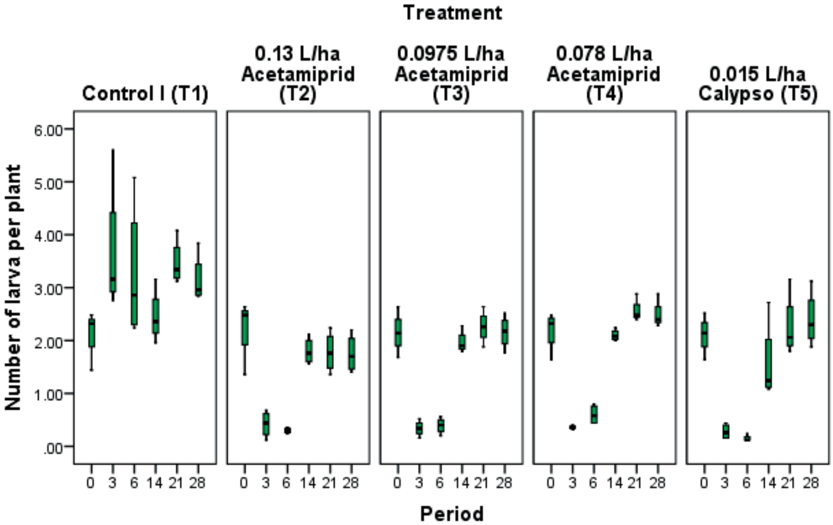

Figure 8. Boxplots of *Myzus persicae* number larvae/25 leaves for different treatments

At 21 DAA, the minimum average of 1.78 larvae was recorded in the variant treated with 0.015 L/ha Calypso and the maximum of 3.47 larvae in the control variant.

The last assessment, the one from 28 DAA, showed that the number of larvae in the experimental variants presents significant differences, and the minimum value (1.75 larvae/25 leaves) was established in the variant treated with 0.13 L/ha acetamiprid and the maximum (3.15 larvae/25 leaves) in the untreated version (Table 3, Figure 8).

In the case of the five variants, significant differences were obtained: between the control and all the other variants, respectively between acetamiprid 0.13 l/ha and acetamiprid 0.078 l/ha (Table 3). In the case of biological insecticides, it was found that both the period and the treatment had a distinctly significant influence on the number of aphids, throughout the study, in conditions of homogeneity between repelicates. The biological treatments showed the highest contribution to the variability of the number of aphids, significantly superior to the effect of the period. Likewise, the interaction between the two factors showed significant influences on the variation in the number of aphids, but considerably less than the separate effects of the factors (Table 4). Analysing the number of aphids in the variants treated with biological preparations, it was found that there are significant differences, depending on the treatment applied at 5 days, the minimum average, 1.85 aphids/10 plants, was recorded in the variant treated with Konia K Plus and the maximum in untreated control variant (6,275 aphids/10 plants). Bitoxybacillin and Konia K Plus did not lead to significant differences for the average amount of adults/plant.

Tabel 4. ANOVA for adults number on surface of 25 plants

Source of variation	DF	Adults number	
		MS	F value
Period	2	5.2589	38.93***
Treatment	3	45.9541	340.22***
Period x Treatment	6	3.3773	3.37***
Error	36	0.1350	

*Significant at $p<0.05$; ** Significant at $p<0.01$;
*** Significant at $p<0.001$

The maximum control of the *Myzus persicae* population at 10 DAA was recorded in the plots treated with Konia K plus (2.37 aphids/10 plants) (Table 5). The most abundant population was recorded, at 10 DAA, in the untreated variant (7.20 aphids/10 plants. The efficiencies recorded in the variants treated with Kabon and Bitoxybacillin showed significant differences in the number of aphids/10 plants, only in relation to the control variant (Table 5 and Figure 9).

Throughout the evaluation period, significant differences were recorded between all biological treatments, the minimum number (1.91 aphids/10 plants) being obtained in the case of the plot treated with Konia K Plus, and the maximum in the untreated (6,225 aphids/ 10 plants) (Table 5). The maximum effectiveness of biopreparations was recorded at 5 DAA. Comparing the results between the two trials, it was found that there are significant differences between the number of individuals registered in the case of applying the treatments Calypso 0.015 l/ha, Kabon 0.9 l/ha, Bitoxibacillin 1.0 l/ha, Konia K Plus 0.9 l/ha (F=8.707, p=0.000).

Tabel 5. Variation of aphids number /10 plants under the effect of different treatment during different assessments time

Treatment	Days after treatments			Treatment mean
	0	5	10	
Control II (T6)	5.20±0.35a	6.275±0.50a	7.20±0.32a	6.225±0.9W
KABON(T7)	2.65±0.26b	2.85±0.34b	3.175±0.46b	2.90±0.40X
BITOXIBACILIN(T8)	1.95±0.34c	2.10±0.35c	3.10±0.39b	2.38±0.62Y
KONIA K PLUS(T9)	1.50±0.29c	1.85±0.35c	2.37±0.39c	1.91±0.49Z
Period mean	2.83±1.50A	3.26875±1.87B	3.96±1.99B	3.35±1.82

Values represents mean ± SD. Values with different letters (a, b, c) in the column indicate a significant variation at p<0.05. For comparisons of period's means (A, B, C) and treatment's means (W, X, Y, Z) capital letters were used.

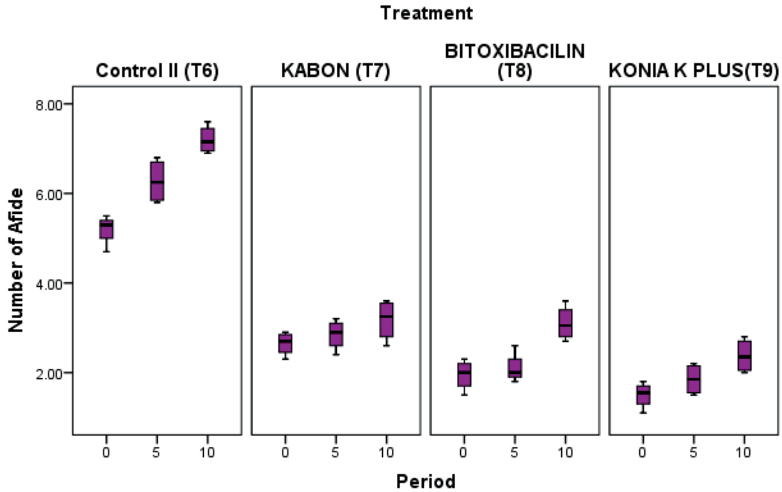

Figure 9. Boxplots of *Myzus persicae* aphids/10 plants for different treatments

Tabel 6. Mean difference of *Myzus persicae* between Calypso and biological treatments

Dependent Variable: Number of adults/plant					
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
0.015 L/ha Calypso (T5)	Kabon (T7)	-1.3700***	0.0000	-2.1202	-0.6198
	Bitoxibacilin (T8)	-0.8616*	0.0180	-1.6118	-0.1115
	Konia K Plus (T9)	-0.3866	0.5260	-1.1368	0.3635
*The mean difference is significant at the 0.05 level.					

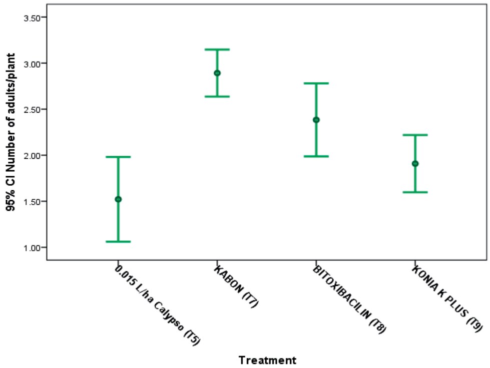

Figure 10. Mean and 95% CI for number of aphids for treatments T5, T7, T8, T9

The statistical analysis between the nine variants (found in the two fields) shows that the results regarding the reduction of the population of *Myzus persicae* in the variant treated with Calypso 0.015 l/ha are superior to those obtained in the variants treated with Kabon (T7) (p=0.000) and Bitoxybacillin (T8) (p=0.0180) (Table 6, Figure 10).
The Tukey test shows that there are no significant differences, regarding the control of the *Myzus persicae* population, between acetamiprid 0.0975 l/ha, acetamiprid 0.078 l/ha, thiacloprid 0.015 l/ha and Konia K Plus 0.9 l/ha (Tukey test, p=0.062) (Figure 11).
The results obtained are consistent with those published by Oprișiu et al. (2023), Wafula et al. (2017), Mohamad et al. (2013), Dheeraj et al. (2013), Liu et al. (2000).

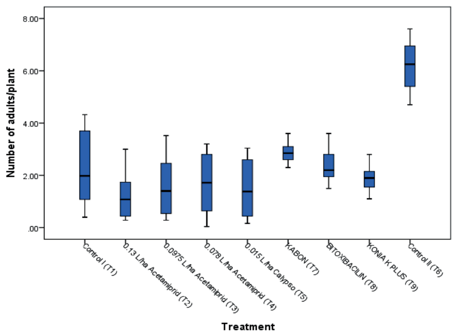

Figure 11. Comparison of the population level of *Myzus persicae* from all experimental variants

Comparing the results between the two fields, significant differences were found in the number of individuals recorded in the case of applying the treatments Calypso 0.015 l/ha, Kabon 0.9 l/ha, Bitoxibacillin 1.0 l/ha, Konia K Plus 0.9 l /ha (F=8.707, p=0.000).
Statistical analysis among the nine variants (which are present in the two trials) highlights that the results regarding the reduction of the population of *Myzus persicae* in the variant treated with Calypso 0.015 l/ha are superior to those obtained in the variants treated with Kabon (T7) (p=0.000) and Bitoxybacillin (T8) (p=0.0180) (Table 6, Figure 10).

CONCLUSIONS

Acetamiprid applied at a dose of 0.13 l/ha had the best effectiveness in reducing the population of *Myzus persicae*, during the entire observation period, the maximum protection of the potato crop was at 6 DAA.
Thiacloprid 0.015 l/ha came in second place, this product causing high mortality of green peach aphid in the first 6 DAA, after which the effectiveness decreased.
The effectiveness recorded, at 28 DAA, in the variants where acetamiprid was applied at a dose of 0.975 l/ha was similar to the Calypso product 0.015 l/ha.
Reducing the dose of acetamiprid to 0.078 l/ha led to poor management of the *Myzus persicae* species.
The biopreparations used in the control of the *Myzus persicae* species had high efficacy at 5 DAA.
The effectiveness of the biopreparation Konia K Plus is like that recorded in the variant treated with Calypso 0.015 l/ha.
The biopreparation Bitoxibacilin (1.0 l/ha) based on *Bacillus thuringiensis*, controls the *Myzus persicae* species well, but over a short period of time (5 days).

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EVALUATION OF SOME NUTRITIONAL COMPOUNDS OF GARLIC *(ALLIUM SATIVUM* L.) PEEL WASTE

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Abstract

Garlic (Allium sativum L.) is known as a valuable spice and common medicine for various diseases. The bulb of garlic is a rich source of nutritional compounds (carbohydrates, proteins, minerals, fibre), vitamin and other biologically active natural compounds, with beneficial effects on the body's health. The skins obtained when peeling the garlic bulb also contain important amounts of proteins, fibres, minerals, carbohydrates and essential phytochemicals. The purpose of this work is to evaluate the content of nutritional compounds from the peel of garlic bulbs sold in local agri-food markets. The obtained results show that the analysed garlic peel contains important amounts of nutritional compounds whose value varies depending on the origin of the garlic: 4.21-5.36% moisture, 15.18-16.53% minerals, 7.38-8.11 protein, 58.77-63.46% fibres, 20.56-21.64%, 0.52-0.78% fat. The preliminary results suggest that the investigated garlic peel could be considered for obtaining products with additional content of nutritional compounds. Also, the superior valorization of garlic peels can be an ecological method of garlic peel waste resulting from peeling garlic bulbs.

Key words*: garlic****,*** *garlic peel, by-products, nutritional parameters.*

INTRODUCTION

Garlic (*Allium sativum* L.), belonging to the *Amaryllidaceae* family, is one of the most widespread aromatic food plants (Lasalvia et al., 2022). It is used to obtain traditional products such as stews, mayonnaise, sausages, ketchups and salads, but it can also be used for health purposes to reduce blood pressure, cholesterol and triglyceride levels in the blood, to control the aggregation of platelets; to inhibit cancer cells or as an antimicrobial agent (Prakash and Prasad, 2023; Essa et al., 2023).
Consumption of garlic and its supplements reduces the risk of diabetes and cardiovascular disease and strengthens the immune system with antibacterial, antifungal, anti-aging and anti-cancer properties (Sunanta et al., 2023; Verma et al., 2023); it was used as a spice, but also a remedy during various epidemics (dysentery, typhoid fever, cholera, flu) and other diseases (Yusuf et al., 2018). Due to its biologically active component, allicin and its derivatives, garlic has long been used as a medicine to prevent and treat a variety of diseases and disorders, including high blood pressure, high cholesterol, coronary heart disease, and cancers such as the colon, rectal, stomach, breast, prostate and bladder cancers, as well as lung cancer and cardiovascular diseases such as antilipemic, hypotensive, enlarged prostate, diabetes, osteoarthritis, allergic rhinitis), traveler's diarrhea, preeclampsia, in the treatment of some epidemics (dysentery, typhoid fever, cholera, influenza) (Azmat et al., 2023; Verma et al., 2023; Sunanta et al., 2023; Prakash & Prasad, 2023; Prakash, et al., 2023; Essa et al., 2023; Lyngdoh and Ray, 2022; Tosin et al., 2017; Saif et al., 2020; Martins et al., 2016; Tesfaye, 2021). Analyzing seven varieties of garlic, Prakash and Prasad observed that they contained variable amounts of moisture (64.16-74.57%), crude protein (4.23-7.39%), crude fat (0.18-0.91%), ash (0.92-1.7 1%), carbohydrate (18.94-29.18%), crude fiber (0.58-0.84%), total soluble solids (28.93-37.93^{0}Brix), acidity (0.73-1.24%), and pH = 6.08-6.77 (Prakash and

Prasad, 2023). The pungency factor as pyruvic acid was found to be in the range of 34.84 - 86.69 µmol/mL, whereas anti-nutritional factors such as saponin (6.63-13.98 g), phytic acid (0.03-0.6 g), and tannin (0.18-0.39 g CE) per 100 g on a fresh weight basis were present (Prakash & Prasad, 2023).

The chemical composition and content of bioactive compounds in garlic products varies with genotype, cultivation practices, growing conditions, plant density, soil type, fertilizer application rate, and processing method of these products (Chen et al., 2019).

Garlic peel is one of the crucial unit operations for the use of garlic at an industrial scale. It has been reported that this by-product contains significant amounts of nutritional compounds and phytochemicals (dos Santos et al., 2022; Azmat, et al., 2023). The quantities and qualities of the compounds included in the composition of garlic peel give this by-product numerous biological properties, such as: antioxidant, anticancer, antidiabetic, antimicrobial, antiviral, anti-inflammatory, cardioprotective, neuroprotective, hepatoprotective which have allowed its use to treat and to prevent various diseases due to its medicinal properties (Azmat et al., 2023). Garlic peels is rich in cellulose content (Prakash et al., 2023). In addition, it contains important quantities of functional ingredients and can be used as a functional material for the production of high-added value products (Kallel et al., 2017; Kim et al., 2010). Garlic peel is also known to have excellent physiological activity and is consumed at home in the form of broth, tea, etc. (Kim et al., 2010). According to Kallel et al., the skin resulting from the peeling of garlic bulbs, initially considered as garlic waste, is an inedible by-product of garlic that has a moisture content of 3.52%, a high protein content of 8.43%, a low content of lipids of 0.86% and a high total fibre content of 62.23% (Kallel et al., 2017). Zhivkova, was found that garlic wasted peels were characterized by the highest content of total dietary fibre (62.10%), total sugars (6.51%), dry matter (80.8%), total ash (7.37%), (Zhivkova, 2021). In addition, the garlic peel also contains 8.5% digestible carbohydrates, 1.36% reducing sugars, 2.61% crude protein and 0.22% free fat (Zhivkova, 2017). Analyzing quality characteristics of garlic peel, Min et al., have identified the following contents of chemical compounds: 10.97 g/100 g moisture, 3.39 g/100 g crude protein, 0.56±g/100 g crude fat, 5.83/100 g crude ash, 79.25 g/100 g carbohydrate, 6.25% total pyruvate, 57.77 mg/100 g total polyphenol, 3.68mg/100g total flavonoid, 57.45% DPPH radical scavenging ability (Min et al., 2020). Experimental studies of Lyngdoh and Ray showed that garlic peel was a very rich source of ash, crude fibre and protein content: 16.34% ash, 21.71% carbohydrate, 60.57 crude fibre, 0.83% fat, 3.36% moisture content and 8.24% protein (Lyngdoh and Ray, 2022). Data regarding moisture and mineral content (ash) from garlic peel, were also reported by Pathak et al.: 5.84% moisture and 8.47% ash content (Pathak et al., 2016). Also, in raw garlic peel powder, notable amounts of dry matter (13.35%), protein (1.30%), fat (1.14%) fat, ash (5.41%), pectin (16.71%), lignin (8.26%), cellulose (31.36%), hemicellulose (22.47%) and holocellulose (53.83%) (Prakash et al., 2023). Lyngdoh and Ray showed that garlic peel can be a very rich source of ash crude fibre, protein and antioxidants content and can also be used to fortify some food products in order to formulate functional foods (Lyngdoh and Ray, 2022). Furthermore, garlic peel it is a valuable source of phytochemicals and medicinal substances (Azmat et al., 2023), which have various beneficial effects including antioxidant activities, antithrombosis activities and cancer inhibition (Min et al. 2020).

Due to the high protein content garlic peels are use as animal feed additives (Chen et al., 2019). Garlic peels have a high content of polyphenols and fibers and have antibacterial activity similar to garlic, so they can be fed to animals and in some cases, they can be used as an alternative to antibiotics (Kim et al., 2010). Recently, the garlic by-products as alternatives to antibiotics, have been used to manipulate the rumen ecology to achieve better growth performance, reduce methane emissions, and improve the quality of ruminant products and the control of parasite infections (Ding et al.,2023). According to reports garlic peel extracts exhibit significant antioxidant activity and can be used as natural antioxidant in nutraceutical preparations to prevent human diseases. (Mounithaa et al., 2023; Kim et al., 2016). Garlic peel extract contains phenolic compounds, as does the bulb (dos Santos et al., 2022). Garlic peel extracts have been reported to possess antifungal effects and antiviral properties, such that they could be used in the management of drug-resistant microbial diseases (Azmat et al., 2023). Based on the evidence provided by the above studies, there could be the possibility of using garlic peel extract as a natural food additive or even as a functional food. The evidence provided by studies on the nutritional composition of garlic peels shows that this product resulting from peeling garlic bulbs contains important amounts of nutritional compounds, especially carbohydrates, fibres, minerals and proteins. Therefore, the problem arises of using this by-product as a potential alternative food source in the creation of dietary supplements or functional meals. The purpose of this paper is to evaluate the content of nutritional compounds in the peel of garlic bulbs sold by domestic producers on the agri-food markets in Timișoara (Romania) in view of the possibility of using them as additional nutritional sources in obtaining products with added food value.

MATERIALS AND METHODS

Plant material consisted of garlic peels (GPs) obtained by peeling the bulbs of white garlic (*Allium sativum*) taken from three domestic producers from different agro-food markets in Timisoara (Figure 1).

Figure 1. The bulbs and cloves of white garlic

In the context of the present study, the outer and inner peels resulting from the peeling of bulbs and cloves were considered as garlic peel waste. Three batches of average samples of garlic bulbs marked P1, P2 and P3 were formed, corresponding to the three providers from which they were collected. Peeling was done manually, and the obtained peels were washed in running tap water first, then in distilled water, to remove adhering debris and dried in an oven at 60^0C for 10 hours (Figure 2).

Figure 2. The peels of white garlic

The dried garlic peels, three samples for each batch were then ground using a kitchen grinder (Figure 3) and used to determine the concentrations of the nutritional parameters of interest: moisture, minerals (ash), protein, fat, fibre, and carbohydrate. Until the time of analysis, the garlic peel powder samples were kept in polyethylene ampoules in the refrigerator, at 4^0C.

Figure 3. Peels and powder of white garlic

The determination of the nutritional composition of the powder of GPs studied was performed according to AOAC Official Methods of Analysis, 2000 (AOAC, 2000) and according to the recommendations of Velciov et al. and Satti et al. (Velciov et al., 2022; Satti, et al., 2018). For moisture content, dried garlic peel powder was dried in an oven at 105 ^{0}C to constant mass (Velciov et al., 2022). The ash content was determined by the calcination method at 550°C (Velciov et al., 2022). The protein content was determined by the Kjeldahl method, using a conversion factor for nitrogen of 6.25. (Velciov et al., 2022; Satti, et al., 2018). The crude fat was determined using the Soxhlet method with hexane as solvent (Velciov et al., 2022; Satti et al., 2018). Crude fibers were determined by using the method of acid - base digestion (Satti et al., 2018). The carbohydrate content was obtained by difference (Velciov et al., 2022). All the data was statistically analyzed for variance (ANOVA) using Statistica 10. The comparisons for means were done using Duncan's Multiple Range Tests (DMRT). Duncan's Multiple Range Tests or Duncan's New Multiple Range Tests provide significance levels for the difference between any pair of means, regardless of whether a significant F resulted from an initial analysis of variance. The Shapiro-Wilk test was used to assess the normality of the data (Ghosh & Mitra, 2020).

RESULTS AND DISCUSSIONS

The experimental results obtained for the determination of moisture, minerals (ash), protein, fibres and carbohydrates from GPs are presented in table 1.

The results obtained, based on the official methods of analysis, show that the analysed GPs contains significant amounts of nutritional compounds whose value varies depending on the origin of the garlic: 4.21-5.36% moisture, 15.18-16.53% ash (minerals), 7.38-8.11 protein, 0.52-0.78% fats, 58.77-63.46% fibres, 20.56-21.64%.

The moisture content of the product provides information on the amount of water contained in a certain product, respectively the amount of dry substance of the respective product. Furthermore, the moisture is important for food preservation and in food processing therefore (Velciov et al., 2022).

It has been established from various researches that foods with low moisture content (especially those with less than 10%) have a longer shelf life with limited deterioration of quality due to microbial activities; those with a moisture content of more than 10% cannot be stored for a long time (Tosin et al., 2017).

As can be seen from Table 1 and Figure 4 the GPs present low amounts of moisture, between 4.21% (GP1)-5.34% (GP2). The highest value has been determined in GP2 (5.36±0.34%): lower values were identified in GP3 and GP1 (4.78±0.33, respectively (4.21±0.23%). In this order, the dry matter content increases. Having a water content of less than 10%, the GPs obtained and analyzed can be kept for a longer period of time.

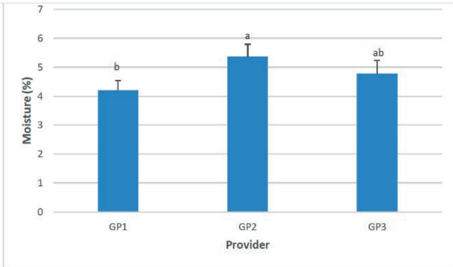

Figure 4. The moisture content in garlic peel

The assumption of normality moisture content distributions was assessed by using the Shapiro-Wilks test. Results indicated that the moisture contents are normally distributed (for GP1: W= 0.987, p=0.78; for GP2: W=0.987, p=0.78; for GP3: W=1, p=1). A one-way ANOVA was performed to compare the effect of the three different providers on moisture contents. It revealed that there was a statistically significant difference in mean moisture contents between at least two groups (F = 6.001, p = 0.037). Duncan's MRT for multiple comparisons found that the mean value of moisture content was significantly different between provider 1 and provider 2 (p = 0.0156, 95% C.I. = [0.30, 2.00]). There was no statistically significant difference in mean moisture contents between provider 1 and provider 3 (p=0.136) or between provider 2 and provider 3 (p=0.131). GPs humidity values determined in this experiment are comparable to those determined by Pathak et al., and Ifesan et al.: 5.84, respectively 5.50% (Pathak et al., 2016; Ifesan et al., 2014). Higher values have been reported by Hagag et al., Ding et al., Min et al., Prakash et al., and Zirkova et al.: 9.44, 8.2-9.4, 10.97, 13.35, respectively 19.2% (Hagag et al., 2023; Ding et al., 2023; Min et al., 2020, Prakash et al., 2023; Zirkova et al., 2021); Kallel et al., and Lyngdoh et al. they determined small values: 3.52, respectively 3.36% (Kallel et al., 2017; Lyngdoh et al., 2011).

Table 1. The nutritional parameters of garlic peel (mean values, reported to dry matter)

Specification	Nutritional values (%)*					
	Moisture	Ash	Protein	Fats	Fibres	Carbohydrates
Provider 1 (GP1)	4.21±0.23b	16.53±0.63a	7.38±0.20a	0.52±0.07b	58.77±1.61b	21.64±0.82a
Provider 2 (GP2)	5.36±0.34a	15.18±0.74a	8.11±0.39a	0.78±0.10a	63.46±1.83a	20.56±0.98a
Provider 3 (GP3)	4.78±0.33ab	16.22±0.71a	7.63±0.37a	0.60±0.04ab	61.86±1.31ab	21.24±0.92a

Ash content indicates the amount of inorganic matter and oxides present in the sample, respectively the sum of the mineral elements that are part of the analysed samples (Velciov et al., 2022). Minerals are indispensable components of our dietary intake, performing a diverse array of functions: serve as the fundamental elements for our skeletal structure, influence muscle and nerve activity and regulate the body's hydration balance, are integral parts of hormones, enzymes, and other biologically - active substances and some play a critical role in optimizing the functionality of the immune system (Stefanache et al., 2023). A high ash content is an indication of high inorganic mineral content (Tosin, 2017). According to Zhivkova et al., 2021 garlic peel contains important amounts of essential macro and microelements (mg/kg), as: Ca (20610), Ca, K (9081), S (635), Mg (950), Al (826), P (721), Fe (682), Na (123), Mn (35.4), Cr (18.40), B (18.0), Zn (12.9), Cu (2.09), Se (0.058) (Zhivkova et al., 2021).

The results obtained when determining the ash content (table 1 and figure 5) show that samples of GPs contain increased amounts of minerals between: 15.18-16.53%, depending on their provider.

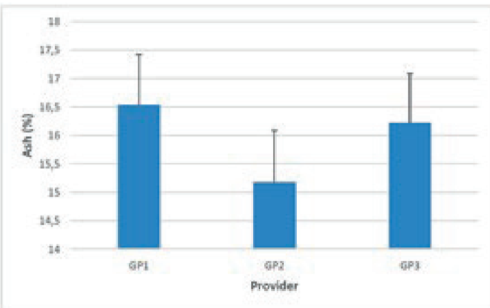

Figure 5. Ash (minerals) content in garlic peel

The richest in minerals are GPs from garlic bulbs P1 and P3, which contain amounts relatively close to ash: 16.53±0.63, respectively 16.53±0.63%. Slightly smaller amounts were determined in GP1 (15.18±0.74%).

The assumption of normality ash content distributions was assessed by using the Shapiro-Wilks test. Results indicated that the ash contents are normally distributed (for GP1: $W = 1$, $p=0.98$; for GP2: $W=0.996$, $p=0.87$; for GP3: $W=0.99$, $p=0.91$).

A one-way ANOVA was performed to compare the effect of the three different providers on ash contents. It revealed that there was not a statistically significant difference in mean ash contents between the groups ($F = 1.938$, $p = 0.224$). Experimental data (table 1) shows that the GPs analyzed contain high amounts of minerals and therefore they could be considered as mineralizing products. Therefore, GPs obtained from the peeling of garlic bulbs, after a preliminary processing (cleaning, drying, grinding, possibly extraction in hydroalcoholic solvents) could be used as a mineral additive to obtain food with the added value or in animal feed. This statement is supported by other studies (Lyngdoh et al., 2022; Ding et al., 2023).

Proteins are essential component of the diet needed for the survival of animals and human, they serve as source of nitrogen in the body system along with the amino acids; good skin, increase in growth and ability to replace the worn-out cells are the quality of protein in the body (Tosin et al., 2017). Protein deficiency is closely related to a number of diseases such as Kwashiorkor, Marasmus (energy deficiency), mental disorders, insufficiency of different organs, edema and immune system weakness and increased protein intake plays an important role in diets intended especially for athletes or diseases related to diabetes and the cardiovascular system (Velciov et al., 2022). There are 67 proteins in both the inner and outer garlic protein in peel (Azmat et al., 2023). The GPs analyzed in this experiment contain significant amounts of protein, the protein

content values being within relatively close limits: 7.38-8.11% (table 1 and figure 6). However, it can be observed that GP2 has a slightly increased content (8.11±0.39%), compared to GP1 and GP3 (7.38±0.20%, respectively 7.63±0.37%).
The assumption of normality protein content distributions was assessed by using the Shapiro-Wilks test. Results indicated that the protein contents are normally distributed (for GP1: W= 0.992, p=0.83; for GP2: W=0.857, p=0.25; for GP3: W=0.983, p=0.74).
A one-way ANOVA was performed to compare the effect of the three different providers on protein contents. It revealed that there was not a statistically significant difference in mean protein contents between the groups (F = 2.375, p = 0.174).

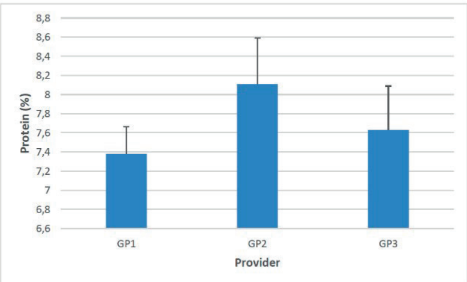

Figure 6. Protein content in garlic peel

These values are consistent with those obtained by Lyngdoh & Ray and Kallel et al.: 8.24, respectively 8.40% (Lyngdoh & Ray, 2022; Kallel et al., 2014); much higher values have been reported by Ding et al. and Hagag et al.: 13.10-13.65%, respectively 18.6% (Ding et al., 2923; Hagag, et al., 2023). Lower and very low concentrations have been reported by Prakash et al., Zhivkova, 2021, Min et al., Kim et al.: 1.3, 2.61, 3.39 and 2.61% (Prakash et al., 2023; Zhivkova, 2021; Min et al., 2020; Kim et al., 2010), respectively Nagorao and Ifesan et al.: 0.4 and 0.57% (Nagorao, 2014; Ifesan et al., 2014).
Fats are essential macronutrients that play an important physiological and biochemical role in the function of the human body, such as energy storage, structural components of biological membranes, electron carriers, enzyme cofactors light-absorbing pigments, hydrophobic anchors for proteins and emulsifying agents in the digestive tract (Baeza-Jiménez et al., 2017). Furthermore, lipids are also used as food ingredients, thus improving texture, taste and flavour of new formulations (Velciov et al., 2022). The analyzed GPs contain low amounts of total fats (Table 1 and Figure 7) the concentrations presenting close values included in concentration limits between: 0.52-0.78%.
The assumption of normality fat content distributions was assessed by using the Shapiro-Wilks test. Results indicated that the fat contents are normally distributed (for GP1: W= 0.993, p=0.84; for GP2: W=0.959, p=0.60; for GP3: W=0.997, p=0.90).
A one-way ANOVA was performed to compare the effect of the three different providers on fat contents. It revealed that there was not a statistically significant difference in mean fat contents between the groups (F = 5.107, p = 0.0507).

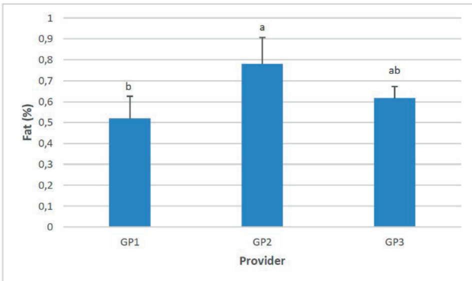

Figure 7. Fat content in garlic peel

These values are comparable to the values determined by Lyngdoh et al., Min et al. and Kallel et al.: 0.83, 0.56, and 0.86% (Lyngdoh et al., 2022; Min et al., 2020; Kallel et al., 2017). Higher values were determined by Prakash et al., and Kim et al.: 1.30%, respectively 1.26% (Prakash et al., 2023; Kim et al., 2010). Much lower and very low values of the fat content were reported by Zhivkova and Ifesan et al.: 0.22 and 0.05% (Zhivkova, 2022; Ifesan et al., 2014). Therefore, the GPs analyzed contain low amounts of lipids, a fact also recorded by Azmat et al., who reported that GPs have a high protein content and a low lipid content (Azmat et al., 2023). According to Lyngdoh et al., the incorporation of garlic peels exhibited increasing protein content but decreasing fat content in the valued-added product (Lyngdoh et al., 2022).

The term crude fibre refers to the insoluble dietary fibre fractions and comprises the constituents of the cell walls found in plant tissues, including lignin, cellulose and hemicellulose (Urban et al., 2023). Cellulose, hemicellulose and lignin are well known for water absorption and regulation of the intestinal tract, while pectin and gums are important in cholesterol reduction and glucose regulation (Velciov et al., 2022). Crude fibre helps maintain the normal peristaltic movement of the intestinal tract, therefore, aids in the digestion of food and can be widely used in the food industry when they are incorporated into many foods in order to enrich their nutritional and sensory properties (Velciov et al., 2022). The increased content in raw fibres is due to the increased content of cellulose and lignin from garlic peel. The components of crude fibre are as follows: 50-80% of the total cellulose, 10-15% lignin and only 20% hemicellulose (Urban et al., 2023). The values obtained when determining the fibre content of the investigated GPs batches show close values between 58.77-63.46% (Table 1 and Figure 8). However, comparing the fibre content values between the three batches of GP, it can be seen that batch GP2 is slightly richer in fibers (63.46±1.83), compared to batches GP1 and GP3 (58.77±1.61, respectively 61.86±1.31%).

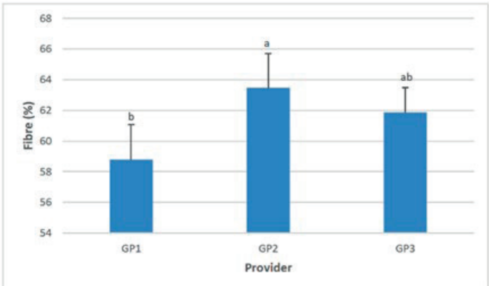

Figure 8. Fibre content in garlic peel

These values show that the garlic peel resulting as waste from the processing of garlic bulbs contains increased amounts of fibre. This statement is confirmed by some previous studies that showed that garlic peel, obtained as a by-product during the processing of garlic, after a preliminary processing, can be used as an additive in obtaining products with added value (Azmat et al., 2023; Lyngdoh and Ray, 2022).

The assumption of normality fibre content distributions was assessed by using the Shapiro-Wilks test. Results indicated that the fibre contents are normally distributed (for GP1: W= 1, p=0.96; for GP2: W=1, p=0.97; for GP3: W=0.999, p=0.95).

A one-way ANOVA was performed to compare the effect of the three different providers on fibre contents. It revealed that there was not a statistically significant difference in mean fibre contents between the groups (F = 3.96, p = 0.0801).

The values obtained when determining the fibre content of the analyzed GPs are close to those obtained by Lyngdoh and Ray., Zhivkova, Lyngdoh et al., and Ifesan et al.: 60.57, 62.1 and 63.23 - when analyzing similar products (Lyngdoh et al., 2022; Zhivkova, 2021; Kallel et al., 2014).

Carbohydrates are one of the three macronutrients in the human diet, along with protein and fat. They play an important role in the human body: act as an energy source, help control blood glucose and insulin metabolism, participate in cholesterol and triglyceride metabolism, and help with fermentation (Holesh et al., 2023). Although there are different types of carbohydrates, only total carbohydrates are taken into consideration in food and remains when the protein, fat, moisture and ash of the food have been removed (Velciov et al., 2022). The weight of the garlic peel is roughly 26.58% total carbs and 18.62% cellulose; it contains a variety of sugars, including rhamnose, trehalose, mannitol, and sorbitol (Azmat et al., 2023). As shown by the Table 1 and Figure 9, the GPs analyzed contain important amounts of carbohydrates, between close concentration limits: 20.56-21.64%. Slightly higher values, but very close, were determined in GP1 and GP3 (21.64±0.82, respectively 21.24±0.92%; compared to these, the GP2 samples contain slightly reduced amounts of carbohydrates (20.56±0.98%).

These values show that the GPs sample analyzed are rich in carbohydrates, a fact also recorded by Prakash et al., Min et al., and Ifesan et al., which, when analyzing similar products, determined the following values:

78.80, 79.78, and 93.26% (Prakash et al., 2023; Min et al., 2020; Ifesan et al., 2014).

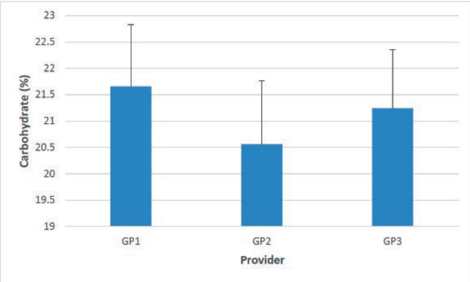

Figure 9. Carbohydrate content in garlic peel

The assumption of normality carbohydrate content distributions was assessed by using the Shapiro-Wilks test. Results indicated that the carbohydrate contents are normally distributed (for GP1: W= 0.999, p=0.93; for GP2: W=0.825, p=0.17; for GP3: W=1, p=0.98)

A one-way ANOVA was performed to compare the effect of the three different providers on carbohydrate contents. It revealed that there was not a statistically significant difference in mean carbohydrate contents between the groups (F = 0.676, p = 0.544).

Comparing the values obtained in this study with the values reported by different authors, it can be seen that they are comparable to those obtained by Lyngdoh et al. și Nagorao: 21.71, respectively 26.58% (Lyngdoh et al., 2022; Nagorao, 2014), but smaller than those determined by Prakash et al., Min et al., and Ifesan et al.: 78.80, 79.78, and 93.26% (Prakash et al., 2023; Min et al., 2020; Ifesan et al., 2014). Therefore, the analyzed sample GPs, due to the content and beneficial activity of carbohydrates, could be used, as such or their extractions, to obtain food products with added value.

It can be concluded that the garlic peel resulting from the peeling of native garlic bulbs, analyzed in this experiment, contains important amounts of nutritional compounds, which play an important role in the human body. Powder of garlic peels analyzed contain increased amounts of minerals, fibers and significant amounts of proteins and carbohydrates. They also contain small amounts of water and fat. These qualities, to which is added the increased content of bioactive compounds determined in previous studies, recommend their use as additives to obtain food with added value. The hydro-alcoholic extracts of garlic peel powders could be used in the future to obtain functional foods. Finally, it can be stated that the garlic peels, this precious by-product, after a prior processing, can be used in the development of new products with high added value in the food and fodder industry due to their improved nutritional profiles. In addition, the recovery of garlic peels as a material for obtaining products with added food value could be an ecological way of reducing the waste resulting from the processing of garlic.

CONCLUSIONS

It can be concluded that the garlic peel resulting from the peeling of native garlic bulbs, analyzed in this experiment, contains important amounts of nutritional compounds, which play an important role in the human body. Powder of garlic peels analyzed contain increased amounts of minerals, fibers and significant amounts of proteins and carbohydrates. They also contain small amounts of water and fat. These qualities, to which is added the increased content of bioactive compounds determined in previous studies, recommend their use as additives to obtain food with added value. The hydro-alcoholic extracts of garlic peel powders could be used in the future to obtain functional foods. Finally, it can be stated that the garlic peels, this precious by-product, after a prior processing, can be used in the development of new products with high added value in the food and fodder industry due to their improved nutritional profiles. In addition, the recovery of garlic peels as a material for obtaining products with added food value could be an ecological way of reducing the waste resulting from the processing of garlic.

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YIELD AND QUALITY EVALUATION OF L 548, VITAMINA, THE NEW TOMATO VARIETY OBTAINED AT BRGV BUZĂU

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Abstract

The researchers of BRGV Buzau had the preservation and breeding of local old autochthonous varieties and landraces as their purpose. In 2022, through repeated individual selection, the variety for fresh consumption with indeterminate growth registered at ISTIS with the provisional name of Vitamina was obtained. The strong distinguishing character of the variety is given by the fruit color. It is yellow, slightly orange. The fruits are large, round, slightly ribbed, with a weight that varies between 96-315 g. In addition to the high production potential, the variety presents an added quality given by the fruits taste and aroma and their richness in beneficial and healthy substances, leading the authors to propose the name Vitamina. It has the U gene, which express medium green cap immature fruit, but also specific taste and aroma given by the SGLK2 gene complex. The variety shows main pathogens attack high resistance and also has the nematode repellence mi gene. The market tests carried out so far with more than 1000 growers, have been favourable to the variety, and it enjoys a lot of appreciation.

Key words: *aroma, autochthonous, expressiveness, local landrace, repellence.*

INTRODUCTION

Spanish conquistadors were the first to record seeing tomatoes being cultivated for food in Mexico in the 16th century. Tomato seeds were then dispersed to the Philippines, the Caribbean, Italy, and Spain and from there to the rest of continental Europe and Southeast Asia (Bland, 2005).

Over time, although it is a crop introduced relatively recently in Romania, in the nineteenth century, tomatoes have gained popularity among growers and consumers, thus writing history in the cultivation and use of this species (Vînătoru et al., 2019). If at the beginning were cultivated trans-generational preserved local populations that were inherited from family-to-family, after 1990, concomitantly with the development of research in crop plant breeding, high-performance varieties and later top hybrids were created. Heirloom vegetables are nonhybrid varieties that have been preserved from generation to generation as seeds (Coolong 2009). Over time, these organic creations have replaced the traditional classic tomato due to their superior production and quality characteristics. Heirloom tomatoes are defined as varieties, which have been passed down through multiple generations of a family (Rowland, 2019).

One of the main problems of modern vegetable farming is the creation of new economically efficient varieties with complex valuable characters adapted to different growing conditions (Mihnea N., 2011).

A wide diversity of traditional varieties, modern cultivars, crop wild relatives and other wild-plant species. They are the basis for food security, as their loss results in reduced crop genetic diversity or genetic erosion, increasing the vulnerability of the future food supply (Harlan, 1992).

Consumers demonstrated their positive reception of heirloom tomatoes, especially when they were cultivated in the open-field and near their area of selection where they express their full potential in the nutrient synthesis and sensory properties (Lázaro A., 2018).

The term landrace has received numerous definitions and several synonyms refer to the same concept, including local variety, local population, traditional cultivar, farmer variety and farmer population (Zeven, 1998) or traditional variety and primitive variety (Negri et al., 2009).

MATERIALS AND METHODS

The genetic material that was used for intensive breeding works comes from a local population identified in Jilava, Ilfov County, in 1986. It bore the code name L 548.

Four lines from the tomato variety for fresh consumption were studied from the phenotypic, biochemical, production and quality point of view: L 28, L 532, L 548 and L 80. Through repeated individual selection works, stabilization in descent of the genotype L 548.

The comparative crops were established in the greenhouse, on an area of 300 m^2 in palisade system. Both years, the tomatoes were planted in high tunnels 1 month earlier and harvested 3 weeks earlier than the field.

Greenhouse soil was prepared with great care as it follows: the removal of previous crop debris, disinfection of the soil in the greenhouses being the most effective method of disease and pest prevention was done chemically. Before the crop was established, organic fertilizers were applied to the soil (80-100 t/ha).

Planting was done by hand at 70 cm between rows and 30 cm between plants per row and 120 cm between strips. The crop planting plan is the same as previously used. Immediately after planting the gaps were filled in. Soluble chemical fertilizers were used for fertilization, which were administered with irrigation water through the drip irrigation system. The special works used were the staking of the plants with string to the greenhouse support system at 200 cm, the pruning of the plants, the removal of the leaves below the first inflorescence, the shearing of the inflorescences when required.

The biochemical analyses carried out were: determination of the dry matter by oven weighing method, acidity, content of soluble substances (sugar, °Brix) and lycopene content.

The phenotypic and production observations were carried out according to the UPOV and IPGRI international descriptors.

The statistical analysis was performed in the SPSS statistical program, using analysis of variance, Anova followed by Duncan's post hoc test.

RESULTS AND DISCUSSIONS

The main parameters were chosen to follow the evolution of plant growth and development as follows: plant height (cm), leaf length (cm), fruit weight (g), number of fruits/plant (pcs), fruit height (cm), fruit diameter (cm) (Table 1) (Figure 1).

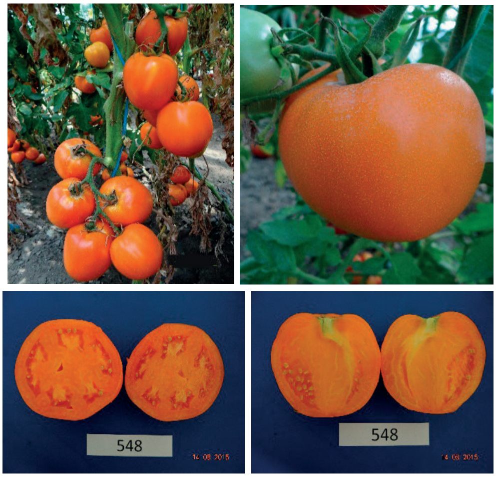


Figure 1. Vitamina variety: plant, fruit detail, fruit section

Table 1. Average data on the main plant growth parameters

Accession / Parameter	Plant height (cm)	Leaf lenght (cm)	Fruit weight (g)	Number of fruits/plant (pcs)	Fruit height (cm)	Fruit diameter (cm)
L 28 S	215±1.17 [a]	36±3.58 [a]	221±4.65 [a]	26.4±3.20 [a]	11.4±2.50 [a]	5.82±1.93 [a]
L 2000 S	196±52.8 [a]	39±4.58 [a]	485.2±328.6 [a]	12±3.74 [a]	6.8±1.65 [a]	7.2±1.46 [a]
L 80 S	220.4±14.7 [a]	36.22±4.98 [a]	18.84±2.36 [a]	317.6±93 [a]	3.64±0.56 [a]	2.12±0.8 [a]
L 532 S	164.6±42.7	33.16±6.08	75.64±5.4	43.4±10.57	7.28±1.31 [a]	2.74±0.63 [a]

*Letters represent Duncan test results with confidence interval of 95% and p<0.05

The measurable characteristics of L 548 are clearly superior to the other lines in the tomatoes assortment for fresh consumption, average values were recorded in terms of fruit weight of 385 g, reaching a maximum of 500 g.
In terms of fruit production per plant, they are significantly reduced than the other lines, but considering their size, the production remains significantly high, recording an average of 9.2 kg of fruit per plant in greenhouse.
The biochemical analyses carried out revealed the nutritional and medicinal importance of this line, being obvious that the line meets essential biochemical characteristics for human body health but also a chemical composition that gives it a special taste and aroma. Regarding the total dry substance, it has a rather low value, recording an average of 5.6% S.U.T., which indicates that the line is intended for fresh consumption and for salads.
Regarding the ratio between sugar and acidity, it is perfectly balanced, a very important characteristic in the case of tomatoes, a characteristic that supports the taste and aroma specific to the Romanian tomato.
The lycopene content reaches the highest values in the case of line 548, of 7.5 mg/100 g, which indicates exceptional antioxidant properties (Table 2).

Table 2. Biochemical analysis of tomato assortment for fresh consumption

Accession	S.U.T. (%)	Acidity (as citric acid) (%)	Sugar (%)	Acidity/sugar ratio	Ascorbic acid, mg/100 g^{-1}	Lycopene, mg/100 g^{-1}
L28	4.5±1.1 ^{b}	0.3±0.2 ^{b}	3.9±0.8 ^{b}	13.1±5.8 ^{a}	7.7±0.8 ^{b}	4.6±1.1 ^{b}
L80	4.16±0.43 ^{b}	0.22±0.04 ^{b}	3.6±0.40 ^{b}	35.0±8.24 ^{a}	5.8±1.64 ^{b}	5.6±1.12 ^{a}
L548	5.6±1.06 ^{a}	0.5±0.07 ^{a}	2.6±1.06 ^{b}	10.5±1.89 ^{b}	8.2±2.13 ^{a}	7.5±1.06 ^{b}
L532	6.3±0.7 ^{a}	0.4±0.1 ^{a}	4.3±0.5 ^{a}	17.1±4.6 ^{a}	7.3±1.5 ^{a}	3.4±1.5 ^{a}

*Letters represent Duncan test results with confidence interval of 95% and $p<0.05$.

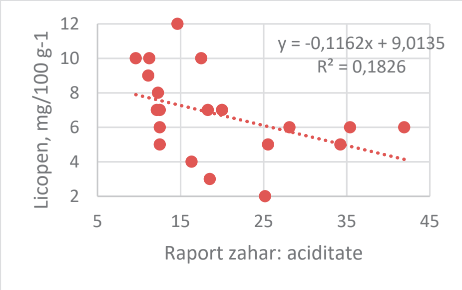
In order to establish a correlation between the characteristics determined by laboratory analysis, the Pearson correlation test was performed, and the correlation matrix was created that reveals the relationships between these parameters and their positive or negative influence (Table 3).

Table 3. Correlation coefficients of the analyzed biochemical composition

Features	**s.u.t. %**	**A**	**Z**	**Z/A**	**Aasc**	**L**
s.u.t. %	**1**					
Acidity (as citric acid) (%) - A	0.394	**1**				
Sugar total (%) - Z	-0.027	-0.028	**1**			
Sugar-acidity ratio - Z/A	-0.147	**-0.530**	**0.839**	**1**		
Ascorbic acid, mg/100 g-1-Aasc	**0.589**	**0.453**	-0.099	-0.290	**1**	
Lycopene, mg/100 g-1-L	**0.503**	**0.486**	-0.258	-0.427	0.420	**1**

According to the matrix above, you can see the negative values colored in red and the positive values colored in green.
The two trends, both negative and positive, are of major interest for breeders because they can determine the relationship between the measurable parameters.

The ratio between lycopene and the sugar/acidity ratio is -0.427, which indicates that the lower the lycopene content, the lower the sugar/acidity ratio. Also, a major influence is observed between the vitamin C content and the total dry matter content, this being 0.589, from which we can conclude that as the vitamin C content increases, the total dry matter content also increases.
These trends can be seen in Figure 2.
The four lines were harvested staggered. Thus, the harvests were carried out in the greenhouse starting from June 15 to October 15 (Table 4).
The crops were planted and treated as a prolonged cycle to highlight the production capacity of the lines chosen for the study (Figure 3).
For each harvest, quality I (STAS I), quality II (STAS II) and SUBSTAS (non-compliant fruits) were calculated. Also, for each line in the two years of study, 2022 and 2023, the early production was calculated.
For a more detailed and easy exemplification, a histogram was made showing the peak moments of the maximum quantities harvested throughout the harvesting period, for each individual line, in the two years of the study, as well as the average of these harvests per year (Figure 4).



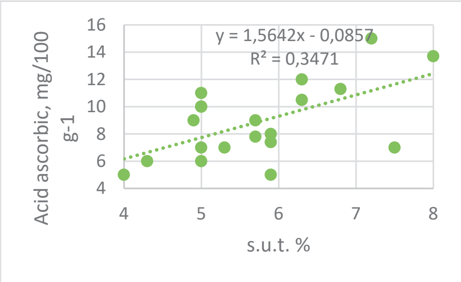


Figure 2. Lycopene/sugar/acidity ratio; ascorbic acid/s.u.t.% ratio

Figure 3. Fruit details on plant and immature/mature fruits

Table 4. Harvest dynamics (g/plant)

Line	I'st harvest (15.06)	II'nd harvest (30.06)	III'rd harvest (15.07)	IV'th harvest (30.07)	V'th harvest (15.08)	VI'th harvest (30.08)	VII'th harvest (15.09)	VIII'th harvest (30.09)	IX'th harvest (15.10)	TOTAL	Early yield
L28 average	174.6	814.8	1164	1571.4	756.6	582	291	232.8	174.6	5820	989.4
L28-2022	186	868	1240	1674	806	620	310	248	186	6200	1054
L28-2023	163.2	761.6	1088	1468.8	707.2	544	272	217.6	163.2	5440	924.8
L548 average	233.2	524.7	1574.1	1457.5	757.9	583	349.8	233.2	116.6	5830	757.9
L548 -2022	157.6	354.6	1063.8	985	512.2	394	236.4	157.6	78.8	3940	512.2
L548 -2023	308.8	694.8	2084.4	1930	1003.6	772	463.2	308.8	154.4	7720	1003.6
L80 average	181.65	847.7	1211	1634.85	787.15	605.5	302.75	242.2	181.65	6055	1029.35
L80-2022	132.3	617.4	882	1190.7	573.3	441	220.5	176.4	132.3	4410	749.7
L80-2023	231	1078	1540	2079	1001	770	385	308	231	7700	1309
L532 average	331	628.9	595.8	496.5	397.2	331	297.9	132.4	99.3	3310	959.9
L532-2022	265	503.5	477	397.5	318	265	238.5	106	79.5	2650	768.5
L532-2023	397	754.3	714.6	595.5	476.4	397	357.3	158.8	119.1	3970	1151.3

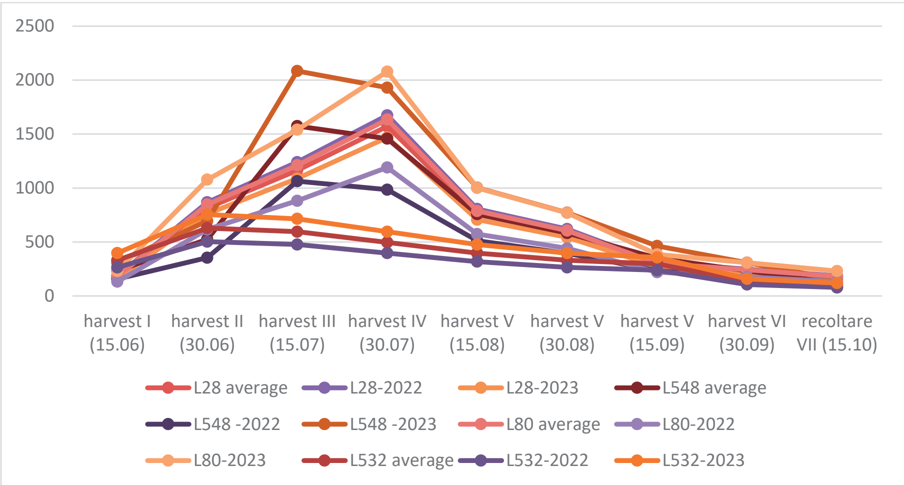


Figure 4. Yield recorded during the harvest period of the greenhouse crop

Regarding the dynamics of harvesting in the greenhouse, they were staggered over a longer period of time, the greenhouse being the one who allows this. Thus, harvesting began on June 15 and continued until October 15, which in the open field is not possible due to the arrival of early frost or frost.
The peak moments quantified in high production were registered by most of the lines in the months of June-July. L 548 is a semi-early line but which can record significantly high productions.
As a result of the studies and research carried out, L 548 has been genetically stabilized and is undergoing annual DUS testing at ISTIS to be approved and patented under the name of Vitamina.
According to the UPOV and IPGRI international descriptors, the variety has the following characteristics: plants with indeterminate growth, immature fruits with a medium green cap, mature fruits of yellow-orange color, round, slightly ribbed, with a weight varying between 96-315 g. Inflorescences are dense, on average 8 and a height of over 2 m of the plant. In addition to the high production potential, the variety presents an added quality given by the taste and aroma of the fruits and their great richness in substances beneficial to health, leading the authors to propose the name Vitamina.
It has the *U* gene, which gives the immature fruit a medium green cap and the *SGLK2* gene complex that accentuates the specific taste and aroma.
The variety shows high resistance to the main pathogens attack and has the nematode repellence gene.
The foliage is rich, well balanced on the plant, it has an average number of 38 leaves/plant, up to a height of 200 cm, with a leaf length varying from 18 to 36 cm. The pistillate point is star-shaped, the number of seminal lobes in the cross section is 5, the thickness of the pericarp is 6 mm.
The fruits are juicy, they can also be used for processing, in obtaining tomato pasta or juice. However, their firmness is medium-weak, making them easily perishable.

CONCLUSIONS

An assortment of tomatoes for fresh consumption from the BRGV Buzau germplasm collection, which has over 4.200 tomato lines, was involved in the study.
Among these, 4 lines were chosen for in-depth research, being characterized phenotypically, biochemically and from the point of view of production quality.
Thus, emphasis was placed on Line 548, originating from a traditional local population, which was genetically stabilized through repeated individual selection and improvement works.
Line 548 presents valuable distinctive characteristics, having large, yellow-orange fruits, indeterminate growth and genetic resistance to the attack of pathogens.
Also, following the biochemical analyses, they indicated the nutritional value of the studied line, relevant to an exceptional chemical composition, a balance in terms of the sugar/acidity ratio, as well as a net content superior to the other lines in terms of the percentage of lycopene, an important and primary antioxidant for the species *Solanum lycopersicum*.
Following this study, line 548 was registered for the purpose of homologation and patenting, meeting the characteristics of the DUS test, distinctiveness, uniformity and stability in descent.
It has the provisional name of Vitamin, a name that indicates the superior nutritional quality of the variety intended for fresh consumption.

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NEW CHERRY TOMATOES VARIETIES OBTAINED AT BRGV BUZĂU

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Abstract

BRGV Buzău owns a rich germplasm collection of tomato, consisting of over 3600 genotypes, over 35% of which are cherry cultivars. The research aimed to obtain new cherry type varieties with high yield potential and superior quality, especially taste and aroma, with high resistance to the main pathogens attack. The research was completed with obtaining many lines, and 4 of them were registered for homologation and patenting. These received the provisional names of Serena, Simila, Rapsodia and Monalisa. Simila variety has round, slightly juicy, tasty dark brown fruits, with an average weight of 12 g. Serena variety presents red, round, slightly ovoid, firm, tasty fruits, with an average weight of 17 g. Rapsodia variety presents brindle, green with burgundy-red fruits, round, firm, tasty, brown-pink pulp, with an average weight of 23.5 g. Monalisa variety has red, ovoid, crunchy, tasty fruits, with an average weight of 15 g, being very productive. Research will continue with the promotion of these varieties as crops and by increasing the seeding surfaces and obtaining new valuable varieties.

Key words: *directions of use, interspecific, kumato, local landraces, selection.*

INTRODUCTION

Tomato ranks first among processed vegetables in the world (Prema, G. et al., 2011). Botanically classified as a berry, tomatoes originated in the South American Andes, ranging from northern Chile in the south, through Bolivia, Peru to Ecuador and Colombia in the north (Grubben and Denton, 2004; Bai and Lindhout, 2007). Initially, Peru had been proposed as the center of domestication of tomato, thus coinciding with its center of origin and genetic diversity. However, genetic evidence points to Mexico as the center of domestication, as modern cultivars appear to be closely related to a cherry tomato-like cultivar grown widely in Mexico and throughout Central America at the time of discovery by the Spanish (Ebert A.W. and Chou Y.Y., 2015). Cherry tomato (*Solanum lycopersicum var. cerasiforme*) is a botanical variety of the cultivated tomato. It is thought to be the ancestor of all cultivated tomatoes (Renuka D. M. et al., 2014). Cherry tomato is grown for its edible fruits; they are perfect for making processed products like sauce, soup, ketchup, puree, curries, paste, powder, rasam and sandwich. They also have good nutritional and antioxidant properties. The size of cherry tomatoes range from thumb tip to the size of a golf ball. And can range from being spherical to slightly oblong in shape. The possible exploitation of hybrid vigour in cherry tomato has been taken up at few research centres however very little systematic attention has been paid by plant breeders to study performance for yield and its components in cherry tomato. The genotypes performing well can be used further in heterosis breeding programme (Renuka D. M. et al., 2014). Tomato (*Solanum lycopersicum*, formerly *Lycopersicon esculentum*) is a highly autogamous species. Cultivated tomato shows a low genetic diversity, but higher phenotypic diversity compared to *S. pimpinellifolium* (Miller and Tanksley, 1990) due to intensive human selection (Xu. J. et al., 2002). Although it is well known that cultivated germplasm resources provide an important genetic basis for both breeding and genetic research

(Thornsberry et al., 2001). Conventional tomato (*Solanum lycopersicum* L.) descriptors are of great utility for gross morphological characterization but may not be practical for the precise fruit description required for distinguishing closely related cultivar groups (Figàs, M. R. et al., 2015). Most species within the *S. lycopersicum* complex can reciprocally hybridize with cultivated tomato, with the exception of *S. habrochaites* (Robertson and Labate, 2007).
The loss of genetic diversity due to the replacement of local tomato varieties by improved cultivars has been in many cases mitigated by the collection and safe storage of germplasm in genebanks (Figas R. M. et al., 2015). It is a self-pollinating species with high genetic variability (Aguirre N. C. et al., 2017). Agricultural biodiversity is fundamental to production and food security, as well as for environment conservation (Corrado G. et al., 2014). Although it is well known that cultivated germplasm resources provide an important genetic basis for both breeding and genetic research (Flint G. et al., 2005). The evaluation of the diversity of a given collection can be based on phenotypic traits (Yan et al., 2007).

MATERIALS AND METHODS

BRGV Buzău owns a rich collection of tomato germplasm, consisting of over 3600 genotypes, over 35% of which are cherry cultivars.
Among the cherry tomato species that BRGV Buzău has in its collection, we mention: *Solanum lycoperiscum var. lycopersicum, S. lycopersicum var. cerasiformae, S. lycopersicum var. pimpinelifolium, Solanum cheesmanie, Lycopersicum hirsutum, S. peruvianum, Solanum spontaneum, S. sisymbrifolium.*
From the assortment of cherry tomatoes, the Flaviola variety was chosen as control, a variety obtained by BRGV Buzau specialists and registered in the Official Catalog of Cultivated Plants in Romania in 2018. Compared to the control, 4 lines with distinct phenotypic expressiveness and genetically stable were introduced into the study, which received the provisional names of Serena, Simila, Rapsodia and Monalisa (Figure 1).
Control variety has red, ovoid, crunchy, tasty fruits, with an average weight of 11.5 g.
Simila variety presents round, slightly juicy, tasty fruits with an average weight of 12 g, dark brown in color.
Serena variety presents red, round, slightly ovoid, firm, tasty fruits, with an average weight of 17 g.
Rapsodia variety presents brindle, green with burgundy-red, round, firm, tasty fruits, brown-pink pulp, with an average weight of 23.5 g.
Monalisa variety presents red, ovoid, crunchy, tasty fruits, with an average weight of 15 g, being very productive.

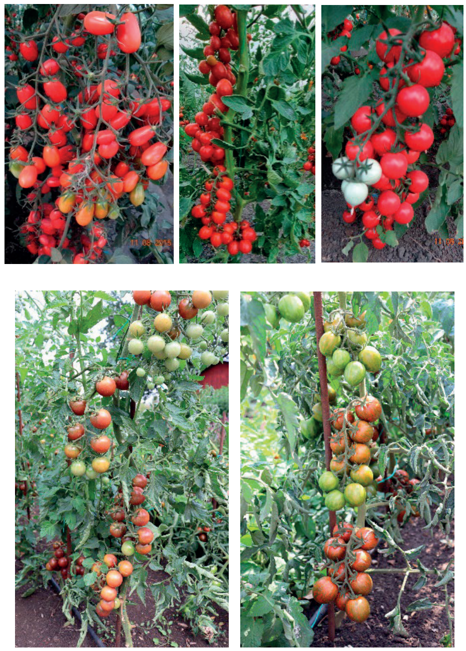

Figure 1. Tomato cherry varieties: Flaviola (control), Monalisa, Serena, Simila and Rapsodia

Statistical analysis was performed using SPSS, ANOVA followed by Duncan's post-hoc test with 95% confidence interval and p-values < 0.05. Evaluation of the germplasm core collection was carried out by performing biometric measurements for the main characters based on the international UPOV and IPGRI descriptors. The amount of total soluble solids was measured by refractometer.

RESULTS AND DISCUSSIONS

After the biometrical evaluation of cherry tomato assortment, the following quantitative and qualitative features were identified:

The 5 lines taken in the study showed distinct phenotypic traits (Table 1, Figure 1). The accessions have indeterminate growth.

Monalisa (Figure 5) is a cherry tomato with rich, intense green foliage, with an average of 8 inflorescences on the main stem, with an ovoid, intense red fruit, weighing on average 9.8 g, good firmness, high production. The inflorescences are mixed, branched and very rich, compact and with concentrated ripening. They can be harvested as such and marketed for fresh consumption.

Simila (Figure 4) is a black cherry tomato, with a distinctive, strawberry-like aroma and low firmness. The plant has small leaflets, medium green in color, fruits weighing 15 g on average, round, brownish pink. They are part of kumato tomatoes group known to be rich in antioxidants and vitamins. The fruits come off the pedicel very easily. The inflorescences are mixed, branched with concentrated ripening.

Flaviola (Figure 3) has cherry tomatoes with high yields, reaching 1500 g per plant. The fruits are small, ovoid, with an average weight of 18 g. The fruits are very crunchy, show great firmness, the inflorescences reach 318 fruits per plant, a value that no other line reaches.

Rapsodia (Figure 2) is a medium tricolor cherry tomato, with high firmness. The variety has round fruits, 10.7 g on average, with a specific aroma and taste, very fragrant and intensely aromatic.

This variety is also part of the group of kumato tomatoes. It presents specific resistance to diseases and pests. Also, the superior nutritional quality is indicated by the sugar content (TSS) of 15.6°Brix, the highest value recorded compared to the other varieties.

Table 1. Main biometric characteristics of the cherry varieties, average values

Accession	Plant height (cm)	Leaf lenght (cm)	Petiole lenght (cm)	Florets no./flowering stem (unit)	Fruit weight (g)	Fruit no./plant (unit)	Fruit height (cm)	Fruit width (cm)	TSS (°Brix)
Monalisa	195.8±2.6 e	26.6±1.1 b	2.22±0.2 bc	8±0.7 b	9.82±0.1 a	65.2±1.3 c	2.8±0.2 d	2.9±0.2 d	8.18±0.2 c
Simila	235±1.6 a	34±1.6 c	8.66±0.2 a	10±1.6 a	15±0.2 b	117±1.6 a	2.84±0.1 d	2.86±0.1 a	6.14±0.1 e
Flaviola (control)	210±1.6 d	44±1.6 a	1.26±0.2 d	7.8±0.8 b	18.74±0.1 c	318±1.6 a	3.68±0.1 bc	2.14±0.1 b	11±0.2 b
Serena	215.2±1.3 c	32.6±0.2 cd	2±0.2 b	7.4±0.5 bc	18.56±0.2 b	312.6±1.1 b	2.62±0.2 c	2.7±0.2 a	7±0.7 d
Rapsodia	218±1.6 b	32.2±0.8 d	-	10.8±0.8 a	10.78±0.9 a	87.8±1.3 d	4.12±0.1 a	1.6±0.5 c	15.6±1.1 a

Note: Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$, 95% confidence level)

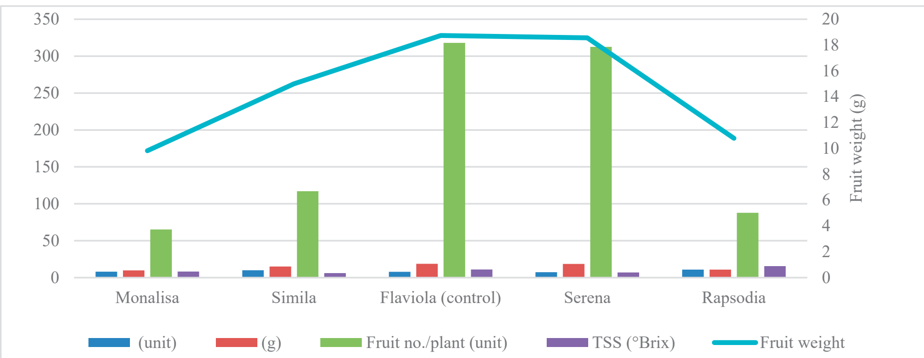

Figure 1. Main biometric characteristics of the cherry varieties

Figure 2. Rapsodia plant detail, immature and mature fruits, fruit cross-section

Figure 3. Flaviola (control) plant detail, immature and mature fruits, fruit cross-section

Figure 4. Simila plant detail, immature and mature fruits, fruit cross-section

Serena variety (Figure 6) is characterized by cherry fruits whose strong point is the very high firmness, the 4 mm crispy and very tasty pericarp. The fruits are slightly truncated ovoid with a mucronate tip.

Also, this variety is distinguished by the strong and characteristic glossy fruits, which gives it a pleasant commercial appearance. The inflorescences are linear, bifurcated, compact, competing in production with the Flaviola variety, recording a value of 312 fruits per plant.

Figure 5. Monalisa plant detail, immature and mature fruits, fruit cross-section

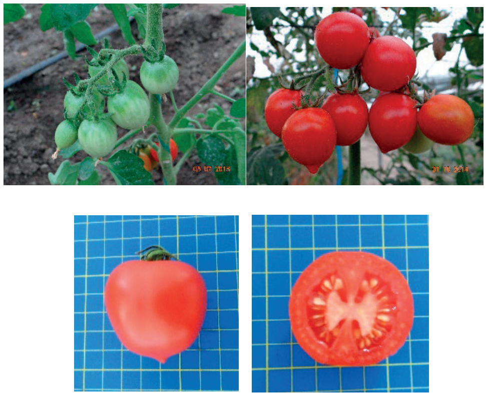

Figure 6. Serena plant detail, immature and mature fruits, fruit cross-section

As a result of the phenotypic and descriptive studies as well as the biometric measurements carried out, it is found that, unlike the Flaviola control, cherry tomato varieties with superior characteristics have been identified.

Thus, in terms of plant height, the Simila variety registered an average height of 235 cm, as opposed to 210 cm, the value recorded by the control variety Flaviola.

Regarding the length of the petiole, also the Simila variety registers a maximum value of 8.6 mm.

Rapsodia variety has the maximum value of 11 inflorescences, unlike the control, which has an average of 7 inflorescences per plant.

Many market requirements for the weight of cherry tomato fruits are that they fall within the range of 8-10 g, average weight recorded by the Monalisa variety with 9.8 g.

And in terms of total soluble substances, Rapsodia surpasses the average value of the control Flaviola, registering 15.6°Brix compared to the control variety, with only 11°Brix.

Therefore, the varieties under homologation and patenting of BRGV Buzău meet superior qualities compared to the Flaviola control, both from the phenotypic point of view, the commercial aspect, but also the biochemical and nutritional quality.

The new varieties have multiple directions of use, being intended for both industrialization and fresh consumption.

CONCLUSIONS

An assortment of cherry tomatoes intended both for fresh consumption and for processing from the germplasm collection of BRGV Buzău, which has over 4,200 tomato lines, was involved in the study.

From these, 4 varieties were chosen that are in the process of approval and patenting and a control variety, Flaviola, approved and patented and entered in the official Catalog of Cultivated Plants since 2018 by the research team of BRGV Buzău.

The cherry varieties were researched, being characterized phenotypically and biochemically, thus emphasizing the nutritional quality, the commercial aspect and the direction of use.

Thus, we conclude that the four varieties present distinct phenotypic and biochemical characteristics in contrast to the control variety, Flaviola, being clearly superior to it, registering much higher values of the main quantitative characters.

The varieties under homologation and patenting by BRGV Buzău have the provisional names of Monalisa, Serena, Simila and Rapsodia.

Monalisa is distinguished by high production, great firmness and very rich and branched inflorescences.

Serena's strong point is cherry-type glossy fruit very firm and mucronate.

Simila is part of the group of kumato tomatoes, having pinkish-brown fruits, with a special raspberry-like aroma.

Rapsodia variety is a kumato-type but tricolor cherry tomato, with a taste and aroma specific to traditional tomatoes, with exceptional nutritional quality.

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AN OVERVIEW OF RECENTLY DISCOVERED INTRA AND INTER-GENERIC ORCHID HYBRIDS AS NEW ADDITIONS TO ROMANIAN FLORA (Part II)

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Abstract

Nine orchid hybrids, previously unrecorded in Romanian flora, have been identified. These hybrids include both intra-generic (within the same genus) and inter-generic (between different genera) crosses, showing the extraordinary biodiversity within the region. Such hybrids are extremely rare, not only in Romania but throughout temperate Europe, making this discovery particularly significant. During our eight-year study (2017-2024), we encountered several hybrids belonging to various orchid genera (Anacamptis, Ophrys, Gymnadenia, Dactylorhiza, Epipactis) and nothogenera (× Dactylocamptis and × Dactylanthera). Of these nine orchid taxa, eight are regarded as new to science, being documented for the first time. These findings are part of an ongoing research project – Orchids of Romania Project, which continues to gather additional data on these unique hybrids. The study includes detailed information on the hybrids' distribution, habitat, ecology, phenology and conservation status as assessed by the IUCN.

Key words: *orchids, hybrids, conservation, intrageneric, Romania.*

INTRODUCTION

Over millions of years of evolution, the process of hybridization has significantly impacted the history of life on Earth (Anghelescu et al., 2020). In the natural environment, hybridization serves as an essential evolutionary mechanism that, in the case of the Orchidaceae family, transcends reproductive barriers between different species and even between different genera (Anghelescu et al., 2020). Following cross-pollination, new hybrid lines are formed through the recombination of parental genomes (Anghelescu et al., 2021a). The concept of nothotaxon refers to the totality of hybrids resulting from the crossing of two distinct parental taxa (natural species, excluding hybrids). These nothotaxa can subsequently evolve into higher taxonomic entities such as nothogenus, nothospecies, or nothosubspecies (Anghelescu et al., 2021a). Thus, hybridization represents a crucial element in the global speciation process, significantly contributing to the formation of new species with enhanced genetic and adaptive variation (Kretzschmar et al., 2007). The Orchidaceae family is unique in producing natural hybrids between species belonging to the same genus (intrageneric hybrids), as well as hybrids resulting from the crossing of two or more species from different genera (intergeneric hybrids) (Anghelescu et al., 2023a). Hybridogenic populations, also known as nothopopulations, can be very numerous and widespread over large areas, sometimes competing with parental populations. In this article, we document the first reported natural occurrence of seven intrageneric hybrids belonging to several orchid genera: *Anacamptis* - *Anacamptis* × *menosii* nothosubsp. *angelliana* N. Anghelescu, N. Kigyossy, L. Balogh & Mih. Balogh, 2024 nothosubsp. *nov*. (*Anacamptis coriophora* subsp. *coriophora* × *Anacamptis papilionacea*), *Anacamptis* × *nicodemi* nothosubsp. *caucasica* N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosubsp. *nov*. (*Anacamptis morio* subsp. *caucasica* × *Anacamptis papilionacea*), *Dactylorhiza* - *Dactylorhiza* × *subalpina* N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosp. *nov*. (*Dactylorhiza* *cordigera* ssp. *siculorum* × *Dactylorhiza incarnata*), *Dactylorhiza* × *carpathiana* (Soó) Soó N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosp. *nov*. (*Dactylorhiza cordigera* × *Dactylorhiza majalis*) and *Epipactis* - *Epipactis* × *romanensis* R. Maalouf, N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosp. *nov*. (*Epipactis distans* × *Epipactis atrorubens*), *Gymnadenia* - *Gymnadenia* × *montana* N. Anghelescu, L. Balogh, M. Balogh & N. Kigyossy, 2024 nothosp. *nov*. (*Gymnadenia winkeliana* × *Nigritella nigra* subsp. *bucegiana*) and *Ophrys* - *Ophrys* × *minuticauda* nothosubsp. *cornuta* R. Maalouf, N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosubsp. *nov*. (*Ophrys apifera* × *Ophrys scolopax* subsp. *cornuta*). Additionally, we included two rare intergeneric hybrids, members of the nothogenera × *Dactylocamptis* - × *Dactylocamptis suzana* N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosp. *nov*. (*Anacamptis coriophora* × *Dactylorhiza maculata*) and × *Dactylanthera* × *Dactylanthera transsilvanica* L. Balogh & Mih. Balogh, N. Anghelescu, N. Kigyossy, 2024 nothosp. *nov*. (*Dactylorhiza maculata* × *Platanthera bifolia*).

The likelihood of these specific hybridization events occurring in the wild and successfully producing viable individuals is exceedingly rare. This rarity highlights the exceptional nature of their discovery and underscores the unique conditions that must align for such hybrids to form in the wild. Out of these nine newly described hybrids, eight are regarded as new-to-science taxa, hereby described for the first time. The intrageneric hybrid *Epipactis* × *romanensis* R. Maalouf, N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 *nothosp. nov*. (*Epipactis distans* × *Epipactis atrorubens*) featured under the name *Epipactis* × *gauckleri* in two previous publications (Weigel & Riechelmann, 2002), as mentioned in Günther Blaich's List of Hybrids of European Orchids (2024). However, the taxon *Epipactis* × *gauckleri* does not feature as an accepted taxon on Kew's database, Plants of the World Online (POWO, accessed 2024), the database which we take as a reference for all our taxonomical studies. Consequently, we chose to make a formal description of this taxon with the confidence that it will be listed shortly on the international plant databases (POWO, WFO, 2024). At the same time, two Romanian endemic orchid taxa (intrageneric hybrids), *Anacamptis* × *menosii* nothosubsp. *angelliana* N. Anghelescu, N. Kigyossy, L. Balogh & Mih. Balogh, 2024 nothosubsp. *nov*. (*Anacamptis coriophora* subsp. *coriophora* × *Anacamptis papilionacea*) and *Ophrys* × *minuticauda* nothosubsp. *cornuta* R. Maalouf, N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosubsp. *nov*. (*Ophrys apifera* × *Ophrys scolopax* subsp. *cornuta*), described previously by Anghelescu et al. (2023a), are reconsidered in this paper and redescribed as new to science taxa, following the kind and helpful advice of IPNI editors at Royal Botanic Gardens, Kew.

During our eight-year study from 2017 to 2024, we carried out extensive fieldwork across various protected areas, including national and natural parks, to document and map the orchid flora found in the newly identified remote hybridogenic zones (Anghelescu et al., 2020; 2023a; 2023b; De Anghelli & Anghelescu 2020, 2023).

Studying orchid hybrids presents numerous challenges due to their complex genetic compositions and varied phenotypic expressions. Many hybrids exhibit a wide range of morphological variations, making it difficult to establish clear identification criteria (Anghelescu et al., 2020). Additionally, the often-limited availability of individuals for study, particularly in natural habitats where rare orchid hybrids are found, further complicates research efforts. Environmental factors, such as habitat conditions and pollinator interactions, also play a significant role in the success of hybridization, adding layers of complexity to the investigation. Furthermore, the legal protections surrounding many orchid taxa restrict access to specimens, which can hinder comprehensive research and the development of a complete understanding of hybrid dynamics. These obstacles necessitate a meticulous and adaptable approach to studying orchid hybrids, requiring both patience and innovative methodologies. It is important to highlight that collecting rare plant taxa, including our rare orchid hybrids from strictly protected areas and national parks, is illegal, even if a study or work permit is obtained. These taxa are safeguarded by both international laws (CITES) and regional Romanian regulations. Additionally, for laboratory research or herbarium voucher deposits, the collection is limited to only 1-3 individual flowers and, in exceptional cases, a single leaf from the holotype. In recent years, photos of the holotype(s) have also been accepted as herbarium vouchers to further ensure the protection and preservation of these extremely rare and vulnerable specimens (Anghelescu et al., 2024).

The objectives of the current study are as follows: (1) to provide a detailed description of the primary morphological traits of the two nothospecies, particularly their floral characteristics, in comparison to their parent species; (2) to offer insights into various biological aspects that are crucial for understanding their ecological needs, including distribution, habitat, community interactions, phenology, reproductive requirements, and conservation efforts; and (3) to report on the IUCN (2021) conservation status of all the considered taxa, accompanied by photographs of the holotypes. Given the significance and rarity of these newly identified nothotaxa, we strongly advocate for the inclusion of these two nothopopulations as new additions to the Romanian flora.

MATERIALS AND METHODS

Sites Studied

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 Prahova, Harghita and Mehedinți Counties:

(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 meters a.s.l (Ielencz, 2005; Herdren et al., 2022).

(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). 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) 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

Particular emphasis was placed on taxonomically informative characteristics, especially those that differentiate the morphology of the leaves, labellum, and tepals. Measurements of both vegetative and floral parts were taken from living plants and fresh flowers (Anghelescu et al., 2021a; 2023a). Our observations indicate that the primary floral traits of the nothotaxa are generally intermediate between those of the parent species.

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., 2021b, 2023a, 2024).

Taxonomical classification

The taxonomy is structured according to the most recent databases in line with the International Plant Names Index n.d. (IPNI, 2024), Plants of the World Online | Kew Science, n.d. (POWO Kew, 2024), The WFO Plant List | World Flora Online, n.d. (WFO, 2024), New Taxa | Euro+Med-Plantbase, n.d. (2024), De Angelli & Anghelescu (2020, 2023).

RESULTS AND DISCUSSIONS

This overview examines the recent discoveries of intra- and inter-generic orchid hybrids that have been identified as new contributions to the flora of Romania. These hybrids, resulting from the complex interplay of various species within the Orchidaceae family, not only enrich the botanical diversity of the region but also highlight the evolutionary dynamics at play within these fascinating plants. Recent studies have focused on documenting these hybrids and their morphological characteristics, ecological requirements, and distribution patterns (Anghelescu et al., 2020; 2021a; 2021b; 2023a). Intra-generic hybrids have emerged from crossings within the same genus, showcasing unique adaptations and traits that distinguish them from their parent species. On the other hand, inter-generic hybrids, formed by the combination of distinct genera, reveal the remarkable reproductive flexibility and resilience of orchids in natural conditions. As a result, hybridization in natural conditions serves as an evolutionary catalyst, breaking down the reproductive barriers that divide different species. By definition, hybridization involves the mating or cross-breeding of two parental lineages with distinct genetic backgrounds. In the first generation of offspring, known as F1 or primary hybrids, their genes combine to create intermediary forms between the parent taxa (Scopece et al., 2007). This phenomenon can have significant impacts on genetic variation within populations and the evolution of species, leading to hybrids with unique combinations of traits (Marques et al., 2014). In some cases, these hybridization events may even result in the formation of entirely new nothotaxa, such as nothogenera, nothospecies, and nothosubspecies (Mayr, 1942).

Description of the nine nothotaxa studied

Following is the full description of the nine intra- and intergeneric orchid hybrids included in this study, named according to POWO (2024). The included nothotaxa are all terrestrial, perennial, rhizomatous, autotrophic, sympodial herbaceous geophytes (Figures 1-9).

Anacamptis* × *menosii* nothosubsp. *angelliana* N. Anghelescu, N. Kigyossy, L. Balogh & Mih. Balogh, 2024 *nothosubsp. nov. (Figure 1).

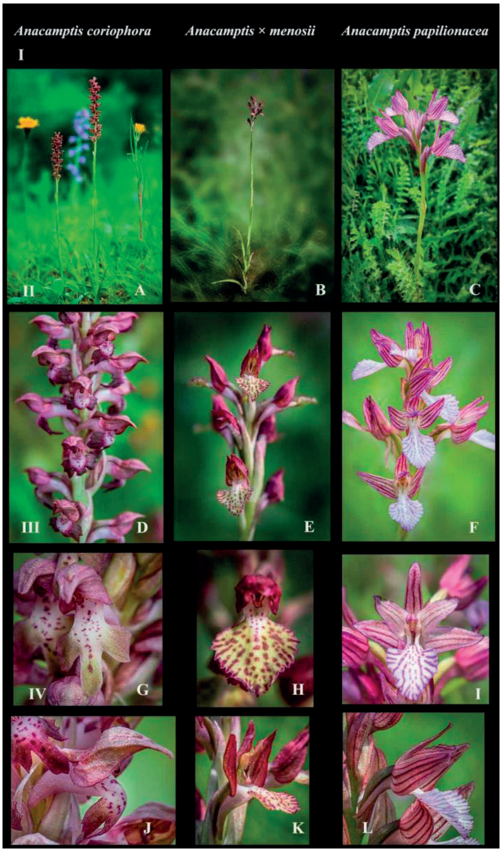

Figure 1. I. Full plants in their natural habitats; II. Inflorescences details; III. Individual flower detail; IV. Side-view of individual flower details. A., D., G. J. - *Anacamptis coriophora*; B., E., H. K. - *Anacamptis* × *menosii* nothosubsp. *angelliana*; C., F., I. L. - *Anacamptis papilionacea*. Photos © Nora E. Anghelescu, 31 May 2023, Mehedinți County

Hybrid formula: *Anacamptis coriophora* subsp. *coriophora* × *Anacamptis papilionacea*.
Discovered: 31 May 2023, Nora E. Anghelescu.
Locus classicus: Mehedinți County, private property.
Flowering time: May-June.
Native to: Romania, Endemic (En).
Description: Stem (epigeal), 10-14 cm, erect, vivid green, with no purple pigmentation. 1-2 basal leaves sheath the stem, 3-5 cauline unspotted leaves are sessile, vivid green, acuminate, sheathing the stem (resembling *Anacamptis coriophora*; in *Anacamptis papilionacea*, the leaves are veined and purple-tinged at the tips). The inflorescence is a lax raceme (resembling *Anacamptis papilionacea*), less floriferous, with 8-10 medium-sized flowers

resembling *Anacamptis coriophora*. The helmet is deep-purple pigmented and tight, with an elongated tip, resembling *Anacamptis coriophora*. The labellum is entire and heart-shaped (in upper flowers, resembling *Anacamptis papilionacea*) to mildly three-lobed (in basal flowers, resembling *Anacamptis coriophora*), smaller in size than *Anacamptis papilionacea*. Labellar stripes and dots are deep-purple to red (heterosis effect), resembling *Anacamptis papilionacea*. The spur is conical and thick, resembling *Anacamptis coriophora* (correction of the original taxonomical descript-tion in Anghelescu et al., 2023a, pg. 421).

Nothopopulation counts: 1(2) individuals/hybrids, flowering each year.

Herbarium voucher specimen: Deposited at the Herbarium of the Botanical Garden Bucharest, N.E. Anghelescu, barcode: **BUC 410363**.

Proposed conservation status: Endangered (EN).

***Anacamptis* × *nicodemi* nothosubsp. *caucasica* N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosubsp. *nov*.** (Figure 2)

Hybrid formula: *Anacamptis morio* subsp. *caucasica* × *Anacamptis papilionacea.*

Discovered: 22 May 2019, Nora E. Anghelescu.

Locus classicus: Mehedinți County, private property.

Flowering time: May-June.

Native to: Romania, Endemic (En).

Description: Stem (epigeal), 9-15(25) cm, erect, vivid green, with no purple apical pigmentation. 4-6 basal leaves, of which 2-3 sheath the stem; 2-4 cauline unspotted leaves, sessile, vivid green, acuminate, sheath the stem, resembling *Anacamptis papilionacea*. Inflorescence is a lax raceme, resembling *Anacamptis papilionacea,* less floriferous, with 8(10)-15(20) medium-sized flowers resembling either *Anacamptis papilionacea* and/or *Ancamptis morio* subsp. *caucasica*. No hybrid is as variable in the shape and colour of the flowers as *Anacamptis* × *nicodemi* nothosubsp. *caucasica*, since it inherits the enormous colour variability (and genetic plasticity) of its parent, *Anacamptis morio* subsp. *caucasica*. Furthermore, the influence of the morio parent is recognized in the slightly ascending spur and the fan-like labellum adorned with various hues of purple markings. Nevertheless, the radial arrangement of the markings reminds of *Anacamptis papilionacea*. From the latter parent, it also inherited the drop-like shape, the lighter colour and the serrated edges of the labellum. The hood is also tighter, similar to *Anacamptis papilionacea*. The horizontally to upward-oriented spur (a feature inherited from *Anacamptis morio* subsp. *caucasica*) is the main characteristic that differentiates it from *Anacamptis* × *nicodemi,* which typically has a slightly downward-oriented spur (a feature inherited from *Ancamptis morio* subsp. *morio* parent).

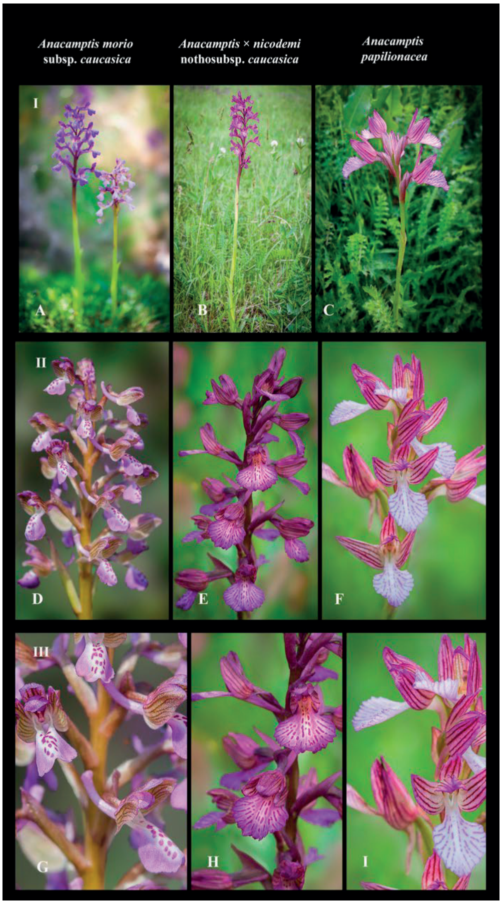

Figure 2. I. Full plants in their natural habitats; II. Inflorescences details; III. Individual flower detail; A., D., G. - *Anacamptis morio* subsp. *caucasica*; B., E., H. - *Anacamptis* × *nicodemi* nothosubsp. *caucasica*; C., F., I. - *Anacamptis papilionacea*. Photos © Nora E. Anghelescu, 22 May 2019, Mehedinți County

Nothopopulation counts: numerous, >100 individuals/hybrids, flowering each year.
Herbarium voucher specimen: Deposited at the Herbarium of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, N.E. Anghelescu, **USAMVB–barcode 40104**.
Proposed conservation status: Endangered (EN).

Dactylorhiza* × *subalpina* N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosp. *nov. (Figure 3).

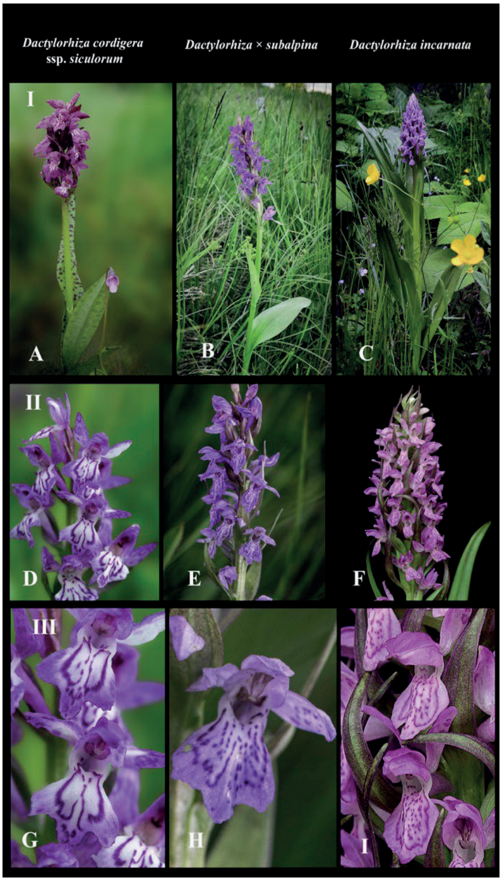

Figure 3. I. Full plants in their natural habitats; II. Inflorescences details; III. Individual flower detail; A., D., G. - *Dactylorhiza cordigera* subsp. *siculorum*; B., E., H. - *Dactylorhiza* × *subalpina*; C., F., I. - *Dactylorhiza incarnata*. Photos © Nora E. Anghelescu, 31 May 2022, Harghita County

Hybrid formula: *Dactylorhiza cordigera* ssp. *siculorum* × *Dactylorhiza incarnate.*
Discovered: 24 June 2023, Nora E. Anghelescu.
Locus classicus: Harghita Mădăraş, ROSCI00090.
Flowering time: June-July.
Native to: Romania, Endemic (En).
Description: The stem (epigeal), 22-36 cm is erect, slender, vivid green, reminiscent of *Dactylorhiza incarnata*, with purple apical pigmentation, resembling *Dactylorhiza cordigera* subsp. *siculorum*. The basal leaf is wide, vivid green and mostly unpigmented, resembling *Dactylorhiza incarnata*. It is broadest in the middle, a feature inherited from *Dactylorhiza cordigera* subsp. *siculorum*. The two sessile, unspotted cauline leaves are vivid green and sheath the stem; the upper one is narrow-acuminate, bract-shaped. The inflorescence is an elongated, lax raceme similar to that of the *Dactylorhiza cordigera* subsp. *siculorum* parent. The flowers are medium-sized, purple-coloured, intermediate between the two parental species. The three-lobed labellum is also intermediate in shape and pigmentation between parents.
Its lateral, scalloped lobes are shorter than the median lobe, spread horizontally, resembling *Dactylorhiza cordigera* subsp. *siculorum*. The labellum is narrower, ovoidal and more elongated than the heart-shaped labellum of *Dactylorhiza cordigera* subsp. *siculorum*, resembling more *Dactylorhiza incarnata* parent. The labellar markings form circular double or triple loops, further resembling *Dactylorhiza incarnata*. The spur is longer than that of *Dactylorhiza cordigera* subsp. *siculorum*, conical, horizontal to slightly downward pointing, resembling *Dactylorhiza incarnata*. Overall, the hybrids phenotypically resemble the *Dactylorhiza incarnata* parent more closely. This nothopopulations is still under study.
Nothopopulation counts: 3 individuals/hybrids, flowering each year.
Herbarium voucher specimen: Deposited at the Herbarium of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, N.E. Anghelescu, **USAMVB–barcode 40105**.
Proposed conservation status: Endangered (EN).

Dactylorhiza* × *carpathiana* (Soó) Soó N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosp. *nov. (Figure 4).

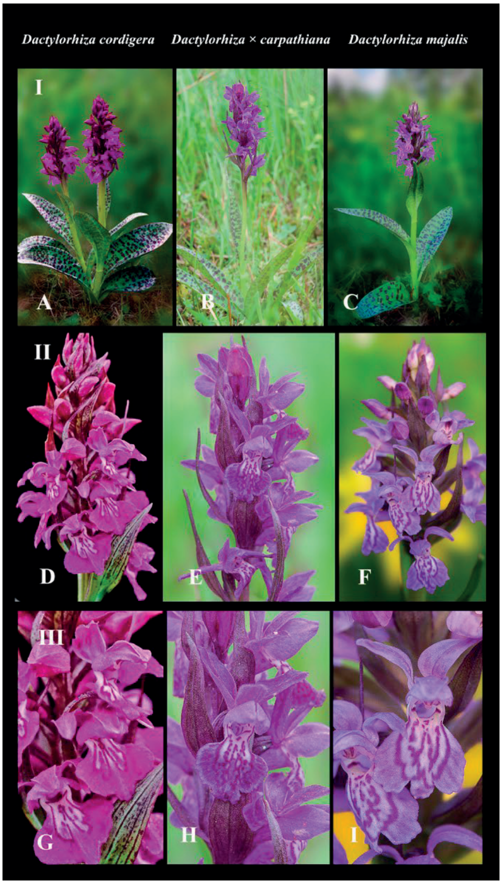

Figure 4. I. Full plants in their natural habitats; II. Inflorescences details; III. Individual flower detail; A., D., G. - *Dactylorhiza cordigera*; B., E., H. - *Dactylorhiza × carpathiana*; C., F., I. - *Dactylorhiza majalis*. Photos © Nora E. Anghelescu, 5 June 2018, Prahova County

Hybrid formula: *Dactylorhiza cordigera × Dactylorhiza majalis*
Discovered: 5 June 2018, Nora E. Anghelescu.
Locus classicus: Bucegi Natural Park ROSCI0013.
Flowering time: May-June.
Native to: Romania, Endemic (En).
Description: The stem (epigeal), 15-32 cm is erect, slender, deep-green, significantly purple-pigmented in the rachis (inflorescence) part, similar to both parents. The lowest/most basal leaf is vivid green and broadest toward the middle, with a more roundish apex, resembling *Dactylorhiza majalis*. The 2-3 basal leaves are strongly purple pigmented on the upper side, with irregular maculae of various shapes and dimensions and form a rosette. They are broadest toward the upper part (middle and apex), resembling both parents. The sessile, maculate cauline leaf is narrow-acuminate, lanceolate and sheaths the stem, resembling *Dactylorhiza cordigera*. The flower bracts are longer than the flowers and purple-pigmented, resembling *Dactylorhiza majalis*. The inflorescence is an elongated, lax raceme, similar in shape and no of flowers to *Dactylorhiza cordigera* parent. The flowers are medium-sized, purple-coloured, intermediate between parental species. The flat, roundish labellum with a white base resembles in shape *Dactylorhiza majalis*, with its lateral lobes scalloped, slightly reflexed upwards. The median lobe is roundish, mildly protruding, another feature inherited from *Dactylorhiza majalis*. Labellar markings also follow *Dactylorhiza majalis*, forming circular loops extended on the lateral lobes. Lateral sepals spread upwards, similar to *Dactylorhiza majalis*. The anther is intermediate between the parents.
Nothopopulation counts: 3-4 individuals/hybrids, flowering each year.
Herbarium voucher specimen: Deposited at the Herbarium of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, N.E. Anghelescu, **USAMVB–barcode 40108**.
Proposed conservation status: Endangered (EN).

× ***Dactylocamptis suzana* N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosp. *nov*.** (Figure 5).

Hybrid formula: *Anacamptis coriophora* subsp. *coriophora* × *Dactylorhiza maculata*
Discovered: 31 May 2020, Nora E. Anghelescu
Locus classicus: Prahova County, private property.
Flowering time: May-June.
Native to: Romania, Endemic (En).
Description: The stem (epigeal), 16 cm, is erect, spindly (resembling *Anacamptis coriophora*), vivid green, with purple apical pigmentation (resembling *Dactylorhiza maculata*). The two basal leaves are narrow, acuminate, vivid green, without pigmentation, a characteristic inherited from *Anacamptis coriophora*. The two cauline unspotted leaves are sessile, vivid green, acuminate, sheathing the stem. The upper one is bract-like.

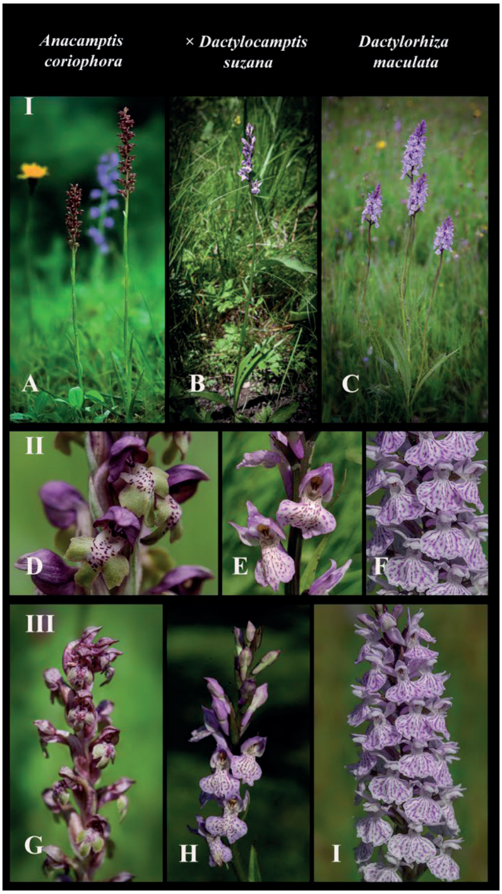

Figure 5. I. Full plants in their natural habitats; II. Individual flower detail; III. Inflorescences details; A., D., G. - *Anacamptis coriophora*; B., E., H. - × *Dactylocamptis suzana*; C., F., I. - *Dactylorhiza maculata*. Photos © Nora E. Anghelescu, 31 May 2020, Prahova County

The inflorescence is an elongated, lax raceme similar to that of the *Anacamptis coriophora* parent. The flowers are medium-sized, light purple-pink, resembling *Dactylorhiza maculata*. The labellum is incurved, three-lobed, dark-spotted, and similar in shape to *Anacamptis coriophora*. The lateral petals and sepals form a tight hood over the gynostemium, resembling *Anacamptis coriophora*. The markings appear as interrupted dots that faintly create elongated loops, reflecting the typical labellar patterns of *Dactylorhiza maculata*. The spur is elongated and downcurved, resembling *Dactylorhiza maculata*, but remains thick, robust, and conical, similar to *Anacamptis coriophora.* The lateral petals and sepals also form a hood over the gynostemium, as seen in *Anacamptis coriophora*.

Nothopopulation counts: 1 individual/hybrid, flowering each year.

Herbarium voucher specimen: Deposited at the Herbarium of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, N.E. Anghelescu, **USAMVB–barcode 40106**.

Proposed conservation status: Endangered (EN).

× *Dactylanthera transsilvanica* L. Balogh & Mih. Balogh, N. Anghelescu, N. Kigyossy, 2024 nothosp. *nov.* (Figure 6).

Hybrid formula: *Dactylorhiza maculata* × *Platanthera bifolia.*

Discovered: 30 May 2020, Lori & Mihaela Balogh.

Locus classicus: Harghita County, private property.

Flowering time: May-June.

Native to: Romania, Endemic (En).

Description: The stem (epigeal), 46 cm is erect, slender/spindly, vivid green, resembling *Platanthera bifolia*, with purple apical pigmentation, resembling *Dactylorhiza maculata*. The basal leaf is vivid green, broad in the middle, with a more roundish apex, resembling *Platanthera bifolia;* the cauline leaves are narrow, acuminate, faintly pigmented, with elongated brownish maculae, a feature inherited from *Dactylorhiza maculata*. The three sessile, unspotted cauline leaves are vivid green and sheath the stem; the upper one is narrow-acuminate, bract-shaped. The inflorescence is an elongated, cylindrical, medium-dense raceme similar to that of the *Platanthera bifolia* parent. The flowers are medium-sized and light pink, intermediate between the two parental species, with a pale/whitish colour reminiscent of *Platanthera bifolia*. The labellum is very faintly three-lobed, with a more prominent, elongated, and downward-pointing median lobe resembling that of the *Platanthera bifolia* parent. The lateral sepals spread laterally, similar to both parental species. The median sepal is more elongated and, along with the narrower acuminate lateral petals, forms a loose hood over the gynostemium, resembling more *Platanthera bifolia* parent. The anther is more elongated than that of *Dactylorhiza maculata*, thus resembling

Platanthera bifolia. The pair of pollinia is light pinkish, resembling in shape and colour that of *Dactylorhiza maculata*. The spur is robust, cylindrical and more elongated, reminiscent of *Platanthera bifolia*, however, slightly downward pointing, resembling *Dactylorhiza maculata*. Overall, the hybrids' stems and leaves phenotypically resemble the *Dactylorhiza maculata* parent more closely, with significant resemblances to *Platanthera bifolia* in the inflorescences.

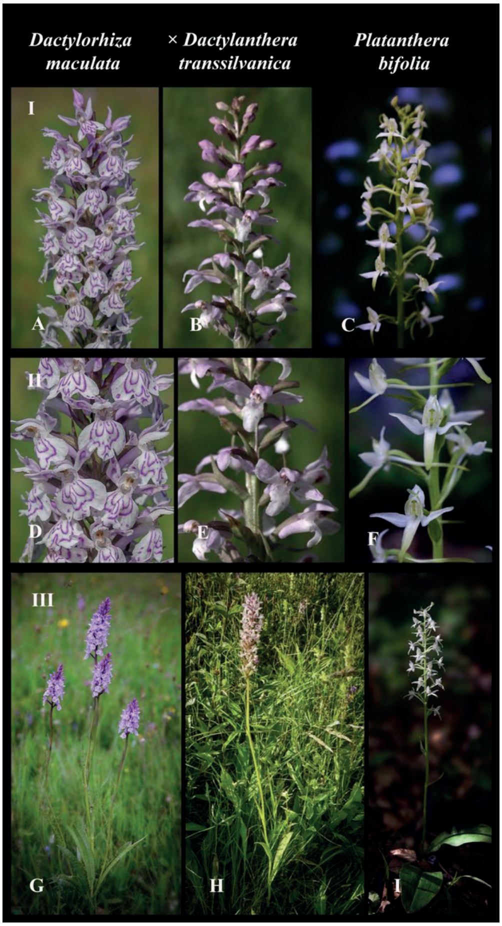

Figure 6. I. Inflorescences details; II. Individual flower detail; III. Full plants in their natural habitats; A., D., G. - *Dactylorhiza maculata*; B., E., H. - × *Dactylanthera transsilvanica*; C., F., I. - *Platanthera bifolia*. Photos © Lori & Mihaela Balogh, 30 May 2020, Harghita County

Nothopopulation counts: 1 individual/hybrid, flowering each year.
Herbarium voucher specimen: Deposited at the Herbarium of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, N.E. Anghelescu, **USAMVB–barcode 40110**.

Proposed conservation status: Endangered (EN).

Gymnadenia* × *montana* N.Anghelescu, L.Balogh, M.Balogh & N.Kigyossy, 2024 nothosp. *nov. (Figure 7).

Figure 7. I. Full plants in their natural habitats; II. Inflorescences details; III. Individual flower detail; A., D., G. - *Nigritella nigra* subsp. *bucegiana* (Hedren et al., 2022); B., E., H. - *Gymnadenia* × *montana*; C., F., I. - *Gymnadenia winkeliana* (Anghelescu et al., 2024). Photos © Nora E. Anghelescu, 29 June 2023, Prahova County

Hybrid formula: *Gymnadenia winkeliana* × *Nigritella nigra* subsp. *bucegiana.*
Discovered: 29 June 2023, Nora E. Anghelescu.
Locus classicus: Bucegi Natural Park ROSCI0013.
Flowering time: June-July.
Native to: Romania, Endemic (En).
Description: The stem (epigeal), 8-12(15) cm is erect, vivid green, with no purple apical pigmentation. 6-10 basal leaves, grass-like, vivid-green; 3-5 cauline unspotted leaves, sessile, vivid green, acuminate, sheath the stem. The roundish, hemispherical-shaped inflorescence (resembling *Gymnadenia winkeliana* parent) is a dense raceme, resembling both parents of the former subgenus

Nigritella. The flowers exhibit an overall, nearly uniform color, blending whitish-pink tones from *Gymnadenia winkeliana* and uniformly dark red hues from *Nigritella nigra* subsp. *bucegiana*, shifted toward violet (bluish) purple. The labellum presents a broader and larger shape than *Gymnadenia winkeliana*, acting as an intermediate form between the parental species, with a tendency towards the features of *Nigritella nigra* subsp. *bucegiana*. The diameter of the labellar opening or tunnel, created by the lateral labellar lobes, is somewhat wider than that of *Gymnadenia winkeliana*, closely resembling the broader and more elongated labellar opening of the *Nigritella nigra* subsp. *bucegiana* parent. The tepals are slightly narrower and more elongated, displaying an intermediate size between the two parental species. The distinctive parallel venation, marked by two parallel veins on the upper side of the lateral petals, is a trait inherited from the *Nigritella nigra* subsp. *bucegiana* parent, absent in *Gymnadenia winkeliana*.
Nothopopulation counts: 4-5 individuals/hybrids, flowering each year.
Herbarium voucher specimen: Deposited at the Herbarium of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, N.E. Anghelescu, **USAMVB–barcode 40103.**
Proposed conservation status: Endangered (EN).

***Epipactis* × *romanensis* R. Maalouf, N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosubsp. nov. (**Figure 8).

Hybrid formula: *Epipactis atrorubens* × *Epipactis distans*.
Discovered: 12 July 2022, Ramy Maalouf.
Locus classicus: Bucegi Natural Park ROSCI0013.
Flowering time: June-July.
Native to: Romania, Endemic (En).
Description: Stem (epigeal) measures 56 cm, erect, and green. It has one basal acuminate leaf and 7-8 unspotted, sessile cauline leaves that are green with parallel venation, ovoid-elongate shape, and acuminate tips, resembling *Epipactis atrorubens*. The phyllotaxis, which refers to the arrangement of leaves on the plant stem (Anghelescu et al., 2023), is alternate, similar to *Epipactis distans*.

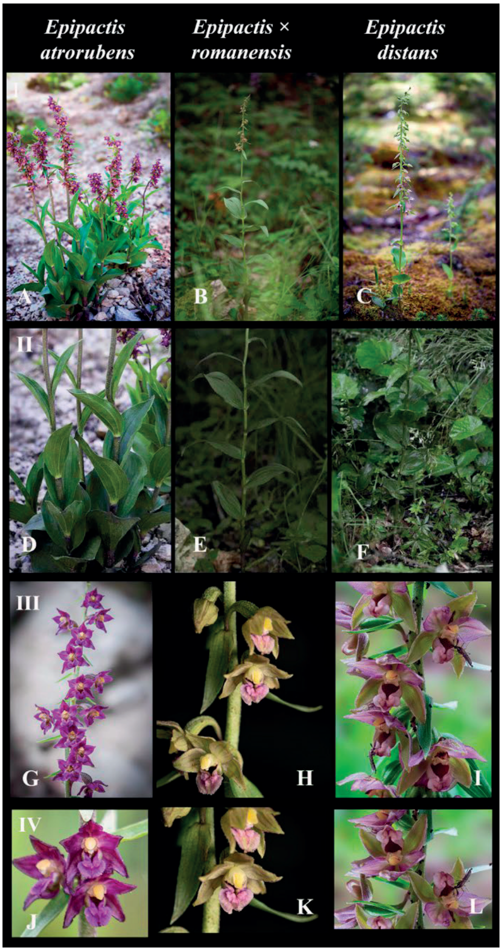

Figure 8. I. Full plants in their natural habitats; II. Details of individual phyllotaxis; III. Inflorescences details; IV. Individual flower detail. A., D., G. J. - *Epipactis atrorubens;* B., E., H. K. - *Epipactis* × *romanensis*; C., F., I. J. - *Epipactis distans*. Photos © Nora E. Anghelescu, 29 June 2022-2024, Prahova County

The inflorescence is a lax raceme, producing fewer flowers - 12-15 medium-sized blooms that resemble those of *Epipactis distans*. The flower colour is an intermediate shade between the parental species. The sepals and lateral petals are rhomboidal-acuminate, greenish with a light pink tint, and centrally green-veined, resembling *Epipactis distans*. The labellum is divided into three segments: the base, or hypochile, contains nectar similar to both parental species; the middle part, or mesochile, has deeply incurved lateral walls, is narrower than that of *Epipactis atrorubens* and is more similar to *Epipactis distans*; the apical part, or epichile, is heart-

shaped, pinkish-green, reflecting the deep red colour inherited from *Epipactis atrorubens*. The labellum features central calli with shapes intermediate between those of the parent species, specifically wrinkled and resembling more closely those of *Epipactis atrorubens*. The flower bracts are lanceolate-elongate, akin to *Epipactis distans*. The flower pedicel and ovary are tinged purple and covered in trichomes (glandular hairs), primarily a characteristic inherited from the *Epipactis atrorubens* parent.
Nothopopulation counts: 2-3 individuals/hybrids, flowering each year.
Herbarium voucher specimen: Deposited at the Herbarium of the University of Agriculture and Veterinary Medicine, Bucharest, N.E. Anghelescu, **USAMVB–barcode 40107**.
Proposed conservation status: Endangered (EN).

Ophrys* × *minuticauda* nothosubsp. *cornuta* R. Maalouf, N. Anghelescu, L. Balogh & Mih. Balogh, N. Kigyossy, 2024 nothosubsp. *nov. (Figure 9).

Figure 9. 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: *Ophrys apifera* × *Ophrys scolopax* subsp. *cornuta.*
Discovered: 5 June 2021, Ramy Maalouf.
Locus classicus: Bucegi Natural Park ROSCI0013.
Flowering time: May-June.
Native to: Romania, Endemic (En).
Description: Stem (epigeal), 25-30 cm, erect, vivid green. 1 basal leaf, sheathing the stem, 3-5 cauline leaves, unspotted, sessile, green, acuminate (resembling *Ophrys apifera*). Inflorescence lax raceme, less floriferous, 4-5 medium-sized flowers, resembling *Ophrys apifera*, smaller than *Ophrys scolopax* ssp. *cornuta.* Sepals light-pink, centrally green veined, resembling *Ophrys* apifera (Anghelescu et al., 2021c). Lateral petals are greenish, villous (resembling *Ophrys apifera*, triangular and equal to those of *Ophrys scolopax* subsp. *cornuta*. Labellum three-lobed, median lobe slightly elongated, speculum bluish, resembling *Ophrys scolopax* subsp. *cornuta*. Lateral lobes are pointed, conical-elongated, shorter, and resemble *Ophrys scolopax* subsp. *cornuta*. Basal field orange-brown, wider, resembling *Ophrys apifera*. Appendix oblique to the labellum, intermediate between parents. Pollinia with shorter caudicle, overhanging resembling *Ophrys apifera* (correction of the original taxonomical description in Anghelescu et al., 2023, pg. 422).
Nothopopulation counts: 1 individual/hybrid, flowering each year.
Herbarium voucher specimen: Deposited at the Herbarium of the Botanical Garden Bucharest, N.E. Anghelescu, barcode: **BUC 410370**.
Proposed conservation status: Endangered (EN).

CONCLUSIONS

This study highlights the intricate complexities and ecological significance of the newly discovered nothospecies within the orchid hybrid populations. Through detailed morphological analysis and comprehensive examination of their biological characteristics, we have provided essential insights into their unique adaptations and ecological requirements. The findings not only contribute to the understanding of hybrid dynamics within the orchid family but also emphasize the necessity for ongoing conservation efforts to protect these rare taxa. As we advocate for their formal recognition in the Romanian flora, we must prioritize the preservation of their habitats and ensure that future research continues to explore the rich biodiversity these hybrids represent. By fostering awareness and protective measures, we can safeguard the future of these remarkable and vulnerable orchid hybrids for generations to come. In conclusion, the recognition of these hybrids as new additions to the Romanian flora underscores the importance of conservation efforts aimed at protecting their habitats and promoting further research. These findings not only contribute to our understanding of hybridization processes but also maintain the ecological balance and genetic diversity within the region's ecosystems. As ongoing investigations continue to unveil the complexities of orchid hybridization, they lead the way for a deeper appreciation of Romania's rich plant heritage.

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A MULTIPLE LINEAR REGRESSION MODEL TO ESTIMATE THE PLANT COVERAGE OF A GREEN WALL SYSTEM

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Abstract

Recently, green walls have come to the attention of researchers from several fields, as a result of the concerted effort to find viable solutions to stop/mitigate urban pollution that produces numerous negative effects. The viability of a green wall consists in the use of plants with a high aesthetic appearance, which are resistant to environmental conditions and ensure a quick and compact coverage. The present paper proposes a multiple linear regression model for the plant coverage of a vertical system in which the soil moisture and temperature are explanatory variables. The questions that we address and answer in this paper are related to the dependence of the plant coverage on the orientation of the wall and the influence of the plant coverage on the temperature inside a green wall system.

Key words*: green walls, multiple linear regression model, plant coverage.*

INTRODUCTION

The use of statistical techniques in landscape research helps researchers to establish links between the parameters used, between the chosen variants, to demonstrate the strength of the connections and to characterize their evolution in a unitary and correct way within the experiment. The mere simple description of the execution steps, the observation of the phenomena and their narration within research do not manage to present in a scientific way all the particularities that appear in the development of the living systems.

To highlight the relationship that exists between two or more data sets, mathematical methods of analysis such as correlation analysis or regression analysis are needed (Wackerly et al., 2007). Any researcher wants to use the results obtained in a project to determine whether or not there are connections between the measured data sets, as well as the strength of the determined connections. The dependence or independence of variables is an important goal in any research and it is necessary to resort to mathematical modeling. A series of studies already published, with applicability in the horticultural field, highlight such connections. Thus, for a good characterization of the chemical composition, pH, ascorbic acid and total phenolics for three species of wild berries according to precipitation, air temperature, atmospheric pressure and wind speed, Tripon and colleagues used multiregression analysis and simple correlation. The conclusion of this research was that only precipitation and air temperature influence wild berries dry matter content (Tripon, 2022).

Another study highlights the variation scanning the binden leaf area and cumulative scan leaf area in relation to the position of the leaf on the shoot and cumulative shoots length was evaluated by regression analysis by Rosu and collaborators. Leaves deviating from the theoretically determined model were determined (Rosu, 2022). Also, a study carried out on tomatoes reveals that productivity and yield in tomatoes can be modeled mathematically using the interaction between humidity and water deficit for three levels of fertilizer. Thus, it was found that the optimal irrigation rate and 73% manure represent a good combination (Stoyanova, 2019). Another research in this sense was carried out in the Jidvei viticultural center, Tarnave region, where the Feteasca regala variety was studied. Using linear regression, it was established that the relationships between global solar radiation and parameters: sugars and global acidity are very strong (Ropan, 2023). Also, the relationships

between global solar radiation and alcohol concentration, total acidity and reduced dry extract have strong relationships with significant statistical correlations.
Different species of plants develop differently in similar conditions; the same species change their way of evolution if they are placed in new conditions of stress. All these changes that occur are determined correctly and quickly by statistical techniques such as correlations and regressions.
In our project, the plants were removed from the traditional way of planting (in soil) and were included in a planting system on a vertical wall made up of 4 facades, each facing a cardinal point. This way of alignment was chosen in order to observe the changes that take place in the evolution of plants, in the way of development on such a system. The aesthetic aspect of green walls is extremely important. and the degree of plant coverage of the wall in a certain period of time contributes significantly to their visual quality.
In urban areas, it is hard to find available land for new parks and other traditional green areas. Thus, vegetation has been extended to other surfaces, such as roofs and facades of buildings (Ghazalli et al., 2019). The use of roofs and facades of buildings for the expansion of green areas could be a remedy for the greening of the habitat, as well as for a high aesthetic aspect of the cities we live in (Pérez et al., 2020; Francis and Lorimer, 2011). Green wall systems are sustainable solutions (Palermo and Turco, 2020; Sheweka and Nourhan, 2012) and could be a component of modern urban design (Perini et al., 2011) with many benefits for city residents (Fan et al., 2011; Perez-Urrestarazu et al., 2015).
During the course of the experiment, the influence of environmental factors and the substrate used on the behavior of the plants used in the vertical system was observed. The monitoring was done with the help of two different devices, every three days, in the same time interval. The research carried out in the experiment is complex, and many of the results have already been published. The behavior of perennial flowering species such as *Heuchera x hybrida* "Fire Alarm", *Festuca glauca*, *Sedum spurium* "Tricolor", *Carex testacea, Polystichum aculeatum* (Cojocariu et al., 2022a) and *Cineraria maritima* was monitored on the experimental structure, as well as annuals such as *Plectranthus forsteri*, *Coleus blumei* (Cojocariu et al., 2022b), *Begonia sempervirens*, and other.
In another work we compared the percentage of plant coverage (PCP) determined by a multilinear regression (MLR) and by a modeling with artificial neural networks (ANN) using confidence intervals. The model obtained by ANN is more accurate in predicting the phenomenon than the model obtained by multilinear regression (Chiruţă et al., 2023).
In this article, in order to be able to answer a series of questions, we had at our disposal the measurements made on the organic mixture used in the system (humidity and temperature), the internal temperature, the system temperature, the ambient temperature (from the Iasi National Meteorological Center) and the measured percentage of plant coverage. The tests we made with linear regression gave interesting results that we have centralized in this material.
Starting from our dataset, our aim was to build the best possible multiple linear regression model, for each cardinally oriented face of the green wall system. Based on these models, we want to explain the variation of the plant cover percentage (PCP) determined by the soil moisture, the soil temperature for each facade of the system and the time in the year these variables were measured.
Because the pH and the moisture content of the organic mixture were highly correlated ($r = -0.9233, p < 0.001$), they were not considered in any of the models. Nutrient substrate moisture and temperature had the greatest influence in predicting percent plant coverage for each system facade. Other important variables for the evolution of the plant coverage percentage were the time of year in which each observation was made and the cardinal direction of each facade of the system were added in the mathematical modelling.
In this article, we try to answer the following questions: Q1: Does the plant coverage percentage depend on orientation of the facade of the GWS [North (N), East (E), South (S), West (W)]? Q2: Does the plant coverage percentage influence the inner temperature of the GWS? Q3: Can one build good regression models of PCP solely on soil Humidity and soil Temperature as explanatory variables? Q4: Will

there be an improvement in the regression models if we also take into account the time of the year when these variables were measured?

In order to determine the influence of environmental factors and the substrate used on the behaviour of plants, the temperatures recorded in the area of the city of Iasi (Temp Iasi) were taken into account - data received from the National Meteorology Center Iasi.

Table 1. Temperatures in the experiment area between 2020-2021

	Temp Iasi 2020	Temp Iasi 2021
Mean	12.16	10.33
Minimum	-4.2	-11.1
Maximum	27.6	27.3
CI mean	12.16 ± 0.87	10.33 ± 0.91

MATERIALS AND METHODS

Experimental setup

Our experiment took place in open air, in the didactic field of the Floriculture department of the Faculty of Horticulture within IULS - Iasi University of Life Sciences (decimal GPS lat. N 47.1941, long. E 27.5555). It observed the behavior of some flower species planted vertically, in local climatic conditions, with the aim of including them in an assortment of ornamental species that can be successfully used in the decoration of green facades. Resistant flower species with minimal maintenance needs and high decorative potential were used such as *H. x hybrida* "Fire Alarm", *S. spurium* "Tricolor", *C. maritima, P. aculeatum, P. forsteri, C. blumei*. The flower species can also decorate by their habitus, or by the shape and color of their leaves (Draghia and Chelariu, 2011). This category also includes ornamental grasses. In Romania, their culture is slowly starting to make its presence felt (Chelariu, 2018), especially for their low maintenance and the high degree of resistance to environmental factors they exhibit. For these reasons, species from the category of ornamental grasses such as: *F. glauca* and *C. testacea* were tested on the vertical structure.

The first set of plants, consisting of the species *H. x hybrida* "Fire Alarm", *F. glauca, S. spurium* "Tricolor", *C. Testacea* and *P. aculeatum* were placed on the vertical structure at the end of autumn 2019. Due to the low rate of survival in the vertical system, *C. testacea* was replaced, between June and November 2020, with the annual species *Begonia semperflorens* ("Big"). Later, the assortment was enriched with new plants, so that in 2021, the following species were on the facades of the experimental module: *B. semperflorens, H. x hybrida* "Fire Alarm", *C. maritima, P. fosteri, C. blumei* and *F. glauca* (Chiruță et al., 2023).

The experimental structure was built out of heat-insulating panels, especially for this study. The facades of the structure are of equal size; each face being composed of four overlapping landings. The layout of the structure on the ground was made so that each facade is oriented towards a cardinal point.

The flower species were planted on each landing in equal and uniform numbers. Plots had identical organic matter within the experimental scheme. During the study, catch/attachment percentage, degree of cover, biometric aspects and visual quality of the mentioned species were monitored and comparisons were made between the cardinal orientations.

Data on the evolution of plant characteristics were collected every 3 days at mid-day. The characteristics of organic matter (moisture, temperature, pH) were also measured with the RZ89 4 in 1 3.5-9 ~ 9 pH Meter Digital Magnetic Soil Health Analyzer Machine Soil Moisture Ammonitor Hygrometer Gardening Plant Tester, as well as the internal and external temperatures of the green wall system, for which the Somogyi Elektronic Home HC 12 device with a resolution of 0.1°C was used.

Multiple linear regression (MLR)

Regression analysis aims to determine the relationship between two (or more) variables of interest, in order to obtain information about one of them from the values of the other(s). The regression is called to be linear when the response variable depends linearly on the parameters. The general equation for a multiple linear regression model with a dependent variable Y^* and m independent variables (or stimuli), denoted by X_k, $k = 1 \ldots m$, is

$$Y^* = b_0 + b_1X_1 + b_2X_2 + \ldots + b_kX_k + b_mX_m \, , \quad (1)$$

where b_k, $k = 1 \dots m$, are called the regression parameters. Each parameter b_k can be interpreted as the expected change in response Y^* associated with a 1-unit increase in X_k, while the other stimuli are held constant. For a given model, the difference between the observed value of Y and the model-predicted value, Y^*, at the same given point, is called the residual.

In a good MLR model, each independent variable explains part of the variation in the dependent variable. If the change in the mean value of Y^* associated with a 1-unit increase in one independent variable (say, X_1) depends on the value of a second independent variable (say, X_2), then there is interaction between these two variables. One can incorporate this interaction into the MLR model by including the product of the two independent variables, $X_1 X_2$.

To quantify how well a multiple linear regression model fits a data set, we calculate the coefficient of determination R^2 and test the utility of the model. For a good model, we want R^2 to be close to 1 and the *F-statistic* for the utility test to be high enough. For more details on the multiple linear regression models, see (Devore, 2012).

The non-parametric one-way ANOVA

The data is grouped according to the cardinal orientation of the facade of the green wall system. Our first task is to check whether there are significant differences between the average plant cover percentage for the four facades. To this aim, we shall perform the Kruskal-Wallis test. This test provides a nonparametric alternative to the one-way ANOVA. We use this test as not all the requirements for the application of the ANOVA test are met. More specifically, the data is not normally distributed. Within the vertical system we have 4 facades from which we randomly sampled the data, so there are 4 categories of data that we have to compare.

The requirements for the application of the Kruskal-Wallis test are: (1) all samples were randomly selected, (2) the observed values in the samples are independent (which is true, as they were collected independently), and (3) all groups should have similar shape distributions. The last condition could be observed from Figure 1, where we have drawn the boxplots for the four groups of data. From Figure 1 we also observe that there are no outliers in the data.

Since it is a nonparametric test, the Kruskal-Wallis test does not assume a normal distribution of the residuals, unlike the parametric version of this test, the one-way ANOVA test.

The hypotheses for the Kruskal-Wallis test are as follows:

H_0: the four samples originate from the same distribution (*the null hypothesis*)

H_1: at least one sample stochastically dominates one other sample (*the alternative hypothesis*).

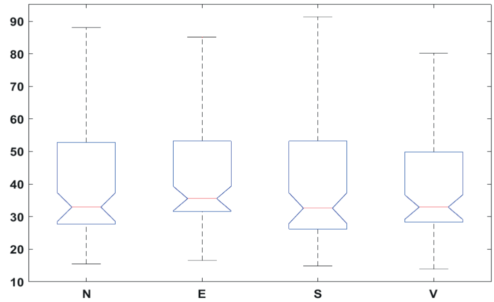

Figure 1. Box-plot for the PCP for each side of the GWS

For the Kruskal-Wallis test and the MLR models derived in this paper we have used the MATLAB software v9.8.0 (R2020a) with the Statistics Toolbox.

RESULTS AND DISCUSSIONS

Dependence of the plant coverage percentage on the orientation of the facade

Our first question is whether the plant coverage depend on orientation (N, E, S, W) of the facade of GWS. To answer this, we have performed the Kruskal-Wallis test, a non-parametric variant of the 1-way ANOVA test. This analysis was carried out for the data collected during the period 2020-2021. The table below shows the Kruskal-Wallis ANOVA test results (Figure 2). The p-*value* for the equality of mean coverage percentages on each of the GWS sides is $p = 0.2755$. The highest observed difference in the mean coverage percentage is 2.74% (between E and W), but it is not significant at the 5% level. In conclusion, the degree of plant cover of the green system does not depend on the cardinal orientation at a significance level of 0.05.

Kruskal-Wallis ANOVA Table

Source	SS	df	MS	Chi-sq	Prob>Chi-sq
Columns	33987.1	3	11329.04	3.87	0.2755
Error	2800140.4	320	8750.44		
Total	2834127.5	323			

Figure 2. The Kruskal-Wallis test table

Correlation between the plant coverage percentage and the inner temperature of the GWS

We are now interested to see whether the plant coverage influence the inner temperature of the GWS. To answer this question, we have calculated the Pearson's correlation coefficient between the inner temperature inside the GWS and the plant coverage percentage for each of the GWS faces. We have carried out this calculation for three types of data: firstly, for the data collected during a 2-year period 2020-2021 (Table 1), secondly, only for the data collected in 2020 (Table 2), and thirdly, only for the data collected in 2021 (Table 3). We observe that, with only one exception, the inner temperature and the plant coverage of the faces are linearly correlated, though not strongly correlated.

Table 1. Pearson correlation coefficients for temperatures and plant coverage percentage for two years (2020-2021)

2020-2021 (2 years)		**Pearson's coefficient**	**Significance** (p-value)
Plant coverage percentage for GWS	*North*	0.0853	0.4488
	East	0.1265	0.2605
	South	0.0988	0.3804
	West	0.0465	0.6800
	total **GWS** *coverage*	0.0914	0.4170

Table 2. Pearson correlation coefficients for temperatures and plant coverage percentage for 2020 only

2020		**Pearson's coefficient**	**Significance** (p-*value*)
Plant coverage percentage for GWS	*North*	-0.2223	0.1681
	East	-0.2707	0.0912
	South	-0.1632	0.3143
	West	-0.2972	0.0625
	total **GWS** *coverage*	-0.2368	0.1413

Table 3. Pearson correlation coefficients for temperatures and plant coverage percentage for 2021 only

2021		**Pearson's coefficient**	**Significance** (p-*value*)
Plant coverage percentage for GWS	*North*	0.3047	0.0527
	East	0.3220	0.0401*
	South	0.2773	0.0793
	West	0.2493	0.1160
	total **GWS** *coverage*	0.2911	0.0649

*Level of significance 0.05

Regression models of PCP on soil Humidity and soil Temperature

For each face of the GWS, we perform linear regression of the plant coverage percentage (PCP) on soil humidity (H) and the soil temperature (T). We shall consider two models, with or without interaction between the independent variables. For the reliability of the models, the data has been transformed to look fairly normal, using the Box-Cox transformation. All the models below are in terms of the transformed variables.

If v is the Box-Cox transformed variable of u (of parameter λ), then:

$$\mathrm{v} = \begin{cases} \ln \boldsymbol{u}, & \boldsymbol{if\ \lambda = 0} \\ \dfrac{\boldsymbol{u^{\lambda} - 1}}{\boldsymbol{\lambda}}, & \boldsymbol{if\ \lambda \neq 0} \end{cases} \qquad (2)$$

The inverse transform is

$$\mathrm{u} = \begin{cases} \mathbf{e}^{\boldsymbol{v}}, & \boldsymbol{if\ \lambda = 0} \\ \boldsymbol{exp}\left(\dfrac{\boldsymbol{ln(1+\lambda v)}}{\boldsymbol{\lambda}}\right), & \boldsymbol{if\ \lambda \neq 0} \end{cases} \qquad (3)$$

In all models below, we denote by x_1, x_2 and y the Box-Cox transformed variables of H, T and PCP (with parameters $\boldsymbol{\lambda_H}$, $\boldsymbol{\lambda_T}$, $\boldsymbol{\lambda_{PCP}}$), respectively. So, from now on we shall work with the transformed variables x_1, x_2 and y.

We are interested in finding regression models (with or without interactions) of the form

$$\boldsymbol{y = a \cdot x_1 + b \cdot x_2 + c \cdot x_1 \cdot x_2}. \qquad (4)$$

In the original variables, this regression model takes the form:

$$\frac{PCP^{\lambda_{PCP}}-1}{\lambda_{PCP}} = a\cdot\frac{H^{\lambda_H}-1}{\lambda_H}+b\cdot\frac{T^{\lambda_T}-1}{\lambda_T}+c\cdot\frac{H^{\lambda_H}-1}{\lambda_H}\cdot\frac{T^{\lambda_T}-1}{\lambda_T} \quad (5)$$

i.e.

$$PCP^{\lambda_{PCP}} = A\cdot H^{\lambda_H} + B\cdot T^{\lambda_T} + C\cdot H^{\lambda_H}\cdot T^{\lambda_T} + D \quad (6)$$

where *A, B, C, D* are depending on the parameters *a, b, c*, λ_H, λ_T, λ_{PCP}. Then, for all the models below, the estimated PCP by the model will be:

$$PCP = (A\cdot H^{\lambda_H} + B\cdot T^{\lambda_T} + C\cdot H^{\lambda_H}\cdot T^{\lambda_T} + D)^{1/\lambda_{PCP}} \quad (7)$$

Model 1 (north side plant coverage percentage model for GWS without interactions among independent variables and no intercept)
The regression model without interactions among the variables and no intercept has the form:

$$y = a\cdot x_1 + b\cdot x_2 \quad (8)$$

The estimated model parameters are given in Table 4.

Table 4. Parameters for linear regression without interactions

	Estimate	SE	tStat	pValue
a	0.11454	0.021727	5.2718	$1.1454\cdot10^{-6}$*
b	0.14164	0.028467	4.9756	$3.7226\cdot10^{-6}$*

*Level of significance 0.05

Other relevant statistics: number of observations = 81, RMSE = 0.86, R^2 = 0.5185, adjR^2 = 0.5125.
Therefore, the model is:

$$y = 0.11454\cdot x_1 + 0.14164\cdot x_2 \quad (9)$$

For example, if H = 4 g/kg and T = 12.5°C, then the estimated model value for PCP = 14.72%.

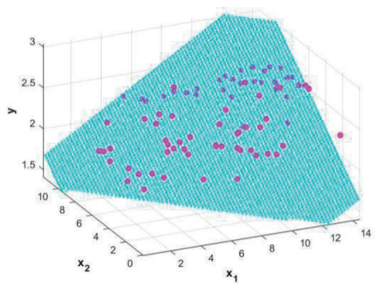

Figure 3. The regression (without interactions) GWS, north side

Model 2 (north side plant coverage percentage model for GWS with interactions among independent variables, no intercept)
The regression model with interactions among the variables and no intercept has the form:

$$y = a\cdot x_1 + b\cdot x_2 + c\cdot x_1\cdot x_2. \quad (10)$$

The estimated model parameters are given in Table 5.

Table 5. Parameters for linear regression with interactions

	Estimate	SE	tStat	pValue
a	0.24612	0.017285	14.239	$2.1406\cdot10^{-23}$*
b	0.30603	0.02221	13.779	$1.3503\cdot10^{-22}$*
c	-0.031526	0.0026874	-11.731	$6.7192\cdot10^{-19}$*

*Level of significance 0.05

Other relevant statistics: number of observations = 81, RMSE = 0.521, R^2 = 0.5034, adjR^2 = 0.4906.
Therefore, the model is:

$$y = 0.24612\cdot x_1 + 0.30603\cdot x_2 - 0.031526\cdot x_1\cdot x_2 \quad (11)$$

For example, if H = 4 g/kg and T = 12.5°C, then the estimated model value for PCP = 46.25%.

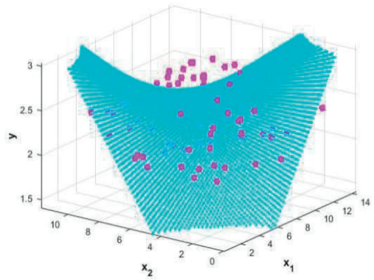

Figure 4. The regression (with interactions) GWS, north side

Model 3 (east side plant coverage percentage model for GWS without interactions among independent variables, no intercept).
The regression model without interactions among the variables and no intercept has the form:

$$y = a\cdot x_1 + b\cdot x_2. \quad (12)$$

The estimated model parameters are given in Table 6.

Table 6. Parameters for linear regression without interactions

	Estimate	SE	tStat	pValue
a	0.22674	0.046315	4.8957	$5.0893\cdot10^{-6}$*
b	0.18859	0.027168	6.9419	$9.6498\cdot10^{-10}$*

*Level of significance 0.05

Other relevant statistics: number of observations = 81, RMSE = 1.19, R^2 = 0.4905, adjR^2 = 0.4840.
Therefore, the model is:

$$y = 0.22674 \cdot x_1 + 0.18859 \cdot x_2 \quad (13)$$

If H = 4 g/kg and T = 12.5°C, then the estimated model value for PCP = 18.51%.

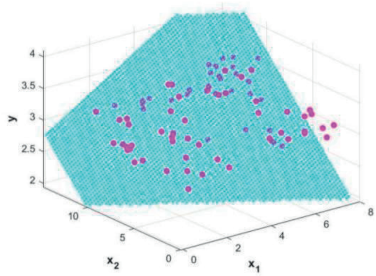

Figure 5. The regression (without interactions) GWS, east side

Model 4 (east side plant coverage percentage model for GWS with interactions among independent variables, no intercept)
The regression model (*with interactions among independent variables*) has the form:

$$y = a\,x_1 + b\,x_2 + c\,x_1 x_2 \quad (14)$$

The estimated model parameters are given in Table 7.

Table 7. Parameters for linear regression with interactions

	Estimate	SE	tStat	pValue
a	0.49591	0.043426	11.419	$2.555\cdot10^{-18}$*
b	0.34883	0.025645	13.603	$2.7549\cdot10^{-22}$*
c	-0.056394	0.0061034	-9.2399	$3.6987\cdot10^{-14}$*

*Level of significance 0.05

Other relevant statistics: number of observations = 81, RMSE = 0.827, R^2 = 0.4612, adjR^2 = 0.4473.
Therefore, the model is:

$$y = 0.49591 \cdot x_1 + 0.34883 \cdot x_2 - 0.056394 \cdot x_1 \cdot x_2 \quad (15)$$

If H = 4 g/kg and T = 12.5°C, then the estimated model value for PCP = 34.95%.

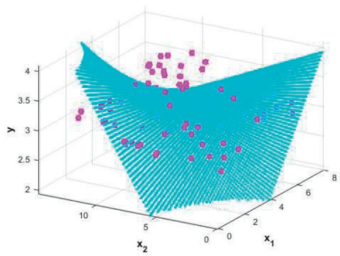

Figure 6. The regression (with interactions) GWS, east side

Model 5 (south side plant coverage percentage model for GWS without interactions among independent variables, no intercept)
The regression model (without interactions among independent variables) has the form:

$$y = a\,x_1 + b\,x_2 \quad (16)$$

The estimated model parameters are given in Table 8.

Table 8. Parameters for linear regression without interactions

	Estimate	SE	tStat	pValue
a	0.06481	0.013107	4.9448	$4.2008\cdot10^{-6}$*
b	0.11704	0.020225	5.787	$1.3813\cdot10^{-7}$*

*Level of significance 0.05

Other relevant statistics: number of observations = 81, RMSE = 0.914, R^2 = 0.5082, adjR^2 = 0.5020.
Therefore, the model is:

$$y = 0.06481 \cdot x_1 + 0.11704 \cdot x_2 \quad (17)$$

If H = 4 g/kg and T = 12.5°C, then the estimated model value for PCP = 10.16%.

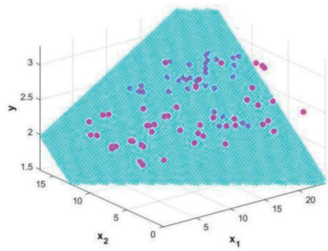

Figure 7. The regression (*without interactions)* GWS, south side

Model 6 (south side plant coverage percentage model for GWS with interactions among independent variables, no intercept)
The regression model (with interactions among independent variables) has the form:

$$y = a\,x_1 + b\,x_2 + c\,x_1 x_2 \quad (18)$$

The estimated model parameters are given in Table 9.

Table 9. Parameters for linear regression with interactions

	Estimate	SE	tStat	pValue
a	0.14085	0.010943	12.871	$5.5242\cdot10^{-21}$*
b	0.23206	0.016748	13.856	$9.8956\cdot10^{-23}$*
c	-0.012987	0.0012055	-10.773	$4.2101\cdot10^{-17}$*

*Level of significance 0.05

Other relevant statistics: number of observations = 81, RMSE = 0.583, R^2 = 0.4623, adjR^2 = 0.4485.

Therefore, the model is:

$$y = 0.14085 \cdot x_1 + 0.23206 \cdot x_2 - 0.012987 \cdot x_1 \cdot x_2 \quad (19)$$

If H = 4 g/kg and T = 12.5°C, then the estimated model value for PCP = 30.54%.

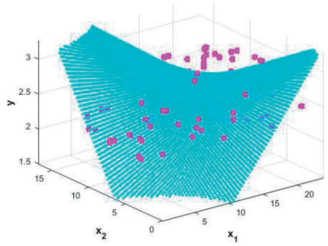

Figure 8. The regression (*with interactions)* GWS, south side

Model 7 (west side plant coverage percentage model for GWS without interactions among independent variables, no intercept)
The regression model (*without interactions among independent variables*) has the form:

$$y = a\,x_1 + b\,x_2 \quad (20)$$

The estimated model parameters are given in Table 10.

Table 10. Parameters for linear regression without interactions

	Estimate	SE	tStat	pValue
a	0.10707	0.01951	5.4881	$4.7567 \cdot 10^{-7}$*
b	0.20832	0.036646	5.6846	$2.1157 \cdot 10^{-7}$*

*Level of significance 0.05

Other relevant statistics: number of observations = 81, RMSE = 1.28, R^2 = 0.5069, adjR^2 = 0.5007.
Therefore, the model is:

$$y = 0.10707 \cdot x_1 + 0.20832 \cdot x_2 \quad (21)$$

If H = 4 g/kg and T = 12.5°C, then the estimated model value for PCP = 16.55%.

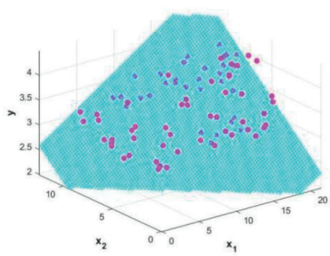

Figure 9. The regression (without interactions) GWS, west side

Model 8 (west side plant coverage percentage model for GWS with interactions among independent variables, no intercept)
The regression model (with interactions among independent variables) has the form:

$$y = a\,x_1 + b\,x_2 + c\,x_1 x_2 \quad (22)$$

The estimated model parameters are given in Table 11.

Table 11. Parameters for linear regression with interactions

	Estimate	SE	tStat	pValue
a	0.23439	0.017735	13.216	$1.3306 \cdot 10^{-21}$*
b	0.40336	0.030502	13.224	$1.2889 \cdot 10^{-21}$*
c	-0.027392	0.0026507	-10.334	$2.8906 \cdot 10^{-16}$*

*Level of significance 0.05

Other relevant statistics: number of observations = 81, RMSE = 0.839, R^2 = 0.4847, adjR^2 = 0.4715.
Therefore, the model is:

$$y = 0.23439 \cdot x_1 + 0.40336 \cdot x_2 - 0.027392 \cdot x_1 \cdot x_2 \quad (23)$$

If H = 4 g/kg and T = 12.5°C, then the estimated model value for PCP = 35.27%.

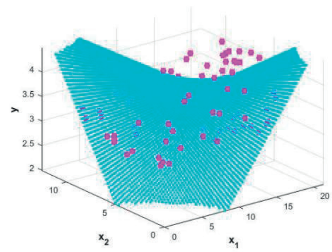

Figure 10. The regression (with interactions) GWS, west side

Regression models of *PCP* on *soil Humidity, soil Temperature* and *WeekNo* (time of the year).

We have available 324 observations that were collected during the years 2020-2021. More precisely, we have 81 values recorded for each face of a GWS.
To be able to give better estimates for the plant coverage percentage for each face of the system, we have introduced a new explanatory variable, called *WkNo*. This variable keeps track of the time of the year when the data was recorded. *WkNo* takes values from 1 (first week of the year) to 52 (last week of the year).
In the following models, we perform linear regression of the plant coverage percentage

(PCP) on soil humidity (Hum), the soil temperature ($Temp$) and the week number (WkNo) for each face of a GWS. We shall consider below a linear regression model of PCP on Hum, $Temp$ and $WkNo$ with an interaction term between $Temp$ and $WkNo$. For each face, the regression models are based on 81 data sets.

In all models below, we shall consider:

Explanatory variables: soil Humidity (*Hum*), soil Temperature (*Temp*), Week number (*WkNo*).

Explained variable: Plant coverage percentage (*PCP*)

The regression model has the general form:

$$PCP = a \cdot Hum + b \cdot Temp + c \cdot WkNo + d \cdot Temp \cdot WkNo. \quad (24)$$

with *a, b, c, d* the regression coefficients to be determined.

Model 1 (plant coverage percentage model for **GWS**, north side):

The estimated model parameters are given in Table 12.

Table 12. Parameters for linear regression with interactions

	Estimate	SE	tStat	pValue
A	8.3459	1.2407	6.7266	$2.7254 \cdot 10^{-9}$*
B	-2.0276	0.52532	-3.8598	0.00023448*
C	0.41093	0.14171	2.8997	0.004865*
D	0.061527	0.016631	3.6996	0.00040312*

*Level of significance 0.05

Other relevant statistics: number of observations = 81, RMSE = 14.1, R^2 = 0.6194, adjR^2 = 0.6046. Therefore, the model is:

$$PCP = 8.3459 \cdot Hum - 2.0276 \cdot Temp + 0.41093 \cdot WkNo + 0.061527 \cdot Temp \cdot WkNo$$

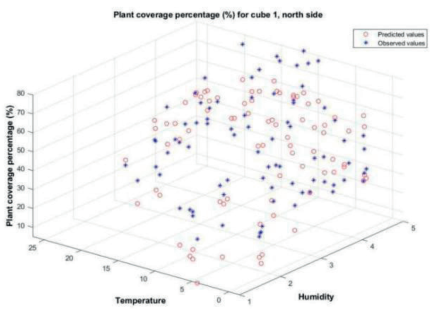

Figure 11. Predicted values vs observed values GWS, noth side

Model 2 (plant coverage percentage model for **GWS**, east side):

The estimated model parameters are given in Table 13.

Table 13. Parameters for linear regression with interactions

	Estimate	SE	tStat	pValue
a	4.4503	1.1693	3.8061	0.00028159*
b	-0.16489	0.50551	-0.32619	0.74516
c	0.82419	0.13345	6.176	$2.8768 \cdot 10^{-8}$*
d	0.010607	0.016575	0.63993	0.52412

*Level of significance 0.05

Other relevant statistics: number of observations = 81, RMSE = 16.6, R^2 = 0.4827, adjR^2 = 0.4625. Therefore, the model is:

$$PCP = 4.4503 \cdot Hum - 0.16489 \cdot Temp + 0.82419 \cdot WkNo + 0.010607 \cdot Temp \cdot WkNo$$

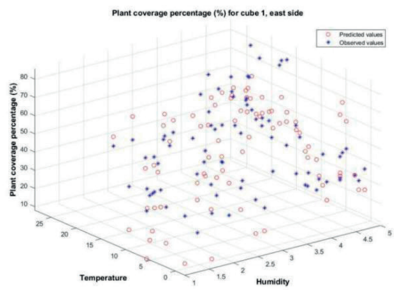

Figure 12. Predicted values vs observed values GWS, east side

Model 3 (plant coverage percentage model for **GWS**, south side):

The estimated model parameters are given in Table 14.

Table 14. Parameters for linear regression with interactions

	Estimate	SE	tStat	pValue
a	5.7456	1.2084	4.7548	$9.0727 \cdot 10^{-6}$*
b	-1.1205	0.47392	-2.3644	0.020581*
c	0.7544	0.1353	5.5758	$3.5032 \cdot 10^{-7}$*
d	0.030779	0.014811	2.0781	0.041031*

*Level of significance 0.05

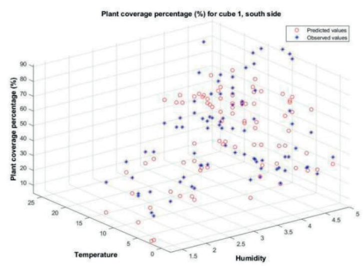

Figure 13. Predicted values vs observed values GWS, south side

Other relevant statistics: number of observations = 81, RMSE = 15.1, R^2 = 0.5812, adjR^2 = 0.5646. Therefore, the model is:

$$\boldsymbol{PCP = 5.7456 \cdot Hum - 1.1205 \cdot Temp + 0.7544 \cdot WkNo + 0.030779 \cdot Temp \cdot WkNo}$$

Model 4 (plant coverage percentage model for **GWS**, west side)

The estimated model parameters are given in Table 15.

Table 15. Parameters for linear regression with interactions

	Estimate	SE	tStat	pValue
a	6.6578	1.0354	6.43	$9.7645 \cdot 10^{-9}$*
b	-1.2003	0.45307	-2.6492	0.0097868*
c	0.54673	0.12459	4.3884	$3.5857 \cdot 10^{-5}$*
d	0.034149	0.014662	2.329	0.022481*

*Level of significance 0.05

Other relevant statistics: number of observations = 81, RMSE = 12.9, R^2 = 0.6074, adjR^2 = 0.5921. Therefore, the model is:

$$\boldsymbol{PCP = 6.6578 \cdot Hum - 1.2003 \cdot Temp + 0.54673 \cdot WkNo + 0.034149 \cdot Temp \cdot WkNo}$$

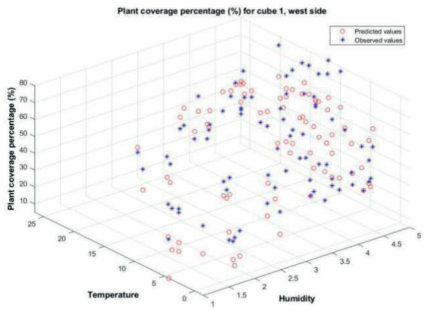

Figure 14. Predicted values vs observed values GWS, west side

CONCLUSIONS

Based on our linear regression models, we can draw the following conclusions:

1. The plant cover percentage of the green system does not depend on the cardinal orientation at a significance level of 0.05.
2. The plant cover percentage of the green system does not influence the indoor temperature of the system at a significance level of 0.05.
3. We have built regression models in which all regression coefficients are significant at level 0.05. These models may help in predicting the plant coverage percentage of the vertical wall using values of soil moisture and soil temperature.
4. By considering the soil Humidity (*Hum*), soil Temperature (*Temp*), and an extra explanatory variable, *WeekNo* (time of the year), we were searching for linear regression models that can explain the percentage of plant cover of each side of the green system. Among all the models we have tried, the best ones (in terms of lowest MSE, and highest R^2 and adjR^2) involved the interaction between the variables *Temp* and *WeekNo*. For example, in the case of the regression model for the plant coverage percentage on the north side of the system, we have got the following equation:

$$\boldsymbol{PCP = 8.3459 \cdot Hum - 2.0276 \cdot Temp + 0.41093 \cdot WkNo + 0.061527 \cdot Temp \cdot WkNo}$$

We can interpret this equation as follows:

- an increase by 1 unit in humidity will determine an increase by almost 8.35% in the plant coverage percentage;
- an increase by 1°C in temperature will determine a decrease by almost 2.03% in the plant coverage percentage;
- the influence of the week number on the plant coverage percentage is 0.41093;
- for each week number, an increase by 1°C in temperature will determine an increase by almost 0.06% in plant coverage percentage

5. Apart from the east side of GWS, all models have significant coefficients, with a coefficient of determination R^2 about 0.5.
6. We have observed a small improvement in the models when considering the time of the year *WeekNo* as a new explanatory variable.
7. The use of mathematical modeling through regression analysis improved and enriched the results of the study, helping to determine more accurate predictions on the development and evolution of a green wall system.
8. We are aware that other factors (such as watering conditions, exposure to sunlight, human intervention, type of soil etc.) may also influence the plant cover percentage of the GWS, thus our models cannot depict the whole evolution of plants on the green system. Should additional data be available, our models can be further improved.

ABBREVIATION

Notation	Explanation
GWS	Green wall system
PCP	Plant coverage percentage
Hum	Soil Humidity
Temp	Soil Temperature
WkNo	Week number
N	Cardinal point North
E	Cardinal point East
S	Cardinal point South
W	Cardinal point West

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VEGETATIVE PROPAGATION SYSTEM FOR *HELICHRYSUM ITALICUM* PLANTS WITH ORNAMENTAL AND MEDICINAL PROPERTIES

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Abstract

Market demand for Helichrysum italicum (Asteraceae) is growing, especially for its valuable essential oils; however, seed quality and crop growth are variable. Among the various methods of vegetative propagation, propagation by stem cuttings is one of the most viable techniques that allow multiplication of genotypes of interest to achieve crop uniformity. Therefore, the aim of this work was to develop a sustainable vegetative multiplication system under controlled growing conditions. For this purpose, H. italicum seedlings were grown on different experimental substrate variants. Growth and vegetative development parameters of the seedlings were monitored, such as: average number of seedlings, average height of seedlings, length and number of roots. The results showed that the best substrates for root growth and rapid seedling production of H. italicum were: perlite and rooting with Radistim 2 (V5-a2b2) followed by perlite with peat and Radistim 2 (V8-a3b2) and perlite with peat and rooting with Atonik solution (V9-a3b3). The results obtained may contribute to the expansion of H. italicum cultivation in Romania for use as a medicinal plant or in the food industry.

Key words: *cuttings, culture conditions, Helichrysum italicum, vegetative propagation system.*

INTRODUCTION

Medicinal and aromatic plants represent a very important pharmaceutical and food raw material, being used since ancient times. However, for their use, scientific knowledge is necessary, the way the species spreads, the identification of plant products, the chemical composition, pharmacological indications, the feeding of medicinal plants, their active principles (Marcelino et al., 2023).

The Food and Agriculture Organization of the United Nations (FAO, IFAD, UNICEF, WFP and WHO, 2021) warns developing countries that 800 million people are malnourished through food and nutritional insecurity (Vassilakou, 2021).

Thus, natural bioactive compounds derived from plants are important resources, which can be sources of both medicines and functional foods (Ghendov-Moşanu, 2018).

The *Asteraceae* family consists of 1,900 genera, 32,000 species spread all over the world (https://en.wikipedia.org/wiki/Asteraceae), growing in various climates, and is also considered the largest family of flowering plants. Important species from this family are used as food, spices or for medicinal purposes, having diuretic, choleretic, anti-inflammatory properties.

The genus *Helichrysum* Miller (family *Asteraceae*) consists of a taxonomic group of plants used in Europe in traditional medicine for their bile-regulating and diuretic effects (Ferraz et al., 2022; Litvinenko et al., 1992). The genus name is derived from the Greek words: "helios" which means sun and "chrysos" which translates to gold, after the color of the yellow-golden inflorescence.

Tutin et al. (1976) mention that the genus *Helichrysum* is a member of the *Asteraceae* family which includes more than 400 members distributed worldwide, with 25 species native to the Mediterranean basin.

Traditional medicine around the world, has been using for about 200 years for medicinal, food and ornamental purposes (Viegas, et al., 2014), some of the species of the *Helichrysum* genus, the best known are those widespread in Europe, Central Asia and China: H arenarium (L. Moench), *H. plicatum, H. italicum* (Roth),

H. bracteatum, H. gymnocephalum, H. splendidum. *Helichrysum* spp., also offers a variety of phenolic extracts and pure compounds, among which are also some species of *Helichrysum* belonging to the Turkish flora: *H. arenarium* (L.) Moench subsp. *aucheri* (Boiss), *H. armenium, H. artvinense* (David and Kupicha), *H. chionophilum* (Boiss and Bal), *H. compactum, H. goulandriorum, H. graveolens, H. heywoodianum, H. noeanum, H. orientale* (L.) DC, *H. pallasii* (Sprengel) Ledeb (Albayrak et al., 2010).
Helichrysum italicum (Roth.) G. Don is an aromatic halophyte plant widespread in Europe in countries such as Italy, Spain, Portugal and Bosnia and Herzegovina, being traditionally used in human nutrition as a spice (Viegas, et al., 2014). The plants of this species especially show antioxidant, antibacterial, antifungal properties. This species in the past was used as a culinary spice due to its curry flavor, having a strong, pungent and spicy smell, and is currently processed to prepare *Helichrysum italicum* essential oil (EO) and to obtain extracts with fragrance compositions and preparations from the cosmetics industry, aromatherapy (Ferraz et al., 2022; Andreani et al., 2019). In the studies developed by Andreani et al. (2019) it is concluded about the species and subspecies of *H italicum* essential oils (EO) that they contain numerous terpenic and nonterpenic constituents, which give a characteristic, aromatic smell, from which the popular name of curry plant.
The species *Helichrysum italicum* (Roth) G. Don, popularly called immortelle or curry plant, has from a food and pharmaceutical point of view numerous therapeutic and seasoning properties in food flavoring, as well as a potential antioxidant that prevents the accumulation of free radicals (Węglarz et al., 2022; Andreani et al., 2019).
Natural antioxidants prevent deterioration of products: food, beverages, pharmaceutical products. In the cosmetic industry, products containing α-pinene prevent the aging process of the skin. The flowers of the species *Helichrysum italicum* (Roth) G. Don, present antibacterial constituents, bitter substances for the treatment of gout, rheumatism of hepato-biliary diseases, gastrointestinal disorders, hepatitis, combating intestinal worms, antifungal. Neryl acetate present in *H. italicum* EO from Italy (Tuscany) has orange blossom, sweet and fruity rose scent.
Authors such as Andreani et al. (2019), as well as Leonardi et al. (2013), mention that the Ar - curcumin present gives a "ginger" aroma, and the italidiones identified are anti-inflammatory agents, which provide protection to the skin against pollution and UV radiation. Also, *Helichrysum italicum* essential oils (EO) Corsican contains oxygenated compounds in greater quantity appreciated in the perfume industry and aromatherapy, (neryl acetate-40%), β-diketones ("italidiones") (5-10%) (Andreani et al., 2019).
According to what was stated in different studies, *Helichrysum italicum* essential oil (EO) has many biological and pharmacological properties (Viegas et al., 2014; Maksimović et al., 2017), collagen regeneration and is used in anti-aging creams (Andreani et al., 2019).
After 2005, the interest in curry plant (*Helichrysum italicum*) increases and at the same time the market price €2000/kg for the essential oil, and the annual production values are estimated at 1.34 tons (France AgriMer, 2017).
Taking into account the previously presented arguments, the growing interest in *Helichrysum italicum* species is also reported in S-E European countries, especially for its valuable essential oils. Reports on the establishment of new crops with *Helichrysum italicum* plants have been mentioned recently by researchers from Croatia (Tomičić et al., 2022) and the Republic of Moldova (Nartea et al., 2023; Cojocaru-Toma, & Nartea, 2022). Thus, the objective of this work was to develop an effective propagation method under controlled culture conditions in a greenhouse with a controlled atmosphere, for curry plant (*Helichrysum italicum*).

MATERIALS AND METHODS

At present, the information with reference to propagation by seeds and by vegetative propagation of *Helichrysum italicum* plant, mentioned in Romania, is only at the level of popularization of offers by commercial companies. To expand the culture, with plants that are marketable, a sustainable production system is needed to ensure a constant supply of good quality biological material.

Among the different methods of vegetative propagation, in this research the propagation by stem cuttings was studied, as it is considered one of the most viable techniques that allow the multiplication of the genotypes of interest and to obtain a uniformity of the culture (Melnic et al., 2022).

The experiments were carried out in a compartment of the block greenhouse for research, an automated unit and with self-control functions of the air conditioning provided by the Research Center for the Study of the Quality of Food Products - QLAB of the USAMV of Bucharest (Romania), during the period October-December 2023. The compartments of this greenhouse are modern constructions with a height of 6.2 m, recently equipped with all the facilities for monitoring and maintaining the climatic factors through the funding obtained for the ClimaGreen project. (Project Cod: RO-NO-2019-0420, https://www.climagreen.eu/), held in the period 2021-2023. ClimaGreen is a multidisciplinary research project between the Research Center for the study of the quality of agro-food products from the University of Agronomic Sciences and Veterinary Medicine (USAMV) of Bucharest, the research institute SINTEF Energy Research in Trondheim (Norway) and the SME Gether AS in Oslo (Norway).

The source of mother plants necessary for the vegetative propagation experiments with Immortelle (*Helichrysum*) from the *H. italicum* species, originally came from seedlings sold by the "Lavandă de Romania" company, from Timiș county (region located in the west of Romania; https://www.butasidelavanda.ro). Planted seedlings were transplanted and maintained for fortification under field conditions between July and October 2023. The biological material, consisting of cuttings, was taken from the level of the mother plants, both from the top of the main shoots and the side branches, as well as from the length underlying it with standard dimensions of 4- 5 cm long.

Rooting pots with dimensions of 10x10 cm, soil, peat, perlite and rooting hormones (Atonik and Radi-Stim no. 2) were used as materials for this experiment. Radi-Stim no. 2 produced in Romania, is a biopreparation, marketed in powder form, intended for rooting lignified and semi-lignified cuttings, which can be used for rooting vine cuttings, fruit tree rootstocks and ornamental plant cuttings (https://viasigradina.ro/radi-stim-nr-2-pudra-10-gr-radistim.html).

For the choice of this rooting preparation, the manufacturer's recommendation was taken into account, which mentions that due to the complex composition, the cuttings are protected during the period of callusation and the emission of roots against the attacks of various pathogens, thus reducing the mortality caused by molds. Atonik biostimulator is a systemic product formulated as an aqueous solution with the following chemical composition: sodium orthonitrophenolate (0.2%), sodium paranitrophenolate (0.3%) and sodium 5-nitroguaiacolate (0.1%). The detachment of the cuttings was made with scissors through a straight cut, after which they were immersed for a length of 1 cm at the base through the rhizogenic stimulant variants.

In the experimental scheme applied to *Helichrysum italicum*, two experimental factors were studied: Factor A - the rooting substrate represented by 3 gradations: a1 - peat substrate 100% (the control); - variant control (Vm); a2 - 100% perlite substrate; a3 - perlite mixture substrate 50% + peat 50%; and the experimental Factor B, represented by 2 rooting stimulators 3 gradations: b1 - no rooting treatment (Vm - Control); b2 - rooting treatment with powder-Radi-Stim 2; and b3 - rooting treatment with Atonik solution. In order to estimate the effect of the B-experimental Factor represented by the stimulating products on the rooting process and after rooting, the cuttings were studied on the 3 types of substrate used for rooting, represented by the A-experimental Factor, in greenhouse cultivation conditions. Growth and vegetative development parameters were monitored at the level of the cuttings, such as: the average number of shoots, the average height of the shoots, the length and the number of roots.

Throughout the 80 days of monitoring rooting and shoot growth development at the level of *Helichrysum italicum* cuttings, controlled conditions in terms of environmental factors (temperature, humidity, light) were ensured in the greenhouse compartment.

Statistical analysis. The bifactorial experiments with siminoc cuttings were placed according to the method of subdivided plots, in 5 repetitions within each experiment, the influence of the substrate on the rooting of the cuttings and the growth dynamics of the stems, leaves and shoots at the plant level were tested. As a result of the combination of the two experimental factors Factor A x Factor B, 9 experimental variants resulted in 5 repetitions. The values of the presented determinations represent the averages of the 5 repetitions related to each experimental variant, and the calculation and interpretation of the experimental results was carried out by the variance analysis method for P 5%, P 1%, P 0.1%, in accordance with the field placement method, respectively in subdivided plots.

RESULTS AND DISCUSSIONS

The biometric determinations carried out on *Helichrysum italicum* plants highlighted the fact that treatments with biostimulators had a direct influence on the number of differentiated roots on the plants, all experimental variants surpassing the control variant V1-a1b1 (Control) in which it was used as a culture substrate peat and no rooting stimulation treatments were performed.

Table 1. Influence of experimental factors on the number of differentiated roots on *Helichrysum italicum* plants

Experimental Variant	Shoots number/plant (No.)	Difference (No.)	Significance degrees
V1-a1b1 (Control)	**2.0**	Mt.	Mt.
V2-a1b2	4.0	2	*
V3-a1b3	3.0	1	-
V4-a2b1	8.0	5	***
V5-a2b2	11.5	9.2	***
V6-a2b3	9.5	7.3	***
V7-a3b1	6.5	4.2	***
V8-a3b2	10.9	8.9	***
V9-a3b3	9.0	7	***
$DL_{5\%} = 1.883$; $DL_{1\%} = 2.745$; $DL_{0,1\%} = 4.181$			

Legend for Statistical significance: (-) - insignificant, (*) - significantly positive, (**) - distinctly significant positive, (***) - very significant positive

Thus, based on the results summarized in Table 1, it is observed that the number of roots formed on the plants varied between 2 roots/plant in the case of the control variant (a1b1) and 11.5 roots/plant in the case of the variant in which perlite was used as a culture substrate in proportion to 100%, against the background of the administration of treatment with Radistim-2 (powder) as a rooting biostimulator (V5-a2b2).

It is observed, in an overall analysis of the values in Table 1, that the highest number of roots was determined in the experimental variants in which stimulation treatments with Radistim-2 were carried out, against the background of growing the plants in 100% perlite as a substrate of growth. Compared to the control variant not treated with growth biostimulators, the differences recorded in the other experimental variants were, from a statistical point of view, insignificant (-) for the V3-a1b3 variant, dsignificantly positive (*) for the a1b2 variant and very significantly positive (***) in the rest of the experimental variants, treatments with biostimulators stimulating the formation of plant roots, regardless of the culture substrate tested in the research (Table 1). Figure 1 shows *Helichrysum italicum* cuttings from variant V5-a2b2 (a2 - 100% perlite substrate; b2 - rooting treatment with powder-Radi-Stim 2) evaluated for root development 50 days after planting the cuttings *Helichrysum italicum*.

The experimental results summarized in Table 2 reveal the fact that there were no significant differences between the experimental variants in terms of the growth dynamics in the length of the roots, the values of this parameter being between the limits of 2 cm and 3.4 cm, compared to the experimental control the differences recorded in the rest of the variants experimental having insignificant statistical assurance (-).

Figure 1. Immortelle *Helichrysum italicum* cuttings evaluated for root development, after 50 days from the date of planting the cuttings (photo - 7 December 2023)

Table 2. Influence of experimental factors on the length of differentiated roots on *Helichrysum italicum* plants

Experimental Variant	Roots length (cm)	Difference (cm)	Significance degrees
V1-a1b1 (Control)	**2.0**	Mt.	Mt.
V2-a1b2	2.7	0.7	-
V3-a1b3	2.5	0.5	-
V4-a2b1	2.7	0.7	-
V5-a2b2	3.4	1.4	-
V6-a2b3	2.7	0.7	-
V7-a3b1	2.5	0.5	-
V8-a3b2	3.2	1.2	-
V9-a3b3	2.6	0.6	-
$DL_{5\%}$ = 1.507; $DL_{1\%}$ = 2.290; $DL_{0,1\%}$ = 3.765			

Legend for Statistical significance: (-) - insignificant.

Analyzing the growth and development dynamics of the stems, it is found that their length varied from 4.3 cm in the case of the control variant V1-a1b1 (Control) to 7 cm, the maximum length of the stem determined in the case of the V5-a2b2 variant, variant in which perlite was used as a substrate for plant growth, and Radistim treatments were performed as a plant rooting biostimulator (Table 3).

It can be seen in the results presented in Table 3, that the perlite used as a culture substrate potentiated the effect of the biostimulators tested during the research, resulting in the harmonious growth and development of the vegetative organs of the *Helichrysum italicum* plants, the height of the plants exceeding 6 cm.

The differences recorded following the determination of this biometric parameter compared to the experimental control (V1-a1b1) varied between the limits of 0.27 cm and 2.7 cm, with insignificant statistical assurance (-) in the case of the experimental variants in which peat was used (alone or mixed with perlite) as a culture substrate and significantly positive (*) in the experimental variants in which the culture substrate was perlite (alone or mixed with peat).

Table 3. Influence of experimental factors on the length of differentiated stems on *Helichrysum italicum* plants

Experimental Variant	Stems length (cm)	Difference (cm)	Significance degrees
V1-a1b1 (Control)	**4.30**	Mt.	Mt.
V2-a1b2	6.10	1.80	*
V3-a1b3	4.57	0.27	-
V4-a2b1	5.30	1.00	-
V5-a2b2	7.00	2.70	*
V6-a2b3	5.00	0.70	-
V7-a3b1	6.20	1,90	*
V8-a3b2	6.37	2.07	*
V9-a3b3	5.80	1.50	-
$DL_{5\%}$ = 1.689; $DL_{1\%}$ = 2.822; $DL_{0,1\%}$ = 5.349			

Legend for Statistical significance: (-) - insignificant, (*) - significantly positive.

The degree of branching of the stem in the plants belonging to the species *Helichrysum italicum* did not show significant differences between the 9 experimental variants (Table 4), the plants differentiating between 1.2 ramifications/plant in the case of the control variant V1-a1b1 (Control) and 2.4 ramifications/plant in the variant V8-a3b2, variant in which the administration of the biostimulator Atonik, against the background of the use of perlite as a substrate for plant growth and vegetation, stimulated the vegetative development of the aerial vegetative organs of the plants, thus forming the largest number of branches per plant.

Table 4. Influence of experimental factors on the number of differentiated branches on *Helichrysum italicum* plants

Experimental Variant	Branches number/plant (No.)	Difference (No.)	Significance degrees
V1-a1b1 (Control)	**1.2**	Mt.	Mt.
V2-a1b2	2.0	0.8	-
V3-a1b3	1.4	0.2	-
V4-a2b1	2.0	0.8	-
V5-a2b2	2.0	0.8	-
V6-a2b3	1.7	0.5	-
V7-a3b1	1.5	0.3	-
V8-a3b2	2.4	1.2	*
V9-a3b3	2.0	0.8	-
$DL_{5\%}$ = 0.999; $DL_{1\%}$ = 1.402; $DL_{0,1\%}$ = 1.980			

Legend for Statistical significance: (-) - insignificant, (*) - significantly positive.

The differences compared to the control variant not treated with biostimulators were insignificant (-) in all experimental variants, with the exception of the V8-a3b2 variant in which there were significantly positive differences (*) of 1.2 cm, compared to the untreated control (Table 4)

The number of leaves formed on *Helichrysum italicum* plants after 80 days of vegetation varied between 18.9 leaves/plant and 26.2 leaves/plant, the lowest number of leaves (18.9 leaves/plant) being determined in the control variant (variant in which the growth substrate was represented by peat 100%) and in variant a3b1 (variant in which the substrate used consisted of a mixture of peat 50% + perlite 50%), in both experimental variants no treatments were administered to stimulate the growth and vegetative development of plants (Table 5).

It is also observed in the results presented in the significant effect of the use of the biostimulators tested in the research, biostimulators which, based on the use of 100% perlite or a 50% mixture of peat and perlite as a substrate for plant growth: 50% led to the optimal growth and development of the plants, with statistically guaranteed differences from distinctly significantly positive (**) in the experimental variant a2b2 (V5), to very significantly positive (***), a result recorded in the V1 variants, V2,V5 as well as the plants from variants V8 and V9 compared to the untreated control variant (Table 5)

Table 5. Influence of experimental factors on the number of differentiated leaves on *Helichrysum italicum* plants

Experimental Variant	Leaves number/plant (No.)	Difference (No.)	Significance degrees
V1-a1b1 (Control)	**18.9**	Mt.	Mt.
V2-a1b2	21.3	2.4	***
V3-a1b3	20.7	1.8	***
V4-a2b1	19.1	0.2	-
V5-a2b2	26.2	7.3	***
V6-a2b3	19.5	0.6	**
V7-a3b1	18.9	0.0	-
V8-a3b2	22,5	3.6	***
V9-a3b3	20,5	1.6	***
$DL_{5\%}$ = 0.374; $DL_{1\%}$ = 0.561; $DL_{0,1\%}$ = 0.904			

Legend for Statistical significance: (-) - insignificant, (*) - significantly positive, (**) - distinctly significant positive, (***) - very significant positive.

In the case of variants a2b1 and a3b1, insignificant (-) differences were recorded in terms of the number of differentiated leaves on *Helichrysum italicum* plants, compared to the experimental control (Figure 2).

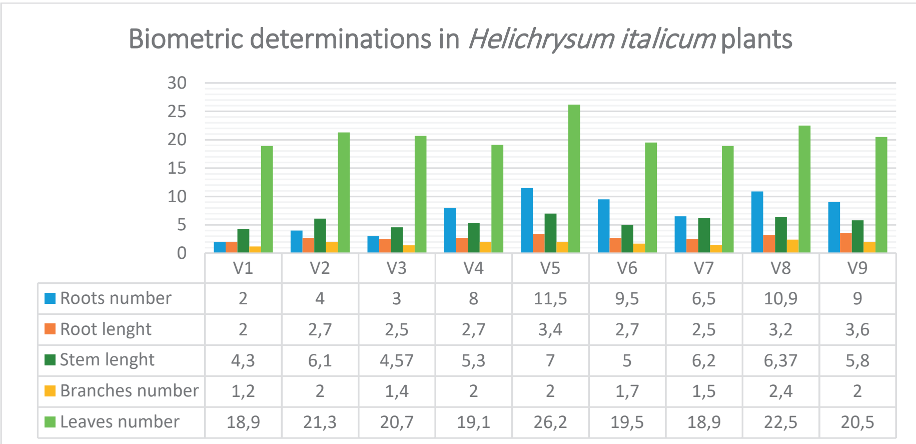

Figure 2. Experimental results recorded for root growth and rapid seedling production of *H. italicum*

CONCLUSIONS

The work brings to attention a special potential of medicinal plants that can be exploited commercially as nutraceutical foods. Through the research carried out, the results of previous research are confirmed, according to which *Helichrysum* sp. species are difficult to multiply by conventional vegetative methods. The arguments for continuing research in order to spread it as a decorative and aromatic plant in Romania are due to the important content of this species in phytochemical compounds.

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LEAF STOMATAL TRAITS AND ASSOCIATED PHYSIOLOGICAL PARAMETERS IN DIFFERENT DECIDUOUS ORNAMENTAL TREES DURING AUTUMN SENESCENCE

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Abstract

Global warming and the climate associated changes generally influence on plant phenology throughout their entire ontogenetic cycle (including autumn phenology), also having an impact on different ecological processes and on ecosystems. Stomata are specialized cellular structures located in the plant epidermis, which have a great importance for plant physiology, evolution, and global ecology. They are known especially for their role in carrying out the gases exchange, but their contribution to the maintenance of optimal leaf temperature, water, and nutrients uptake, as well as to assuring the continuity of their transport throughout the plant cannot be neglected This paper describes: 1) characteristics of stomata in mature leaves of some deciduous ornamental trees grown in the Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania; 2) water use efficiency, quantum yield, and transpiration: stomatal conductance ratio, during autumn senescence. Both indicators' categories can be promising to predict the autumn phenological shifts of the studied species driven under urban area conditions.

Key words: *Botanical Garden, deciduous ornamental trees, leaf stomata, water use efficiency, leaf senescence.*

INTRODUCTION

Plant ontogeny progresses by succession of specific phenological phases in relation to the species, represented as changes of traits expression due to intrinsic and extrinsic determinants, together with their interactions (Barton, 2023). Obviously, especially in recent years, in areas known to have a temperate climate, because of global warming such changes are accentuated. To address this challenge, both in the spring and autumn seasons, plants have adjusted their behavior, which has a significantly impact on different ecological processes (Xie et al., 2018; Tao et al., 2019).

It's worth mentioned that understanding the autumn phenology of deciduous trees species, but also of the climatic and meteorological factors involved can lead to the prediction of the autumn phenological shifts induced by projected climate change (Xie et al., 2018). The studies of Mariën et al. (2018) on beech, birch, and oak in forest trees in Belgium, highlighted that environmental conditions did not affect the onset of leaf senescence in mature trees, suggesting the existence of a conservative strategy. In a broad sense, autumn leaf senescence is a controlled type of programmed cell death that, unlike other stressors inducing cell death, avoids the loss of leaf nutrients (Keskitalo et al., 2005). Perennials specifically recapture leaf nutrients during autumn to relocate them to their over-wintering organs, as this is essential for their growth potential and foliage redevelopment during the subsequent year (Hagen-Thorn et al., 2006).

As previously documented, stomata are specialized cellular structures located in the plant epidermis (mainly in the stem and leaves) (Esau, 1977), having their central importance for plant physiology, evolution, and global ecology (Hetherington and Woodward, 2003; Delian, 2020; Ramakrishnan and Ray-Mukherjee, 2022). It has been extensively studied and well documented their role in carrying out the gas exchange during photosynthesis (Yin et al., 2020), and respectively, that specific to respiration, being named the plant's "breathing" pores (Biscoe, 1872). In addition, due to the transpiration process carried out mainly at the level of the stomatal pore, but also at the level of the cuticle (Kane et al., 2020; Hazlett, 2022), these formations contribute to maintenance of optimal leaf temperature, water, and nutrient uptake, as well as the continuity of their transport throughout the plant (Chen et al., 2022). Really, due to the ability to internally regulation of the stomatal pore diameter and under the action of external factors, plants generally manage the water use efficiency (WUE), especially in drought conditions (Yoo et al., 2009; Ronzhina et al., 2023). Moreover, in-depth genetic studies are intended to increase the tolerance of plants to drought by modulating the stomatal density (SD) and their characteristics to improve WUE (Li et al., 2021; Jiao et al., 2022), also to increase the plant tolerance to drought, in parallel with climate changes (Bertolino et al., 2019). WUE is also influenced by the presence of trichomes, cuticle and cuticular waxes. A high trichome/stomata ratio improved WUE thanks to the increase in the resistance to the water vapor diffusion at the leaf level (Galdon-Armero et al., 2018, cited by Bertolino et al., 2019). Furthermore, stomata are entry gates for pathogens, such as bacteria. At this level, the fight for the plant's immune response is triggered (Wang et al., 2022) and, not least, the stomata are involved in the plant-insect interactions (Lin et al., 2022).

The stomata distribution at the leaf level, their density on a leaf unit area, as well as stomatal traits (e.g., types, size etc.) depend on the ecological conditions of plant growth (Paridari et al., 2013; Kryvoruchko and Bessonova, 2018; Yigit et al., 2019; Hurt and Doğan, 2020; Kou et al., 2023) and are permanently subject to a process of adaptation to the constantly environmental factors changing (Yin et al., 2020; Li et al., 2021; Soheili et al., 2023), in order to successfully fulfill its multiple roles and especially so that the process of photosynthesis can be negatively affected as little as possible (Ronzhina et al., 2023). For example, in *Carpinus betulus* grown at different altitudes (Hyrcanian forest, Iran) a negative correlation of stomatal sizes with altitude was determined, while for SD the correlation was positive. In addition, relative temperature and precipitation strongly influence the morphological characteristics of the leaf (Paridari et al., 2013).

The interactions between stomata's traits and the environmental conditions are also markedly highlighted in the urban environment, especially regarding the changes induced by the temperature increase (Zhu et al., 2020; Markin et al., 2023), carbon dioxide (CO_2) concentration increasing (Gardner et al., 2023), but also by different pollution sources (Petrushkevych and Korshykov, 2020), which leave their mark on the plant's morphology, anatomy, and physiology. Apart from the previously mentioned, SD, as well as the presence of trichomes influence the ability of plant species to reduce the effects of pollutants in urban environments, influencing the accumulation of air contaminants in leaves (Simon et al., 2014; Zhang et al., 2018). Therefore, Green Infrastructure (GI) is one potential passive control system for air pollution in street canyons (Tomson et al., 2021). Marek et al. (2022) highlighted the adaptation of *Pinus sylvestris* plants to the temperature increase, rather than to changes in CO_2 level. Therefore, the intraspecific relationship between SD and climate can characterize SD response to global warming (Marek et al., 2022).

Considering the previously presented, the aim of this study was to describe: 1) stomata characteristics in mature leaves of 19 deciduous ornamental trees species grown in the Botanical Garden of the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV of Bucharest), Romania, using light microscopy; 2) leaves water use efficiency and quantum yield during autumn senescence. Both indicators' categories can be promising to predict the autumn phenological shifts of the studied species driven under urban area conditions.

MATERIALS AND METHODS

Study sites and species

This research was carried out during autumn senescence, on leaves of 19 deciduous ornamental trees species grown in the Botanical

Garden of the Faculty of Horticulture, USAMV of Bucharest, Romania, North latitude of $44^{0}24'$ N and $26^{0}05'$ East longitude, and an altitude which varies between 60 m and 90 m above sea level, with a temperate-continental climate.

The species belong to 19 different families, as follows: (A) Sapindaceae (Aceraceae) - 1. *Acer platanoides* L.; 2. *Acer pseudoplatanus* L.; (B) Anacardiaceae - 3. *Cotinus coggygria* Scop.; (C) Betulaceae: 4. *Betula pendula* Roth (*B. verrucosa*); 5. *Corylus avellana* L.; 6. *Corylus colurna* L.; 7. *Carpinus betulus* L.; (D) Caesalpiniaceae - 8. *Cercis siliquastrum* L.; (E) Calycanthaceae - 9. *Calycanthus floridus* L.; (E) Cornaceae - 10. *Cornus mas* L.; 11. *Cornus sanguinea* L.; (F) Ebenaceae - 12. *Diospyros virginiana* L.; (G) Fagaceae - 13. *Quercus rubra* L. (*Q. borealis* Michx.); (H) Ginkgoaceae: 14. *Ginkgo biloba* L.; (I) Magnoliaceae: 15. *Liriodendron tulipifera* L.; (J) Moraceae - 16. *Maclura pomifera* (Rafin.) C.K. Schneid.]; (K) Oleaceae: 17. *Forsythia x intermedia* Zabel; (L) Tiliaceae: 18. *Tilia tomentosa* Moench (*T. argentea* DC.); (M) Ulmaceae: 19. *Celtis occidentalis* L.

Leaf traits

The stomata density and their characteristics features

Stomatal distribution, stomata density (SD) in leaves and their traits have been analysed on mature healthy still green leaves, in October 2023, by the method of stomatal impression described everywhere in specialized works. A thin layer of nail polish was applied on both sides of the leaves, on an area of about 2 cm^2. After solvent evaporation (about 20 minutes later), the transparent stomatal impression of the leaf epidermis was taken with the help of a sheet of transparent shells and placed on a microscopic slide, which was labelled with the name of the sample.

The observations, images and measurements of the anatomical structures were made with the optical microscope Leica DM1000 LED, Camera video Leica DFC295 the Laboratory of Microscopy and Plant Anatomy of the USAMV of Bucharest. For stomatal density (SD), ob. 40 x. was used, and the photos and measurements of the stomata were made at ob. 20 x.

SD was counted and expressed as number per mm^2. The guard cells length (GcL) (µm), guard cells width (GcW) (µm), stomatal pore length (SPL) (µm) and stomatal pore width (SPW) (µm) were measured. Then, the stoma area (STArea) as well as the stoma pore area (STPArea) were calculated, too (μm^2). Also, photos were taken for the stomatal impressions of the lower epidermis of each sample.

Physiological associated indicators

Based on the net photosynthesis rate (A - µmol CO_2 m^{-2} s^{-1}) and transpiration rate (E - mmol H_2O $m^{-2}s^{-1}$) (data are not shown) measured *in-situ*, for still green leaves, using the portable infrared gas analyser (LCPro-SD-ADC BioScientific Ltd, Hoddesdon, UK), there were calculated two associated indicators, namely: water use efficiency (WUE) (A/E) (µmol CO_2 m^{-2} s^{-1}/ mmol H_2O $m^{-2}s^{-1}$) and quantum yield (φCO_2) (A/ Photosynthetic Photon Flux Density (µmol CO_2 m^{-2} s^{-1}/ µmol photons $m^{-2}s^{-1}$).

Statistical analysis

Data were processed using Microsoft Excel (version 2010) and are shown as average values ± Standard Error (SE). The analysis of variance (ANOVA) was performed. Then, the post hoc Duncan Multiple Range Test (DMRT) by using IBM SPSS Statistics software was carried out to determine where there were statistically significant differences between different species. Statistically significant differences have been considered at the value of $p \leq 0.05$.

Pearson correlation coefficients were also calculated to evaluate the posible relationship between stomatal traits and physiological indicators at 95% confidence level. Graphs were constructed using Microsoft Excel 2010.

RESULTS AND DISCUSSIONS

An overview of the leaves micromorphological characteristics is shown in Figure 1. The variability of the epidermal cells shapes and size of the for the 19 species studied, the type of stomata, as well as the presence of trichomes in some cases can be observed. The stomata are present only at the level of the lower epidermis (abaxial) and in most of the analyzed species, they are of the paracytic and anomocytic type. In the case of 9 *Acer* studied species, most of the stomata were of anomocytic type, but on *A. platanoides* there were observed some that resemble the paracytic type (Toma et al., 2015). As regards the presence of trichomes, the types present and their respective sizes, the species *C. occidentalis* stood out, which at the level of the upper epidermis presented multicellular hairs, with a diameter of 8.43 µm and a length of 106.7 µm. In *C. sanguinea*, they were located on both epidermises, but the length varied (155 µm adaxially, respectively 217 µm abaxially). For *T. tomentosa*, stellate hairs were observed, and in the case of *F. x intermedia* the secretory hairs consisted of 8 cells, with a mean diameter of 22.33 µm. Our results agree with those obtained by Gülz et al. (1991) who observed in the leaves of *T. tomentosa* just emerging from the bud, dense trichomes (consisting of eight stellate cellular hairs) located on the abaxial side, while in mature leaves, because of the growth of differentiated cells, the density of the hairs was reduced. On the adaxial surface there were only solitary stellate hairs, to which solitary glandular trichomes were added. Simon et al. (2014) also found that in *C. occidentalis* leaves, in addition to the stomata size and their distribution as having a major importance in the accumulation of contaminants from the air, the presence of numerous trichomes leaves its mark on the dust deposition. Also, unicellular glandular hairs have been observed by Toma et al. (2015) on the *A. platanoides* petiole epidermis. On *A. platanoides* and *A. campestre* lamina there were observed unicellular (or tricellular on the latter species) eglandular hairs.

Table 1 shows the average values ± SE of the determined indicators, as well as the significance of the differences at the level of $p < 0.05$. Stomata number, as well as their sizes varied widely among evaluated species, being strong significantly different from a statistical point of view. According to ANOVA test, p values were < 0.001 for each studied parameter. Stomatal density per mm^2 leaf area ranged between the lower one – 70.33 mm^{-2} (*A. pseudoplatanus*) to the highest registered for *C. occidentalis* (467 mm^{-2}), with a mean value of 172 mm^{-2}.

The length and width of the guard cells also showed a wide variability. The length was higher than the width, ranging between 8.70 µm (*A. platanoides*) and 22.80 µm (*B. pendula*), with an average value of 15.86 µm. The lowest value for guard cell width was 2.86 µm, (*C. occidentalis*), while the maximum one was 7.80 µm (*G. biloba*). It can be observed that for *A. platanoides* the lowest values were noticed also for stoma pore length (5.26 µm), stoma pore width (1.50 µm), stoma area (74.98 μm^2) and stoma pore area (7.95 μm^2). At the opposite pole was *B. pendula*, with STArea of 418.91 μm^2 and *C. mas* with a SPArea of 63.84 μm^2, species in which SD had a low value of only 85 stomata mm^{-2}.

The low values of the water use efficiency and quantum yield (Table 1) can be explained by the fact that the determinations regarding net photosynthesis and transpiration rate were carried out during autumn, when naturally, physiologically, the rate of net photosynthesis decreases in relation to the onset of leaf senescence, with all the consequences that arise on the physiological processes in plants. However, if the degree of senescence is variable depending on the species, the mentioned indicators also show very different values.

The Pearson correlations between stomatal traits and associated physiological indicators are shown in Figure 2. Green represents a negative correlation, while red represents a positive correlation. Overall, we notice a wide variability in relation to the studied species. SD showed rather a negative correlation with different indicators (more evident in *A. platanoides*), except *C. siliquatrum* and to a certain extent *Q. rubra*. Also, the stomata traits, water use efficiency and quantum yield were strongly positive correlated (r > 0.7) in *A. pseudoplatanus*, and to a lesser extent in descending order of the species *C. betulus*, *C. siliquastrum* and *F. intermedia*. Negative correlations were recorded especially in *C. coggygria*, *B. pendula*, *C. floridus* and to some extent in *C. occidentalis*.

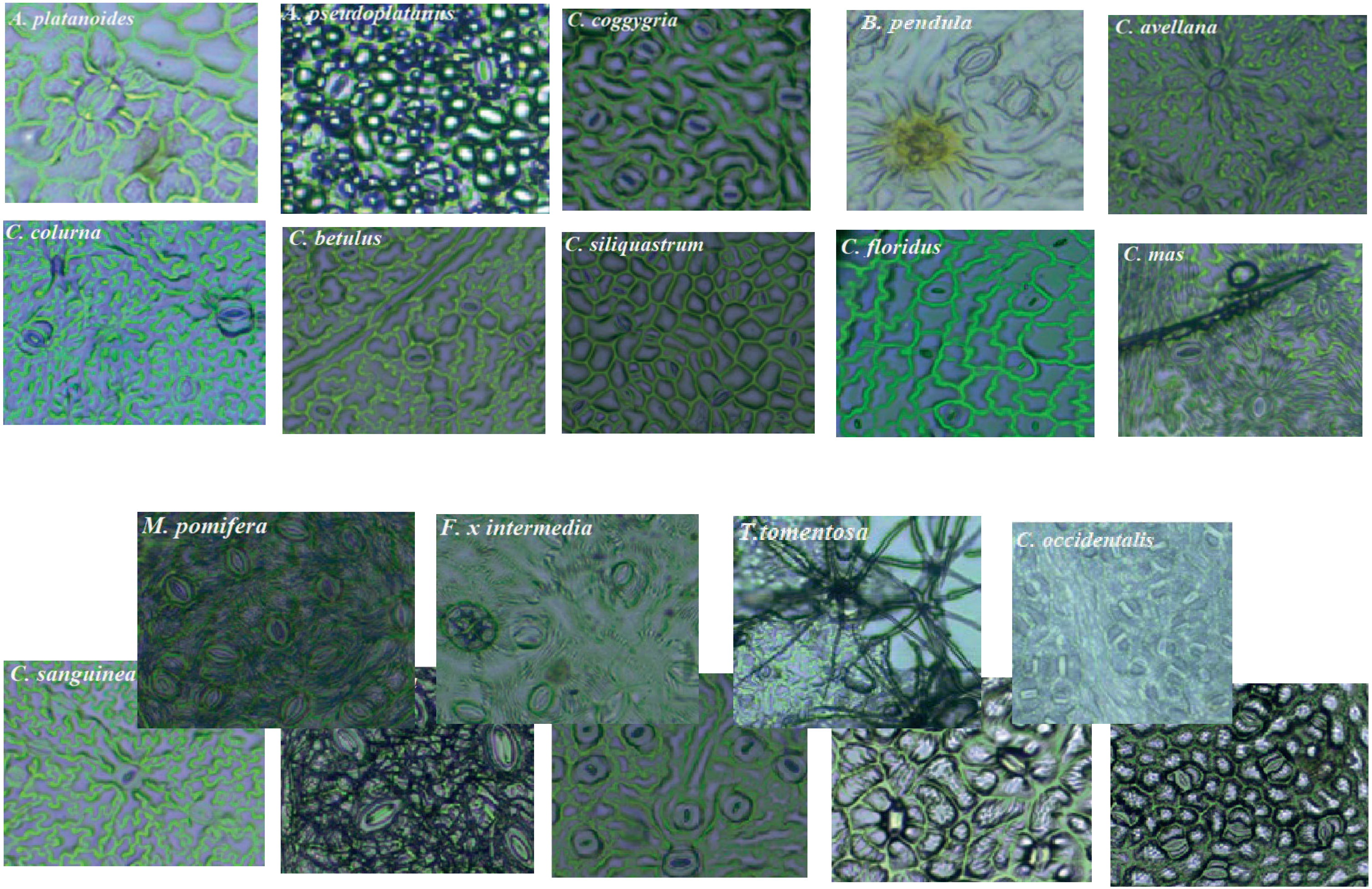

Figure 1. Stomatal photographs (20 x) of the leaves abaxial impressions for different deciduous ornamental trees during autumn senescence

Table 1. Leaf epidermis stomatal traits and synthetic physiological indicators for leaves of 19 woody ornamental trees, during autumn season

Species	Stomata density: No mm^{-2}	Guard cell length (GcL)(μm)	Guard cell width (GcW) (μm)	Stoma pore length (SPL) (μm)	Stoma pore width (SPW)(μm)	Stoma area (StArea) (μm^2)	Stoma pore area (SPArea) (μm^2)	Water use efficiency (WUE)	Quantum yield (φCO_2)
A. platanoides	197±7.02 cde	8.70±1.41 h	3.43±0.43 gh	5.26±0.24 k	1.50±010 h	74.96±17.98 k	7.95±0.86 i	0.14±0.13 e	8.39E-05±7.61E-05 fg
A. pseudoplatanus	70.33±6.14 i	18.33±0.13 bcd	6.60±0.15 cd	10.70±0.82 def	3.30±0.20 cdef	302.59±11.03 bcd	35.65±4.80 cde	1.01±0.25 cde	58E-04±15E-04 cd
C. coggygria	227.67±16.50 c	11.27±0.34 gh	4.53±0.12 f	7.93±0.18 ji	2.60±0.11 gf	131.52±5.38 ijk	20.65±1.25 fg	0.10±0.03 e	11E-04±2.8E-05 efg
B. pendula	102.67±6.74 ghi	22.80±2.25 a	7.53±0.18 b	14.03±0.73 a	3.33±0.23 cdef	418.91±38.37 a	46.53±2.28 b	0.64±0.09 cde	9E-04±1E-04 abc
C. avellana	135.33±11.57 fg	13.53±1.05 fg	5.78±0.30 e	7.27±0.24 j	1.50±0.11 h	177.77±22.22 ghi	10.95±1.18 hi	4.24±0.61 a	6E-04±9.01E-05 cd
C. colurna	92.67±11.05 hi	17.13±0.93 bcde	4.53±0.32 f	9.87±0.69 fg	3.33±0.31 cdef	213.99±26.49 fgh	33.32±5.50 e	-2.11±0.18 fg	-4.9E-04±3.1E-05 h
C. betulus	130.33±10.74 fgh	16.50±1.23 cdef	5.50±0.26 e	10.00±0.64 efg	4.47±0.12 ab	256.21±25.88 def	44.73±3.45 bc	-2.94±0.30 g	-18E-03±14E-04 i
C. siliquastrum	160.33±6.74 ef	16.03±1.32 cdef	4.37±0.18 f	13.33±0.41 ab	3.23±0.22 cdef	193.06±22.99 fghi	43.07±2.95 bcd	0.98±0.21 cde	0.001±2E-04 ab
C. floridus	105±8.66 ghi	15.93±1.38 cdef	5.47±0.18 e	8.40±0.12 hij	2.27±0.12 g	209.89±16.00 fgh	19.03±0.98 fgh	0.91±0.16 cde	0.001±2E-04 a
C. mas	85±5 i	17.27±1.22 bcde	6.00±0.12 cde	12.83±0.32 ab	4.97±0.23 a	296.41±29.25 abc	63.84±4.28 a	1.37±0.35 cd	0.0003±8.5E-05 defg
C. sanguinea	185.33±2.67 de	13.30±0.40 fg	5.90±0.15 de	7.73±0.38 j	2.73±0.23 efg	193.41±9.45 fghi	21.17±2.32 fg	0.74±042 cde	7.7E-05±4.37E-05 fg
D. virginiana	165.33±11.35 ef	18.30±0.26 bcd	3.93±0.18 fg	12.43±0.23 bc	4.50±0.36 ab	226.47±13.08 fgh	55.78±3.60 a	0.26±0.07 de	0.0005±0.00015 cde
Q. rubra	303.33±9.28 b	15.30±0.91 efg	9.27±0.38 a	9.60±0.31 fgh	4.50±0.26 ab	358.35±29.57 b	46.55±3.88 b	-1.60±0.07 f	-0.0055±0.00022 j
G. biloba	85.33±14.07 i	19.87±0.97 b	7.80±0.29 b	9.17±0.34 ghi	4.83±0.26 a	342.88±13.53 bc	15.47±2.10 ghi	1.75±0.22 bc	0.0003±4.72E-05 defg
L. tulipifera	179.67±10.91 de	15.13±0.64 efg	3.85±0.22 fg	7.73±0.29 j	3.40±0.06 cde	168.39±12.40 hij	26.28±0.90 ef	1.02±0.16 cde	0.00086±2.78E-05 bc
M. pomifera	287.67±22.18 b	17.17±0.93 bcde	4.03±0.14 fg	11.20±0.43 cde	3.97±0.12 bc	206.51±11.15 fgh	44.33±0.68 bcd	2.24±0.16 b	0.00066±2.35E-05 bcd
F. x intermedia	210.33±4.33 cd	19.00±0.17 bc	4.40±0.21 f	12.30±0.15 bc	3.57±0.30 cd	235.04±14.38 efg	43.87±3.70 bcd	2.48±1.09 b	0.0003±0.0001 defg
T. tomentosa	77.67±11.05 i	14.83±0.32 fg	6.73±0.24 c	11.40±0.26 cd	2.90±0.21 gfed	243.05±14.08 defg	33.07±2.61 e	0.04±0.40 e	-1.9E-05±0.0001 g
C. occidentalis	467±27.22 a	10.93±0.56 gh	2.87±0.09 h	7.83±0.22 j	4.47±0.29 ab	111.39±5.23 jk	34.99±2.51 de	0.98±0.22 cde	0.0005±0.0002 cdef

Data are shown as mean value ± SE (n = 3). The comparison was done on columns between different species, by the post hoc Duncan Multiple Range Test (DMRT) by using IBM SPSS Statistics software. Statistically significant differences have been considered at the value of $p \leq 0.05$ and are represented by different letters.

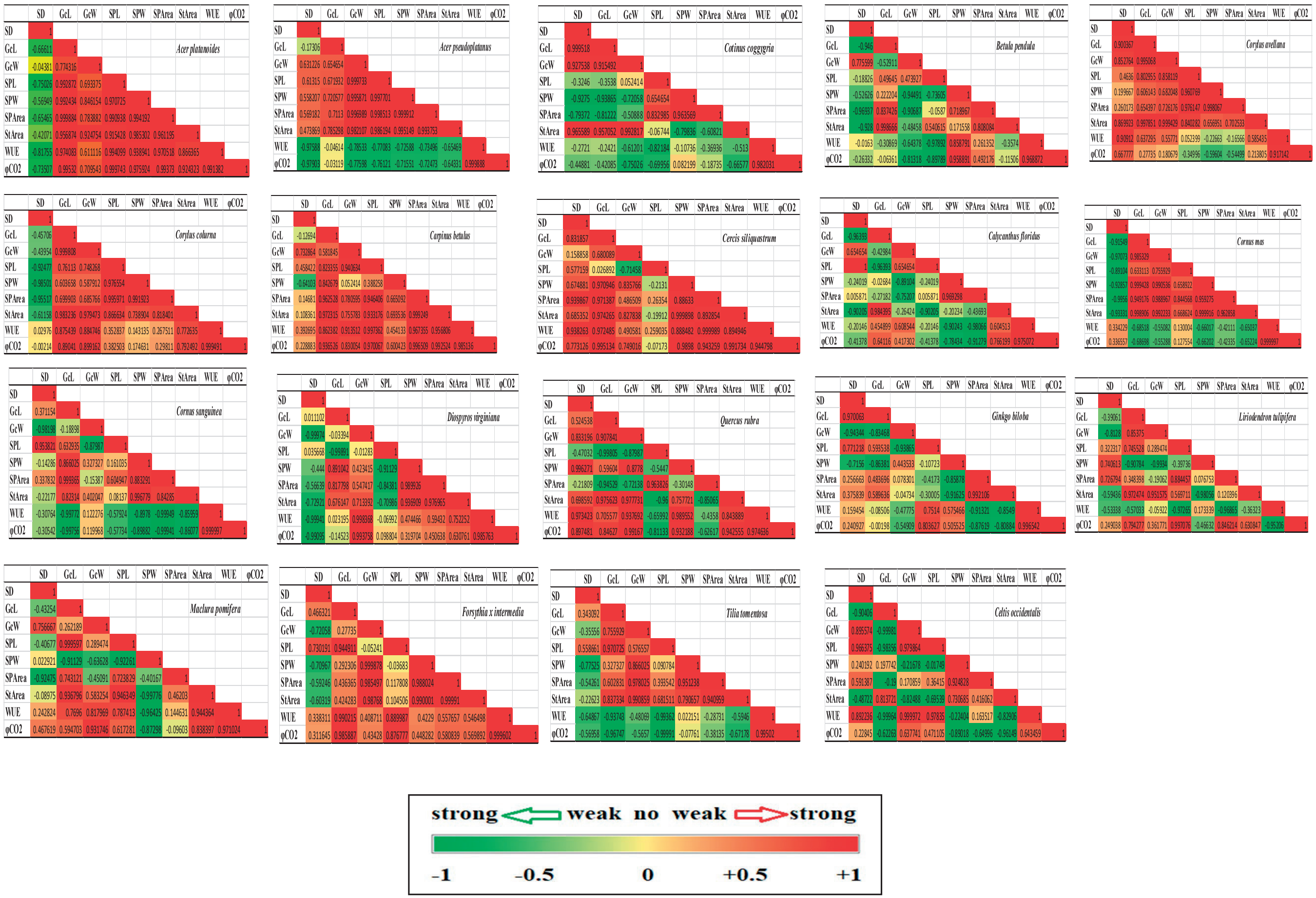

Figure 2. Pearson correlation analysis of stomata traits and associated physiological indicators

We recorded that for WUE there is a very strongly positive correlation with the quantum yield, as logically expected. The exception was *L. tulipifera*, in which case a strong negative correlation was noticed (r = - 0.95).
Although the stomata ostioles occupy a small area of the total leaf surface, through them significant amounts of water pass in the form of vapors (during transpiration), as well as CO_2 (raw material in photosynthesis) (Chen et al., 2022). Regarding their characteristics, Yin's et al. (2020) studies on 45 species of woody plants highlighted the relationship between the maximum rate of photosynthesis and stomatal characteristics (SD, stomatal length, maximum stomatal conductance) and revealed some contradictory results compared to those obtained through previous studies. The authors' conclusion was that plants that possess a smaller number of stomata (but with larger sizes) can have a maximum stomatal conductance with lower values. As a result, under conditions of increased CO_2 concentration and reduced water availability, as is predicted to happen in the future, the rate of photosynthesis can still be maintained at adequate values.
Yigit et al. (2019) highlighted the dependence of the micromorphological characteristics of the leaves on the growing conditions. Thus, in *T. tomentosa*, apart from the length and width of the stomatal pore, where the differences were statistically insignificant (the average values varied from 9.76 - to 10.73 µm; respectively 3.38 – 4.35 µm), for stoma length (14.36 to 18.89 µm), stoma width (9.36 to 15.07 µm) and stomata density (20 to 176) the differences depending on the area were significantly different. In our study we found that SD was 77.67 mm^{-2}, so close to the one recorded in the city of Sivas (Turkey) (72), and the stoma length of 14.83 µm (close to the one measured in Izmir - 14.36 µm). The changes in stomatal characteristics are specific to the species, the cultivar and depend on the climate specific to the plant growth conditions, otherwise confirming the results previously obtained by other authors (Hurt and Doğan, 2020; Sevic et al., 2020). Although a negative relationship was usually found between SD and the concentration of carbon dioxide in the atmosphere, the research carried out by Marek et al. (2022) in *Pinus sylvestris* highlighted the link between SD variation and temperature changes, respectively a response to climate warming.
Naturally, plants can regulate the degree of stomata opening to maximize the CO_2 assimilation, simultaneously with the reduction of water losses. But the studies have also demonstrated that in the case of extreme heat, a decoupling of stomatal conductance - net photosynthesis rate takes place, which allows leaves survival in such conditions, as well as a quickly depletes available water (Marchin et al., 2023). Maximum daytime operating stomatal conductance have been characterized by having lower instantaneous water use efficiency (iWUE), while water potential gradients were highly varied. As a result, the faster stomatal response can be useful for such leaves. Thus, smaller stomata with a faster dynamic feature can be integrated to plants selection for stomata conductance as an adding trait for enhancing photosynthesis performances, as well as to improve agricultural qualities (Drake et al., 2012).
Moreover, genetic manipulation of stomata, with the aim of making their role more efficient is a current concern of researchers. In this context, the proof is in-depth studies carried out by Li et al. (2021) on poplar. It has been demonstrated the role of *PdERECTA* in stomatal modeling with the aim of increasing the efficiency of water use, respectively reducing SD, and increasing their size in the case of overexpression of the mentioned gene. It was thus emphasized that stomatal conductance decreased and implicitly the intensity of transpiration done the same, which increased WUE (without significantly affecting CO_2 absorption). It was appreciated that the *PdERECTA* gene is of interest for the genetic modification of poplar to obtain drought tolerance trees. Jiao et al. (2022) isolated an EPIDERMAL PATTERNING FACTOR (EPF) secreted Cys-rich small peptide *PdEEPFL6* from NE19 [*Populus nigra* × (*P. deltoides* × *P. nigra*)] that was highly induced by dehydration treatment in poplar. Overexpression of *PdEPFL6* determined a significantly decrease of the *PdSPCH* and *PdMUTE* transcription factors expression, which are implicated in stomata development. Thus, stomata density

was reduced and respectively, drought resistance was improved.

Eensalu et al. (2008) highlighted variations in *B. pendula* stomata traits depending on the leaf position in the crown, respectively in relation to the intensity of the light incident on the leaf. In the upper part of the crown, a higher SD and a smaller leaf surface were determined, while no significant changes were noted regarding the epidermal cell density. Also, the length and width of the guard cells at the abaxial leaf level had lower values in the case of reduced light availability. To detect some bioindicators of the state of the urban environment, Petrushkevych and Korshykov (2020) studied different measurements of *B. pendula* exposed to different levels of aerotechnogenic loading. Among them, there was a decrease in the length, width, and stomata area, while the thickness of the leaf and the SD increased. In addition, in *B. pubescens*, in the case of high temperatures and water deficit, the size and number of stomata decreased, while the number of mesophyll cells and chloroplasts per surface unit increased, as measures to counteract the effects of the decrease in leaf conductance (Ronzhina et al., 2023).

Regarding the contribution of the stomatal transpiration has in total transpiration, Kane et al. (2020) underline the existing differences in *Q. rubra* depending on the age of the leaves, respectively the presence or absence of stomata and the presence of the cuticle (and its thickness). The authors obtained different data as compared to those previously reported. It was considered that intense transpiration in young growing leaves is due to keeping the stomatal ostiole open. The recent explanation was that the ability to close would develop as stomata are exposed to low humidity and high concentrations of abscisic acid.

Akinshina et al. (2020) described *L. tulipifera* stomata, regarding the position on the leaf, the specific type, as well as the influence of light on SD. Thus, in the case of leaves exposed to the sun, the number of stomata was by 82% higher compared to the number of stomata in the shade leaf (182 vs. 100 mm^{-2}; length - 39 µm vs 32 µm; width 21 µm vs. 13 µm). The ecological plasticity of this species relative to light and temperature is highlighted, which improves its acclimatization to arid climate conditions. The ability to quickly close the stomata, as the water potential of the leaves decreases (respectively the isohydric response) denotes an ability of *L. tulipifera* to avoid the stress caused by drought, compared to other species such as *Pyrus* and *Quercus* characterized by an anisohydric response (Cregg et al., 2023). In the same vein, the research carried out by Kryvoruchko and Bessonova (2018), in *Q. robur* and *Q. rubra* species grown alone, highlighted an increase in SD by 30.6% and 25.3%, respectively, compared to those grown in groups. At the same time, there were changes in the anatomy of the leaf, which for solitary plants leads to xeromorphism type behavior, as adaptation reagents to the greater shortage of air and soil moisture.

Miller-Rushing et al. (2009) investigated whether the tree species grown individually in the Arnold Arboretum in Boston, Massachusetts underwent changes for 100 years (1893 to 2006), regarding SD, guard cell length, and intrinsic water use efficiency (iWUE), examining leaves from 74 herbarium specimens collected from three genera: *Acer* (maples), *Quercus* (oaks), and *Carpinus* (hornbeams). In oak and hornbeam, a negative correlation was found between SD and the length of the guard cells, respectively, the SD decreased, while the length of the guard cells increased. The WUE values did not undergo significant changes over time. The authors appreciate that iWUE does not respond to changes in CO_2 concentration, probably due to changes in stomatal characteristics, such as their density and the guard cells sizes.

In the species *C. coggygria*, in China, Li et al. (2022) studied the effects of unusual continuous rainy weather and determined a significant positive correlation with the values of some environmental indicators such as sunshine duration, temperature, photosynthetically active radiation (PAR) and daily precipitation (DPD) greater than or equal to 0.1 mm. Also, the transpiration rate was significantly correlated with SD and temperature, PAR, DPD, and the atmospheric CO_2 concentration.

Following the meta-analysis carried out by Gardner et al. (2023), it was suggested that the intensification of the photosynthesis process, rather than the reduction of stomatal conductance, leads to the improvement of iWUE, under conditions of increased CO_2 concentration in all analyzed species. In addition, leaf–air vapor pressure difference (D). factor was also important to compare the different species and the obtained results emphasized that the most responsive to D were angiosperms, as against gymnosperms. The studies carried out by Avci and Aygün (2014) on 18 Turkish hazelnut (*Corylus avellana* L.) revealed that the leaves are hypostomatic and there are statistically significant differences regarding the number of stomata and respectively the size of the stomata, appreciating that these indicators could be used to identify the varieties.

The drought applied to *B. pendula* seedlings significantly affected the anatomy of the leaf (Kou et al. 2023), regarding the length of the stomata ($p<0.05$) and had a highly significant effect ($p<0.01$) on the change in stomatal structure. At the control, SD was 56.33 mm^{-2}, while the higher drought stress level (of 25 % polyethylene glycol) induced an increase of SD to 73 mm^{-2}. At the same time, stomata length, stomata width and stomatal aperture decreased from 44.33 µm to 39.36 µm; 36.53 µm to 32.67 µm, respectively from 5.71 µm to 1.75 µm in the case of the last parameter. Authors appreciate that these data can serve as a theoretical basis for the selection and breeding of new drought-tolerant European birch species and the promotion of new drought-tolerant species in China. According to the previously data, our obtained results of SD – 102.67 mm^{-2} or guard cells length (22.80 µm – evident below 39.36 um as noted before) possible signify an adaptation of this specie to the drought stress.

CONCLUSIONS

Global warming and the climate associated changes generally influence on plant phenology throughout their entire ontogenetic cycle (including autumn phenology), also having an impact on different ecological processes and on ecosystems.

The micromorphological characteristics of the mature leaves of the studied ornamental deciduous trees are very closely related to the species and the specific growing conditions. The striking variability regarding the stomata density, but also their dimensions, as well as the marked differences compared to the existing data in the specialized literature, are the expression of the fact that the biological material was represented by *in situ* mature, old individual trees (not grouped), grown, and adapted along the time to the environmental conditions of an urban botanical garden, located in a temperate climate.

Overall, the results regarding the interrelationships between micromorphological and physiological associated characters, such as water use efficiency and quantum yield, suggest that trees belonging to different botanical families have a specific behavior during leaf senescence, in the autumn season. Some species are less photosynthetically efficient than others, which may mean that a negative photosynthesis can be associated with a higher rate of respiration. In autumn conditions, the intensification of the catabolic processes at the leaves level before they fall should not be considered as a minus for the plant, but a benefit for what means an efficient compounds re-translocation in perennial plant organs before the leaves fall and not ultimately a proper preparation of the plant to get through the winter and start vegetation the following year.

To our knowledge, these are the first results on this topic in Romania. These are added to those previous obtained by other researchers, contributing to expanding our understanding and support the prediction of the autumn micromorphological and physiological shifts driven under urban area conditions, helping also, to understand plants adaptation to the future changing environmental climate.

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INVESTIGATIONS ON THE BIOACTIVE COMPOUNDS AND ORNAMENTAL PROPERTIES OF SOME LAVENDER CULTIVARS

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Abstract

Lavandula angustifolia L. is a perennial plant with multiple uses in the cosmetic, pharmaceutical, food, aromatherapy industry and for ornamental purposes. Six lavender cultivars were used for measurements, laboratory analyses and decorations: L. angustifolia 'Hidcote', L. angustifolia 'Sevstopolis', L. angustifolia 'Nana Alba', L. angustifolia 'Dwarf Blue', L. angustifolia 'Blue Scent', Lavandula x intermedia 'Grosso', grown under field conditions. The ATR-FTIR spectra of various tissues of lavenders used in this experiment have revealed presence of a wide range of biochemical compounds with pharmaceutical importance. The content of lycopene, β-carotene, flavonoids, tannins and the antioxidant activity of each cultivar were determined, using UV-Vis spectroscopy. The best antioxidant activity has been noted in the 'Sevtopolis' cultivar, the 'Blue Scent' has the highest percentage of lycopene (3.47 mg/100 g) and the 'Dwarf White' cultivar the large amount of β-carotene (3.25 mg/100 g). The ornamental qualities have been highlighted by making decorations to create a relaxing space. The data obtained will help to identify the cultivars with the most biologically active principles as well as those with special decorative qualities.

Key words*: Lavender, UV-Vis spectroscopy, ATR-FTIR spectroscopy, ornamental, bioactive compounds.*

INTRODUCTION

The *Lamiaceae* family incorporates a wide variety of plants with biological and medical applications (Uritu et al., 2018). The genus *Lavandula* is an important member of this family comprising 39 species, 30 subspecies and varieties, as well as 17 hybrid taxa (Upson and Andrews, 2004). Lavender is cultivated mainly for its essential oil, with an important role in the pharmaceutical, cosmetic, perfumery and aromatherapy industries (Zuzarte et al., 2009; Prusinowska and Śmigielski, 2014; Tardugno et al., 2019, Haban, 2023). Studies have also confirmed its rich content in phenolic compounds, with strong antioxidant effects (Gallego et al., 2013; Piskernik et al., 2023; Chianese et al., 2023; Batiha et al., 2023).

The main phenolic compounds of lavender flowers are: hydroxybenzoic acids, hydroxycinnamic acids and flavonoids (Dobros et al., 2022), but new phenolic compounds such as lavandunat, lavandufurandiol, lavandufluoren lavandupyrones A and B, lavandudiphenyls A and B were isolated by Yadikar et al. in 2018. The level of bioactive compounds depends on the species, cultivars, geographical origin, climatic conditions, harvest time and extraction method (Dobros et al., 2022).

The most important cultivated species of the *Lavandula* genus are: *L. angustifolia* Mill.; *L. latifolia*.; *L. lanata* and *L. x intermedia* (a sterile hybrid obtained by crossing *L. angustifolia* and *L. latifolia*). The genus also includes numerous subspecies and several hundred cultivars (Passalacqua et al., 2017), very valuable from an ornamental point of view. They are distinguished by their elegant appearance, foliage in contrasting colours, fragrant flowers in distinct shades (white, light pink, shades of blue and purple) and adaptability in the landscape (Bader, 2012; Demasi et al., 2021). Fresh or dried flowers are used in floral art and in making potpourris to flavour interiors. Lavender extracts and essential oils with phytotoxic and insecticidal properties may also be valuable in the agrochemical industry (Pavela, 2005). In addition to the aesthetic and utilitarian aspects, lavender also has an important role in attracting beneficial insects, such as bees and butterflies, being a melliferous plant (Benachour, 2017). It can also add more flavour to syrups, drinks and sweets. All these properties cause a significant increase in interest in the cultivation of lavender, both from an economic and medicinal perspective, as well as from an ecological and ornamental perspective. This study focuses upon the phytochemical profile of lavender extract, involving the flavonoids, tannins, carotenoids content and antioxidant activity. Moreover, our study aims to highlight the aesthetic value of some lavender cultivars as well as their role in alternative therapies.

MATERIALS AND METHODS

The study was carried out on six varieties of lavender grown in the field, in Argeș county. The mother plants were purchased from specialized companies. Specific care works were applied (watering, soil mobilization, shortening of branches to 15 cm every spring, fertilization with natural fertilizers 2 times/ year), for 3 years. In the 3rd year, the plants developed a uniform bush. Leaves and flowers were collected to determine the content of some biochemical compounds by ATR-FTIR and UV-Vis spectroscopy.

Lavandula angustifolia 'Sevtopolis'. Tall bush (50-70 cm), light blue-violet flowers, fragrant. High content in biochemical components, long flowering period and resistance to drought and frost. Suitable for landscaping, floral decorations, essential oil and floral water.

Lavandula angustifolia 'Hidcote'. Medium bush (40-60 cm), dark purple flowers, fragrant, blue-green foliage. It is a perfect choice for low hedges, borders, various decorations, dried flowers, as the flowers retain their colour and fragrance.

Lavandula angustifolia 'Nana Alba'. Attractive cultivar, flowering, with fragrant white flowers. It grows as a compact bush, low waisted (30 and 40 cm). It can be grown in containers, in beds, on terraces, slopes, borders.

Lavandula angustifolia 'Dwarf Blue'. Grows up to 20-25 cm, dense bush, gray-green leaves, purple inflorescences, fragrant. Tolerates partial shade and is drought tolerant. It is recommended for all types of gardens, in beds, borders, as cut flowers.

Lavandula angustifolia 'Blue Scent'. Compact shrub (40-50 cm), with gray-green leaves and slender, straight stems bearing fragrant inflorescences of blue flowers. Prefers sun exposure, well-drained soil. Drought tolerant. Good for borders, slopes, containers, gardens, urban areas, floral arrangements, rustic cottages. Good for drying and flavoring food and drinks.

Lavandula x intermedia 'Grosso'. Tall and vigorous cultivar (60-90 cm), with large and rich deep purple flowers, up to 15 cm long, strongly scented. It lends itself to decorations, as the flowers retain their fragrance and colour when dried. Excellent for all types of gardens.

Determination of the total content of tannins

The tannin content was determined with the methodology proposed by Cosmulescu et al. (2023), with some modifications. For analysis, a volume of 1 mL of aqueous extract was added to a 10 mL flask containing 2 mL of distilled water and 2 mL of Folin-Ciocalteu reagent. After 5 minutes, 5 mL of 10% sodium carbonate solution was added. After 60 minutes of rest, the absorbance of samples was measured at 760 nm and the concentration of tannins was expressed in mg GAE/100 g dry weight of plant material.

Determination of total content of flavonoids

A volume of 1 mL of methanolic extract was added to a 10 mL volumetric flask containing 4 mL of distilled water and 0.3 mL of 5% sodium nitrite. The mixture was allowed to stand 5 minutes, then 0.3 mL of 10% aluminium chloride was added to the volumetric flask. After other 5 minutes of rest, a 2 mL solution of 1 M sodium hydroxide was added and the volume of the sample was adjusted with distilled water to 10 mL. The absorbance of the solution was measured at 510 nm. Total flavonoid content was expressed as mg catechin equivalents per 100 g (mg CE/100 g).

Determination of carotenoids content

The concentration of carotenoids expressed as mg/100 g plant material was determined in the resulting supernatant according to the mentioned procedure by Cosmulescu et al. (2023). The molar extinction coefficients of 184900/M cm at 470 nm and 172000/M cm at 503 nm for lycopene and 108427/M cm at 470 nm and 24686/M cm at 503 nm for β-carotene in hexane were used.

Total antioxidant activity
Total antioxidant activity was determined using the methodology suggested by Lazar et al. (2020) and the results were expressed as a percentage of the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH I%).

Statistical Analysis
All analyses were performed in triplicate and data were reported as mean ± standard deviation (SD). Results were processed by Excel (Microsoft Office 2010) and SPSS Trial Version 28.0 (SPSS Inc., Chicago, IL, USA).

Data were subjected to analysis of variance (one-way ANOVA; $p \leq 0.05$), and Duncan's Multiple Range Test (DMRT) post hoc tests were used to measure specific differences between sample means. The Pearson correlation coefficient was used to measure the strength of the linear correlation between the determined parameters.

ATR-FTIR Analysis
The spectral measurements were made with a FTIR Jasco 6300 spectrometer. An ATR accessory equipped with a diamond crystal (Pike Technologies) allows collection of FTIR spectra directly on a sample without any special preparation. The spectra were recorded in the region of 4000-400 cm^{-1}, detector TGS, apodization Cosine. The spectral data were processed with JASCO Spectra Manager II software. Samples were scanned at 4 cm^{-1} resolution, accumulation: 100 scans. IR bands were identified by comparison with published assignments (Silverstein and Webster, 1998; Mossoba et al., 2005; Movasaghi et al., 2008; Munteanu et al., 2023). The crystal was cleaned between measurements with ethanol and dried with a lint-free tissue. For each sample, three or four spectral data were performed to ensure the spectral reproducibility and assess analytical precision and the average spectrum was done.

Chemometric Analysis
Infrared Spectra were exported from Spectra Manager, in ASCII (dx) format, into the Unscrambler Software (Edition X 10.4, Camo Oslo Norway) for chemometric analysis. Spectra were pre-processed using the second-derivative transformation, the Savitzky-Golay derivation. The use of spectra derivatives with the Savitzky-Golay algorithm as a chemometric pre-processing technique is widely reported in most classifications based on FTIR spectroscopy (Dovbeshko et al., 2000; Topală and Tătaru, 2018; Topală et al., 2020; Vîjan et al., 2023).
The principal component analysis (PCA) model was developed using cross-validation. PCA was performed both on the entire spectral range (4000 to 400 cm^{-1}) and on the MIR spectral region (1680-1400 cm^{-1}), Validation: Cross Validation, Algorithm: Singular Value Decomposition (SDV).

RESULTS AND DISCUSSIONS

Determination of tannins, flavonoids, carotenoids and antioxidant activity
The biochemical compounds of lavender flowers extract are represented in Table 1. According to the results obtained, the mean concentration of tannins was 1479.11 mg GAE/100 g, with a minimum content in 'Nana alba' cultivar (1372.20 mg GAE/100 g) and the maximum in 'Dwarf Blue' (1725.31 mg GAE/100 g). Our results are in line with Costea et al. (2019) that report 1325 mg GAE/100 g tannins for lavender flowers collected in June.

Table 1. The biochemical compounds of studied cultivars

Cultivar	Flavonoids (mg CE/100 g)	Tannins (mg GAE/100 g)	Lycopene (mg/100 g)	β-carotene (mg/100 g)	DPPH I%
'Blue Scent'	1305.91±11.73 b	1372.66±92.08 c	3.47±0.04 a	2.38±0.08 d	82.06±0.35 d
'Nana White'	764.57±6.75 d	1372.20±0.21 c	2.34±0.03 d	3.25±0.04 a	72.39±0.30 e
'Hidcote'	1302.24±7.20 b	1403.75±0.12 b	2.29±0.03 d	2.37±0.04 d	86.37±0.29 b
'Dwarf Blue'	1148.78±1.21 c	1725.31±0.50 a	1.21±0.02 e	1.56±0.04 e	85.89±0.27 bc
'Sevstopolis'	1247.83±4.60 b	1511.33±0.14 b	2.90±0.04 b	2.64±0.08 c	87.91±0.30 a
'Grosso'	1549.80±81.49 a	1489.41±0.13	2.82±0.04 c	3.03±0.10 b	85.63±0.36 c
Total	**1219.86±245.23**	**1479.11±130.06**	**2.51±0.72**	**2.54±0.56**	**83.37±5.38**
Min.	**760.53**	**1319.44**	**1.19**	**1.52**	**72.09**
Max.	**1598.06**	**1725.72**	**3.51**	**3.28**	**88.20**

The average flavonoid content was 1219.86 mg/ 100 g and variation limits between 764.57 mg/ 100 g ('Nana Alba') and 1549.80 mg/100 g ('Grosso').

Galego et al. (2013) reported that the lavender flowers lyophilized extract had a lower polyphenol content (52 ± 2.1 mg gallic acid/g) than the leaves. Radulescu et al. (2016) investigated the chemical composition of *Lavandula angustifolia* extracts obtained by different extraction methods. They reported a content of 78.345±0.982 (μg/mg total extract) total flavonoids obtained by subcritical fluid extraction.

Carotenoids showed oscillations from 1.21 to 3.47 mg/100 g (mean value of 2.51 mg/100 g) for lycopene and from 1.56 to 3.25 mg/100 g (mean value of 2.54 mg/100 g) for β-carotene.

According to the results obtained by Dobreva et al. in 2024, levels of carotenoids in lavender were higher in conventional farming (between 55.5 and 77.3 μg/g) than in organic farming (between 36.9 and 72.2 μg/g).

Measurements of antioxidant activity of lavender plant extracts showed a significant variation between cultivars. The highest antioxidant activity was determined in 'Sevstopolis' and the lowest in 'Nana Alba' cultivars (87.91 and 72.39 %, respectively).

In the study by Galego et al. (2013), the extracts from lavender leaves had the highest antioxidant activity, 1.5± 0.06 (mM Trolox/g lyophilized extract), followed by lavender stems and the lavender flowers 0.2±0.01 (mM Trolox/g lyophilized extract).

According to the data of Radu (Lupoae) et al. (2019), the lavender extract displayed a high polyphenolic and flavonoids content, with an antioxidant activity of 2.28 mmol Trolox/g. D.W. Komes et al. (2011) studied the phenolic composition and antioxidant activity of some medicinal plants, including lavender, as affected by the extraction time and hydrolysis and reported a higher content of 4.94 mg catechin/L in hydrolysed extract of lavender. Lee and Shibamoto (2002) observed low antioxidant activities of volatile extracts of lavender in comparison to thyme, basil and rosemary.

According to Costea et al. (2019) the antioxidant properties of lavender plants were in correlation with the contents of total phenolic compounds and flavonoids in extracts. As shown in Table 2, the antioxidant activity (DPPH I %) correlated positively very significantly with flavonoids (r=0.778***), positively significantly with tannins (r=0.469*), but showed a significant negative correlation with β-carotene (r=-0.521*). Also, there was an increase in tannin content distinctly significantly correlated with a reduction in lycopene content (r=-0.697**) and β-carotene (r=-0.684**). In the case of carotenoids, between lycopene and β-carotene there was a significant positive correlation (r=0.531*).

Table 2. Correlation matrix between the content of flavonoids, tannins, lycopene, β-carotene and antioxidant activity in the lavender cultivars (r values are presented)

		Flavonoids (mg CE/ 100 g)	Tannins (mg GAE/ 100 g)	Lycopene (mg/100 g)	β-carotene (mg/100 g)	DPPH I %
Flavonoids	Pearson Correlation	1	0.115	0.350	-0.157	0.778***
	Sig. (2-tailed)		0.649	0.155	0.535	0.000
Tannins	Pearson Correlation		1	-0.697**	-0.684**	0.469*
	Sig. (2-tailed)			0.001	0.002	0.049
Lycopene	Pearson Correlation			1	0.531*	-0.041
	Sig. (2-tailed)				0.023	0.870
β-carotene	Pearson Correlation				1	-0.521*
	Sig. (2-tailed)					0.026
DPPH I%	Pearson Correlation					1
	Sig. (2-tailed)					

***. Correlation is significant at the 0.001 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

ATR-FTIR Analysis

Leaves are complex assemblages of organic compounds and it might be expected that they would display distinctive spectral features in the infrared range (4000-400 cm^{-1}). Fundamental vibration modes of various

molecular functional groups produce characteristic spectral absorption features that can serve to fingerprint many compounds (Vîjan at al., 2023). Such functional groups and related spectral features include hydroxyl (OH) in alcohols and acids, carbonyl (C=O) in esters, ketones, aldehydes and acids, and methyl (CH_3) and methylene (CH_2) in alkanes.
To compare the lavender samples FTIR spectroscopy was used as an efficient method.
Figure 1 shows the ATR-FTIR spectra of the lavender samples with major high bands and highlights the ornamental potential depending on biometric characteristics and aesthetics qualities.
Typically, the characteristic differences in the FTIR spectral analysis for lavender samples were observed (Tables 3 and 4).

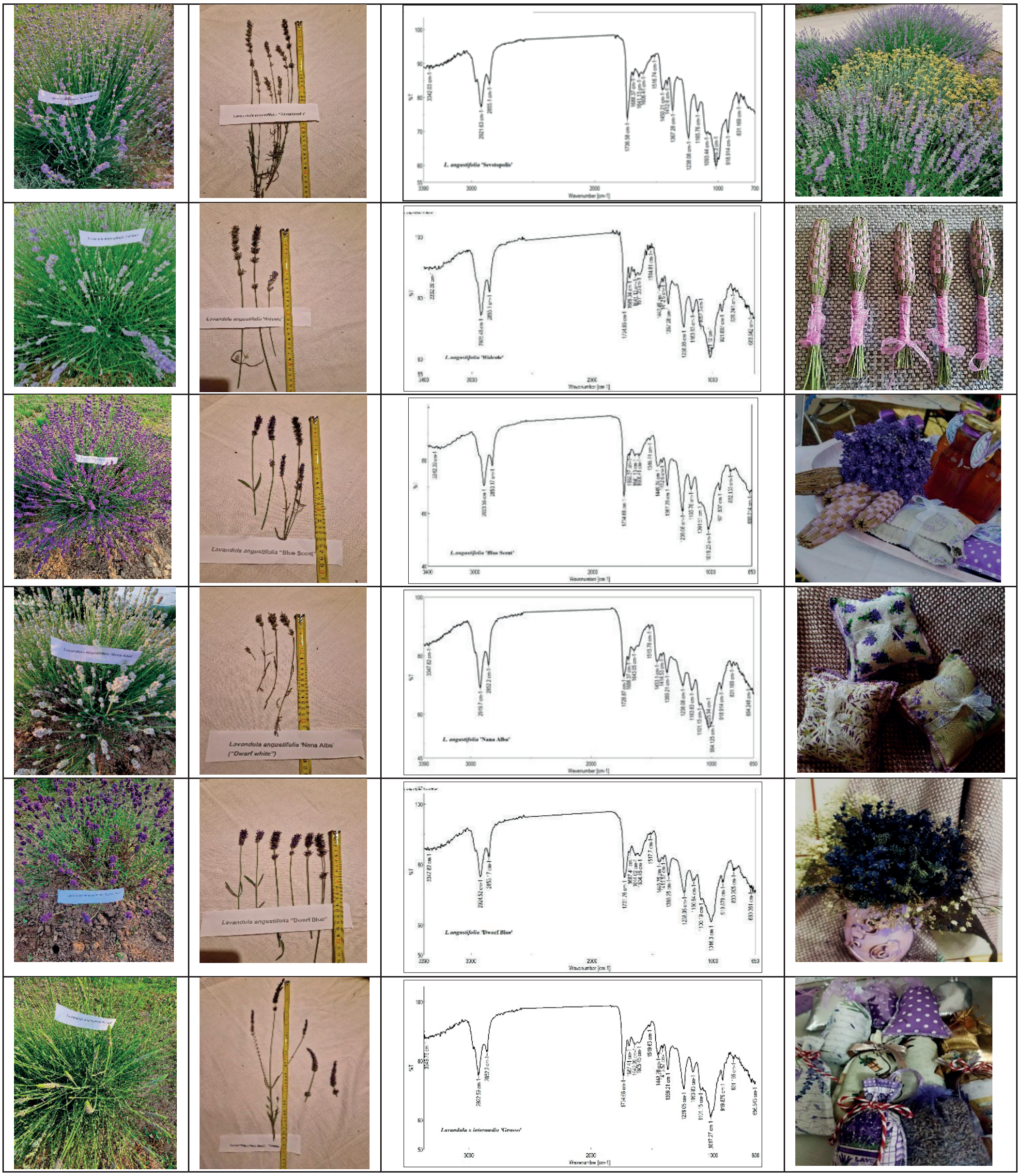

Figure 1. The ATR-FTIR spectra of the lavender samples

Table 3. ATR-FTIR spectra vibrational assignments for lavender flowers

Frequencies [cm^{-1}]						
'Hidcote'	**'Sevstopolis'**	**'Nana Alba'**	**'Dwarf Blue'**	**'Blue Scent'**	**'Grosso'**	**Peak assignment**
3332	3343	3347	**3347**	3342	3349	Stretching O-H, N-H, C-H
2925	2921	2919	2924	2923	2922	Asymmetric stretching vibration of CH_2
2855	2855	2852	2853	2853	2852	Symmetric stretching vibration of CH_2
1734	1736	1728	1731	1734	1734	νC=O stretching due to lipids
1689	1688	1688	**1687**	1688	1687	Stretching νC=O, Amide I
1658 1641	1641	**1643**	**1644**	1641	1642	Amide I band of protein and H-O-H deformation of water (Movasaghi et al., 2008)
1611	1606		**1604**	1606	**1605**	Adenine vibration in DNA (Mossoba et al., 2005)
1514	1516	1515	**1517**	1516	**1519**	Amide II
1442	1450	**1453**	**1446**	1449	1448	Asymmetric CH_3 bending of the methyl groups of proteins (Movasaghi et al., 2008)
1412	1412	**1414**	**1413**	1412	1413	Stretching C-N, deformation N-H, deformation C-H (Dovbeshko, 2000)
1367	1367	1369	**1368**	1367	1369	Stretching C-O, deformation C-H, deformation N-H (Dovbeshko, 2000)
1238	1238	1238	1238	1238	1238	Stretching PO_2^- asymmetric (phosphate I) (Dovbeshko, 2000)
1163	1165	1163	**1160**	1165	**1163**	Stretching ν_{as} O–C–O
1097	1093	**1101**	**1100**	1091	**1101**	Phosphate ester (C-O-P) band (Movasaghi et al., 2008)
1021	1016	**1025**	1016	1018	1017	ν(CO), ν(CC), δ (OCH), ring (polysaccharides, pectin) (Movasaghi et al., 2008)
		994				C-O ribose, C-C (Movasaghi et al., 2008)
921	918	918	919	**921**	919	Phosphodiester region
829	831	831	830	832	831	C_2' endo conformation of sugar (Movasaghi et al., 2008)
683	692	694	690	668	656	OH out-of-plane bend

Table 4. ATR-FTIR spectra vibrational assignments for lavender leaves

Frequencies [cm^{-1}]						
'Hidcote'	**'Sevstopolis'**	**'Nana Alba'**	**'Dwarf Blue'**	**'Blue Scent'**	**'Grosso'**	**Peak assignment**
3342	3351	3344	**3348**	3353	3344	Stretching O-H, N-H, C-H
2918	2917	2918	2918	2923	2917	Asymmetric stretching vibration of CH_2
2850	2851	2850	2850	2853	2849	Symmetric stretching vibration of CH_2
1728	1727	1727	1728	1730	1726	νC=O stretching due to lipids
1687	1690	1690	**1687**	1686	1688	Stretching νC=O, Amide I
1643	1658	1658	**1643**	1641	1642	Amide I band of protein and H-O-H deformation of water (Movasaghi et al., 2008)
1605	1604	**1610**	**1602**		**1612**	Adenine vibration in DNA (Mossoba et al., 2005)
1516	1514	1514	**1516**	1516	**1515**	Amide II
1454	1442	**1453**	**1453**		1453	Asymmetric CH_3 bending of the methyl groups of proteins (Movasaghi et al., 2008)
1413	1412	**1411**	**1414**	1414	1413	Stretching C-N, deformation N-H, deformation C-H (Dovbeshko, 2000)
1368	1368	1368	**1368**	1366	1367	Stretching C-O, deformation C-H, deformation N-H (Dovbeshko, 2000)
1316				**1315**	**1314**	Amide III band components of proteins
1237	1237	1234	1237	1238	1236	Stretching PO_2^- asymmetric (phosphate I) (Dovbeshko, 2000)
1142	1145	1167	1162	1144	**1169**	Stretching ν_{as} O–C–O
			1142			Phosphate & oligosaccharides Oligosaccharide C-O bond in hydroxyl group that might interact with some other membrane components Membrane-bound oligosaccharide C-OH bond (Movasaghi et al., 2008)
1093	1095	1096	**1098**	1096	**1100**	Phosphate ester (C-O-P) band (Movasaghi et al., 2008)
1008	1009	**1010**	1005	1006	**997**	ν(CO), ν(CC), δ (OCH), ring (polysaccharides, pectin) (Movasaghi et al., 2008)
		891		**894**	892	Phosphodiester region
		833	831	832	831	C_2' endo conformation of sugar (Movasaghi et al., 2008)
755 654	800 750	800 754 661	650	799 724 635	765 732 651	OH out-of-plane bend

For the lavender samples considered, the first three principal components (PCs) represent 100% for flowers (PC1= 96%, PC2= 6%, and PC3 = 1%), 99% respectively for lavender leaves. This indicates that these three components were provided a good separation between the groups (Figures 2 and 3).

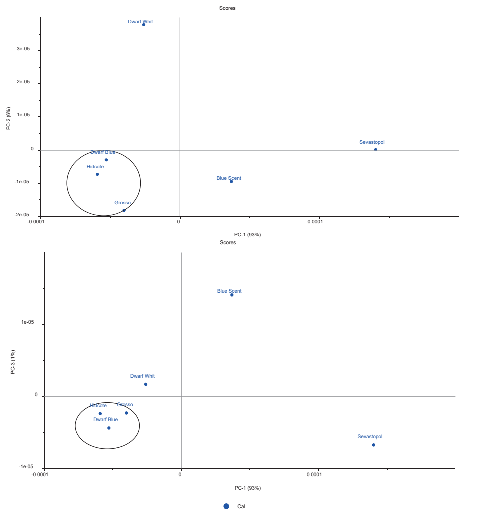

Figure 2. 2-D scores obtained from PCA of FTIR spectra of lavender flower samples for the first two PCs a), and PC3 versus PC1 b).

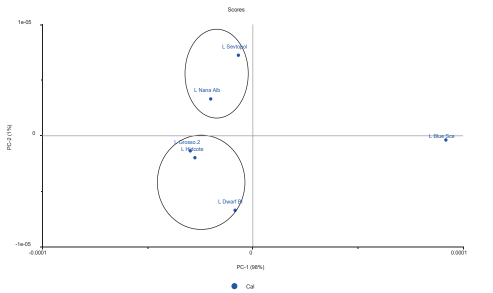

Figure 3. 2-D scores obtained from PCA of FTIR spectra of lavender leaves samples for the first two PCs

Principal component analysis (PCA) includes the region at 1680-1400 cm^{-1}. 'Hidcote', 'Dwarf Blue' and 'Grosso' were separated from the other samples, both in leaves and flowers.

Both the flower stalks and the flowers from the inflorescences, fresh and dry, were used to make specific decorations for a therapeutic space.

CONCLUSIONS

The obtained results revealed a high content of flavonoids, tannins, lycopene and β-carotene in 'Sevstopolis', 'Grosso', 'Hidcote' and 'Dwarf Blue' in contrast to 'Nana Alba' and 'Blue Scent'. The antioxidant activity was correlated positively very significantly with flavonoids and positively significantly with tannins. The lavender flower extract for 'Nana Alba' with low antioxidant activity exhibited relatively low flavonoid content. 'Sevastopolis' cultivar had the highest antioxidant activity, therefore it is the most frequently used in the phytopharmaceutical industry.

ATR-FTIR spectra coupled with chemometric analysis can be effectively used to discriminate lavender species. In our study, 'Grosso', 'Dwarf Blue' and 'Hidcote' were separated by chemometry from the FTIR analysis; they also had a high content of tannins and flavonoids, a fact confirmed by the UV-VIS spectral analysis.

The varieties 'Nana Alba', 'Blue Scent', 'Dwarf Blue' are easy to integrate into the landscape thanks to their elegant port. 'Dwarf Blue' and 'Blue Scent' floral stems are used in floral art as accent points, keeping their colour alive even after drying. 'Grosso' offers the largest floral mass and together with 'Sevstopolis' and 'Hidcote' are suitable for potpourri, flower arrangements, scented bags and small pillows as the flowers retain their fragrance for a long time.

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LANDSCAPE STUDY ON THE USE OF WOOD IN THE REHABILITATION OF THE "BOJDEUCA ION CREANGĂ" MUSEUM GARDEN IN IAŞI

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Abstract

This work presents the idea of arranging the space in the courtyard of the "Bojdeuca Ion Creangă" Museum in Iaşi. The objective is to make the space more efficient and create a favorable environment for specific activities. Through a detailed study of the site and a documentary study on the history of the building, the need for rehabilitation and appropriate landscaping was identified. The project is designed in a predominantly geometric style, combining the geometric elements imposed by the plan form with free plant design elements to give the whole composition a harmonious and aesthetic appearance. By strategically dividing the space into distinct functional areas and equipping them with specific furniture and plant elements, the landscaping will provide an optimal setting for cultural, recreational, and leisure activities within the museum. Thus, by integrating the ideas of landscaping with wooden objects in the proposed context, a new and valuable framework will be created for the experience offered to visitors in the courtyard of Ion Creangă's Bojdeuca Museum in Iaşi.

***Key words**: museum garden arragement, public garden rehabilitation, wood landscape design.*

INTRODUCTION

The present work results from the rigorous and careful work done to realize the Diploma Project of the co-author Sandu Laura-Corina. This project came as an idea in the context of the recent and controversial rehabilitation works of the building and courtyard of the "Bojdeuca Ion Creangă" Museum in Iaşi. Some of the historical sources that give details about the foundation of the museum and then about the first intervention of restoration and rehabilitation of the museum house or about the last intervention are present on sites like historia.ro, dosaresecrete.ro, romania.europalibera.org, and many others.

To support this approach of scientific research on the rehabilitation and restoration process of sites with historical, architectural, and landscape value, several works with the same specificity have been studied, of which we mention the one concerning the rehabilitation of the Metropolitan Park with historical and archaeological vestiges in Târgovişte, Dâmboviţa county (Pascu, Zlati, Calance, 2021), or the one that foresees the restructuring and redevelopment of the green space around the stadium in the city of Orăştie (Pascu et al., 2021). Another work studied is the one that brings into discussion the improvement of the quality of urban life through the introduction of landscape architectural elements in a project proposal within the square of Independence Square in Iasi, whose central point of interest is the monument of the Independence Statue (Cojocariu et al., 2023). The quality of urban life is also brought into question in Grecu's work, by introducing the principles of therapeutic gardens in the redevelopment of the green space of the neurology hospital in Iasi (Grecu C. et al., 2023). Other works mentioned are broader historical studies on the restoration of historical and architectural sites in Romania that include the part of gardens or parks related to them (Comanescu and Raducan, 2016), (Dobrescu, 2013) or a study on the successive rehabilitation interventions of the Herastrau historical monument park in Bucharest (Tudora, 2020). Another work proposed a project design for a private garden for events with multiple purposes in a existing beautiful wooded site in the Iasi city, Romania, on the shores of Lake Ciric II (Istrate et al., 2023).

The second part of the paper will present the idea of the space arrangement in the courtyard of the "Bojdeuca Ion Creangă" Museum in Iaşi. The objective is to make the space more efficient and

create a favorable environment for specific activities. The need for rehabilitation and appropriate landscaping was identified through a detailed site study. The project will be designed in a predominantly geometric style, combining geometric elements imposed by the shape of the plan with elements of free plant design to give a harmonious and aesthetic appearance to the whole composition. By strategically dividing the space into distinct functional areas and equipping them with essential elements, the landscaping will provide an optimal setting for cultural, recreational, and leisure activities (Dascalu and Cojocariu, 2016).
Landscaping aims to create a pleasant and optimal environment for cultural, recreational, and leisure activities in an atmosphere that reflects the theme and spirit of the museum and allows visitors to connect with this place's history and cultural heritage. Thus, integrating the design ideas and preserving the importance of wood in the proposed context will create an authentic and valuable setting for the experience offered in the courtyard of the ”Bojdeuca Ion Creangă” Museum in Iaşi.

MATERIALS AND METHODS

This work focuses on rehabilitating the area surrounding ”Bojdeuca Ion Creangă” Museum in Iaşi. The aim is to introduce wooden street furniture, decorative objects made of the same material, and ornamental plant compositions, enhancing and harmonizing the arrangement. The objective is to create a new green space dedicated to socializing and relaxing for the inhabitants of Iasi.
The rehabilitation of the area is an essential step in preserving and promoting Iasi's cultural heritage. Adding wooden street furniture and decorative objects aims to revitalize the place's aesthetic and provide facilities and comfort for visitors.
These wooden elements will be chosen and placed considering the garden space's functional needs. They will be designed to combine traditional aesthetics with modern utility, providing a pleasant and relaxing atmosphere for those who will frequent the space.
Creating this new green space encourages social interaction and connection among the residents of Iaşi. It will become a place to meet, spend leisure time, and relax, contributing to improving the community's quality of life.
The study material is based on general documentation of the features and typology of landscape design specific to the theme and an analysis of the space's current situation. At the time of the study, the site was under redevelopment and did not offer many opportunities for visitors.
Research methods specific to diploma projects were applied in the preparation of this work:
- Expert documentation from print and electronic sources to ensure a solid knowledge base and a detailed understanding of the subject of study.
- Analysis of existing case studies addressing similar real sites. This stage involved documenting and carefully examining the different approaches and solutions identified in these case studies, designed to address objectives identical to those proposed in the redevelopment process. One of these is the Amphitheatre of the Moscow Polytechnic Museum, whose landscape resolution inspired the introduction of water steps and decorative vegetation in the resolution of the studied slope (https://archello.com/project/museum-park-of-the-polytechnic-museum-in-moscow). Another case study was the garden of the Shakespeare family's birthplace in Stratford-Upon-Avon, which inspired the present project through the variety of decorative and aromatic plants that make up the picturesque compositions of the garden of the famous british museum (https://www.theenglishgarden.co.uk/gardens/visit-the-new-garden-at-new-place-shakespeares-family-home/).
The aim was to identify ideas and strategies relevant to the redevelopment project by analyzing these approaches and solutions.
Site analysis through a site visit and on-site analysis. This direct site analysis was necessary to gain a comprehensive and coherent understanding of the site. Based on the information and observations obtained during the visit, the redevelopment solution could be designed appropriately and adapted to the site's specificity.
- Design and preparation of the project for the redevelopment of the courtyard of the “Bojdeuca Ion Creangă” Museum, using a computer program specific to the garden design

process, carrying out both the written documentation of all the stages of research, case study, and analysis of the existing situation and the actual design part presented in plans and perspectives of the overall and detail of all areas related to the museum courtyard, with the description of plant compositions and the preparation of a general estimate of works and plant materials and furniture.

RESULTS AND DISCUSSIONS

The ”Bojdeuca Ion Creangă” Museum is in a quiet and picturesque settlement in the Sararie district of Iasi, Romania. The land on which the hovel is situated is approximately 2,500 square meters (according to the site plan in Figure 1) and is integrated into a residential setting. The surrounding area is characterized by a pleasant atmosphere and picturesque landscape.

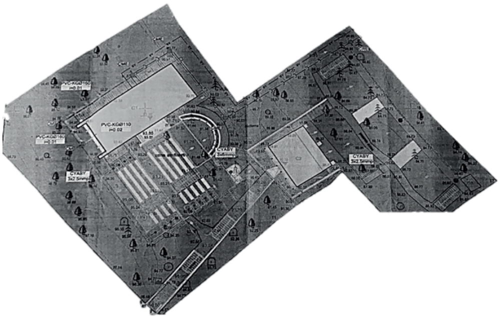

Figure 1. Situation plan of the site of the “Ion Creangă” Museum Complex

At the time of the study, both the outdoor space and the building of “Bojdeuca Ion Creangă” Museum were undergoing rehabilitation and renovation, which included the rehabilitation and modernization of the infrastructure of the grounds as well as the structure of the amphitheater and the building. The museum courtyard has only one access road (Figure 2 left). During the works, the drainage system of groundwater and rainwater from the slope was restored, which required the dismantling and restoration of the amphitheater in front of the “Bojdeuca Ion Creangă” Museum (Figure 2 right).

There is considerable mature vegetation on its surface, predominantly consisting of deciduous trees and shrubs. However, it is essential to note that most trees show signs of decay. The vegetation is affected by various factors, including neglect and lack of proper care (Figure 3).

Figure 2. Existing situation (steps to the main access (left); amphitheater and “Bojdeuca Ion Creangă” Museum building (right)

As part of the rehabilitation, special attention is paid to caring for and restoring damaged vegetation and introducing new and young species. The aim is to preserve the site's authentic character and provide visitors with an attractive and pleasant landscape.

Renovating the museum's back area involved changing the design and rebuilding the existing infrastructure to bring a splash of color to the site (Figures 3 and 4).

Figure 3. The existing situation of the slope behind “Bojdeuca Ion Creangă” Museum

At the time of the investigation, the area at the rear of the woodland was being used as a storage area for waste, and materials from the construction site were still in progress. Intervention was needed to transform this area into a landscaped and functional space (Figure 3).

The S.W.O.T. analysis method was used to effectively assess and make informed decisions on planning and practical solutions for implementing design concepts. This strategic approach is a valuable tool in landscape design, providing a detailed assessment of a site's existing conditions and potential before the redevelopment process.

Figure 4. The existing situation of the slope behind “Bojdeuca Ion Creangă” Museum

Strengths analysis identifies valuable site features such as mature vegetation, architectural elements, or outstanding historic features. These can be enhanced and integrated into the redevelopment project to accentuate the site's beauty and authenticity.

The weaknesses analysis focuses on aspects that need improvement, such as the degradation of existing vegetation or accessibility issues. With the right approach, professional restoration, and care interventions, these weaknesses can be reduced entirely or eliminated.

The installation of appropriate street furniture and efficient lighting systems highlights opportunities. Landscaping and the introduction of proper plant compositions for each area will help create an attractive and safe environment that promotes cultural and recreational activities and social interaction within the site.

Threats are external factors that may affect the redevelopment project, such as neighboring buildings, legal issues, or climate change. Appropriate protection and adaptation strategies can be developed by identifying and anticipating these threats to counter their potential negative impact.

Thus, applying the S.W.O.T. analysis method, a comprehensive site assessment is obtained, which can guide design decisions and practical solutions in the redevelopment process. The evaluation considers favorable aspects and those requiring improvement or additional protection.

Functional zoning

Following the studies and analyses carried out, as well as the identification of specific dysfunctions, a detailed zoning of the space of the ”Bojdeuca Ion Creanga” Museum in Iasi was carried out. This approach aimed to ensure a good organization and development of the various activities taking place within the site.

Through zoning, the aim was to optimize the use of space and create an environment suitable for each activity, thus respecting each area's functional and aesthetic requirements. Therefore, each zone has been planned and developed coherently, considering each area's needs, traffic flows, facilities, and objectives (Figure 5).

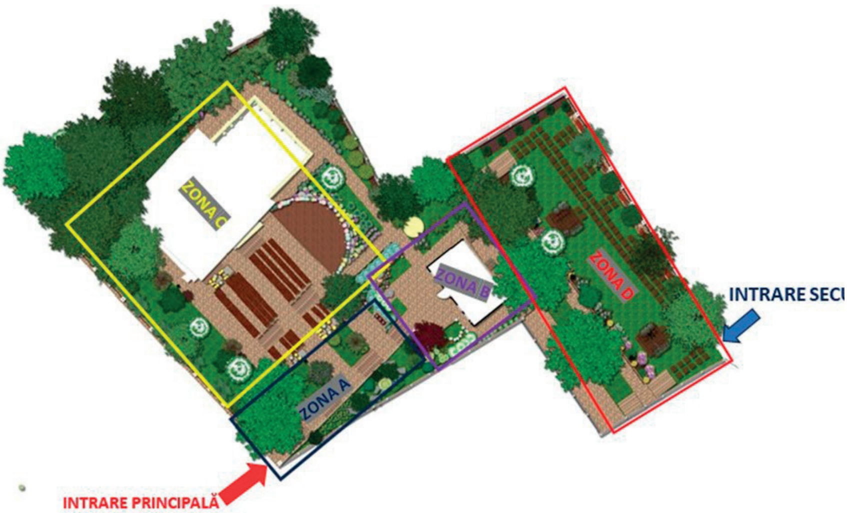

Figure 5. Zoning of the "Ion Creangă" Museum Complex in Iași

Area A has been identified as the current main entrance to the site space. It comprises a set of four steps descending to the front of the box, a bridge with a side walkway to the intermediate area of the amphitheater, and one to the stage area of the amphitheater. Following rehabilitation, the staircase railing is equipped with a wheelchair lift (Figure 6).

Area B, the central area of interest, is marked by Ion Creanga's hovel. Here, a simple layout has been adopted to enhance the beauty of the hovel itself. On the left and right side of the building, we can admire simple plant compositions with a rustic look, made up of various flower species commonly found in traditional courtyards.

This choice has added a unique charm to this area, enriching it with enticing colors and fragrances. The design was intended to create a pleasant and familiar atmosphere, transporting us back to the past of our culture and traditions. (Figures 6 and 7).

As can be seen in Figures 7 and 8 below, the use of various species of flowers creates a playful look from the variety of colors of the plants combined in a rustic style. This option included species such as *Aster novi-belgii*, which reaches a height of between 80 and 140 cm, *Leucanthemum maximum,* a flower that blooms from June to September, with a height of between 50 and 90 cm, *Phlox paniculata,* with heights of 50 and 150 cm, which was planted in groups for a more pleasing visual effect. *Anthurium majus* and *Gladiolus* sp. species were also included to add an exotic and vibrant touch.

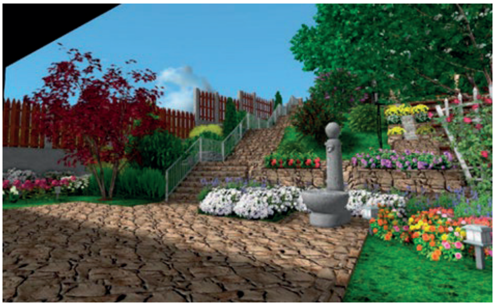

Figure 6. Main access area A

Figure 7. Zone B -"Bojdeuca Ion Creangă" Museum

A beautiful *Acer palmatum*, also known as Japanese Maple, is included as the composition's focal point. Due to its ornamental foliage and vivid colors, its presence makes the composition attractive and elegant and creates a pleasant atmosphere for visitors (Figure 6).

Various annuals were used in the design to create an impressive color spectrum. The composition includes varieties of *Salvia farinacea,* also known as Mealy Sage, a generous selection of *Tagetes erecta, Impatiens walleriana, Begonia semperflorens, Salvia splendens,* and *Zinnia elegans.* With this selection of plants, we have brought a burst of color and created an attractive and lively look to the area. At the back, you can see planters with *Pelargonium peltatum* species (Figure 6).

The existing cist has been retained as a site but given a new look by choosing a rustic-looking object with modern facilities, which successfully fulfills the need for thirsty visitors or their pets to drink water.

Area C, or the amphitheater area, includes a system of wooden terraces and benches, and at the end of these are water basins that have a dual function, both as planters for flowers and as decorative elements.

Figure 8. Zone C - "Ion Creangă" Museum Amphitheatre

The open-air amphitheater becomes an exciting and versatile space for organizing various cultural and educational activities for all age groups. Around the amphitheater is an elegant perimeter planter, and at its base is an impressive flower bed, providing a visually captivating landscape. The stone amphitheater stage has been clad with a wooden floor for better use in dance and stage movement performances while giving the whole area a rustic and authentic look. This charming space is designed to create a pleasant atmosphere and facilitate social interactions, making it the perfect venue for memorable events and performances.

In Figure 8, we can see an impressive floral bed composed of various species such as *Campanula persicifolia, Hypericum* \`Hidcote\`, *Geranium dalmaticum, Viola cornuta, Hosta sieboldiana*, and *Hosta fortunei* \`Albomarginata\`. These plants contribute to a charming and vibrant landscape. In the perimeter garden, *Petunia x hybrida* species were chosen.

Figure 9 shows that the *Chrysanthemum leucanthemum* species on either side of the water add color to the area.

The images in Figure 9 give us four views of the amphitheater area, which includes a fruit tree of the genus *Malus floribunda*, already present in the landscape, in the area to the left of the stage. This area blends harmoniously with a plant composition of *Camellia japonica* and *Berberis x stenophylla*. This landscape arrangement brings aesthetic balance and a diversity of textures and colors to the area. *Berberis stenophylla* complements the composition with its glossy leaves and varied colors. Together, these plants create a pleasant and attractive landscape, providing a relaxing atmosphere and a friendly setting for moments of rest and contemplation in this area (Draghia and Chelariu, 2011).

In Figure 9, we can see the asymmetrical arrangement of the seating areas clad with wooden stencils on two stacks of three and four steps on either side of an access alley to the entrance area of the new museum within the amphitheater. This arrangement was designed to provide a pleasant and comfortable experience for all participants as the museum guide talks to them about the life and work of the great Romanian storyteller.

Figure 9. Overview and details of Zone C - “Bojdeuca Ion Creanga” Museum amphitheater

Zone D was designed to provide a space for rest. Initially, only one main entrance was foreseen. Still, in this project, a second entrance has been proposed to facilitate direct access to this area for those living in the valley area and ensure a flow and smooth movement of visitors, especially on hectic days or events such as the Night of the Museums, when the number of visitors exceeds hundreds or even thousands of people (Figure 5).

The landscaping of this area focuses on visitor comfort, featuring wooden benches and gazebos that add a natural and rustic look. These are strategically placed, providing places to rest and shelter pleasantly and practically (Figure 10).

Wooden slabs were chosen instead of traditional materials to facilitate pedestrian circulation. The choice aims to create a friendlier and more pleasant environment, both aesthetically and in terms of user experience. Wood-framed tiles offer an enjoyable and environmentally friendly alternative to traditional solutions such as concrete or stone.

In the upper part of the area, a simple lawn was created, allowing people to sit in the shade under the *Prunus avium* species. This choice created a relaxing and pleasant space where visitors could enjoy nature (Figure 10).

Similar water pools were decided to be installed to create harmony between the upper and lower areas, adding calm and beauty to the overall landscape. On hot summer days, the waterfalls of the stepped pools in the amphitheater area and those with water jets behind the grotto provide a feeling of coolness and freshness in the air while being a visual highlight. The water games also create a relaxing and pleasant atmosphere, complementing the visitor experience (Figure 10).

Figure 10. Overview and details behind the “Bojdeuca Ion Creangă” Museum

Plant compositions

To enhance the space, vegetation has played a crucial role in fulfilling the aesthetic function. Its careful selection and strategic placement created an attractive and harmonious landscape, adding color and texture to the space (Pascu and Zlati, 2021).

To mask the fence, which acted as a hedge, plant compositions consisting of *Cornus alba, Cornus mas*, and *Corylus avellana purpurea* were decided*. Lavandula angustifolia* and *Hosta sp.* were used along the path (Figure 11).

To mask the area behind the fence of the neighboring property, in front of the amphitheater, a plant composition consisting of *Pyracantha coccinea*, *Ilex aquifolium*, Berber*is x stenophylla*, *Mahonia x media*, and *Juniperus squamata* was chosen. This combination was selected to create an aesthetically pleasing effect and to harmonize the area with the amphitheater's general landscape (Figure 11).

In addition to the existing vegetation in the entrance area, new plant species have been added, such as *Camellia japonica, Viburnum plicatum, Berberis thunbergii, Deutzia gracilis* (Figure 9). *Chamaecyparis pisifera* 'Filifera Aurea' will add a touch of color to the composition; its leaves have a golden hue and offer a slightly prickly appearance, adding diversity and texture.

Figure 11. Detail of perimeter plant compositions

Picea glauca 'Conica' is a slow-growing spruce species with a conical shape; its leaves are silver-green.

Juniperus horizontalis is a climbing and spreading species of juniper. Its bluish-green leaves will add variety and movement to your composition. *Juniperus horizontalis* will spread out on the ground and create a beautiful effect on the left side of the steps.

Juniperus communis is integrated into the composition to add a natural and rustic element. Its dark green leaves will provide a touch of consistency in the landscape.

This plant composition will combine different textures, colors, and shapes, creating a pleasant and harmonious landscape (Figure 12).

Figure 12. Detail of plant compositions along the main access steps

In the back part, it was decided to use *Berberis thunbergii, Buxus sempervirens, Lavandula angustifolia, Hydrangea macrophylla*, and *Hosta* sp. compositions. *Rhus typhina, Spiraea Japonica, Forsythia x intermedia, Hydrangea macrophylla,* and *Thuja occidentalis* (Bernardis, 2010; Bernardis, 2011; Sandu, 2012) (Figure 13).

Figure 13. Detail of vegetation composition along the slope retaining wall

Perennial or annual floral species were also included in the plant compositions, complementing the landscape and adding value. Also, in certain areas, such as the front of the box or near the amphitheater, floral carpets composed of various species were proposed to ensure a long flowering period and provide an attractive decoration. Among the floral species included in the project are *Campanula persicifolia, Hypericum* `Hidcote`, *Geranium dalmatic, Viola cornuta, Hosta sieboldiana,* and *Hosta fortunei* `Albomarginata`, *Tagetes erecta, Zinnia elegans.*

CONCLUSIONS

This work proposes a wide-ranging project to redevelop the space within the ”Bojdeuca Ion Creanga” Museum in Iasi, using wood as the primary material in the transformation process. The proposed reconfiguration considers aesthetic aspects, functionality, and integration into the urban ensemble.
The use of wood in the space's design proved appropriate, providing a warm and authentic ambiance. Thus, wooden walkways were included, giving the composition a pleasant and natural look. Wood was also used to create pieces of furniture, such as pergolas, benches, and tables, to provide visitors with a comfortable place to relax and contemplate during their visit.
In selecting the vegetation, consideration was given to the adaptability of the plants to the type of soil and climate specific to the area. Plants were chosen that would grow harmoniously and create a pleasant atmosphere around the museum; in the case of some existing plants that were in an advanced state of decay, the decision was taken to remove them, while others were strategically integrated into the composition to preserve the authentic elements and incorporate them into the new atmosphere created.
Through the landscape approach applied in this project, the intention was to revitalize the space in the courtyard of Ion Creanga's Bojdeuca museum and transform it into a pleasant and functional environment that would enhance the cultural heritage and offer visitors an authentic and memorable experience. This redevelopment could help attract more visitors and strengthen their connection with the history and traditions of this remarkable place.

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A COMPREHENSIVE STUDY ON TECHIRGHIOL LAKE'S ECOSYSTEM THROUGH CONSERVATION, ECOTOURISM, AND SUSTAINABLE PRACTICES

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Abstract

The paper aims to present the ecological potential of the Techirghiol area, focusing on the lake and its surroundings. Located in south east Romania, being the largest saline lake of the country, it is well known for its diverse ecosystems, habitat variety, and rich flora and fauna. In the introduction phase, multiple analyses were made, such as types of vegetation, species and habitats, trophic networks, and pollution. Regarding the results and discussions, the relationship between ecosystem and various factors was identified, creating a comprehensive synthesis. In terms of recommendations, the integration of ecotourism principles was strongly suggested to harmonise human interaction with lake ecosystems. This study underscores the fundamental importance of embracing conservation and sustainability, while developing an alternative type of tourism. As a conclusion, this study establishes a foundation for making well-informed decisions, thereby promoting a symbiotic coexistence between human activities and the valuable natural resources the lake provides.

Key words: *Techirghiol Lake, ecotourism, landscape sustainability, blue-green strategy, Natura 2000 site.*

INTRODUCTION

Techirghiol Lake is the largest salt lake in Romania, situated in the southeast of the country in the immediate vicinity of the coastline, approximately 15 km south of Constanța County. The site represents a Natura 2000 protected area and a special avifauna protection area (SPA), within the administrative territories of Techirghiol and Tuzla commune, near the national road DN39, connecting the municipality of Mangalia with Eforie.

Figure 1. Map showing the outline of the study area

Lake Techirghiol, formerly a fluvio-marine liman, spans an area of 1226.97 hectares, with a maximum depth of 9 meters and a salinity level of approximately 70 g/l. Positioned in the coastal expanse between Eforie Nord and Tuzla, the lake is divided by two dams into three segments. Among these, the eastern section, the most extensive, boasts high salinity, the middle section is brackish, and the western section, situated at the lake's tail, also known as Lake Zarguzon, is characterized by freshwater (Făgăraș et al., 2008).

Under these circumstances, the biodiversity of Lake Techirghiol is notably delicate, necessitating heightened attention. The long-term preservation of biodiversity involves implementing additional measures, specifically in the form of nature protection areas. Lake Techirghiol holds a crucial role within the Techirghiol Nature Reserve, acknowledged as both a Natura 2000 site and a Ramsar site. In addition to protective measures, effective strategic planning for the lake and its surroundings is paramount for conservation. Human activities, notably tourism, emerge as the primary threats to the biodiversity and natural values of the lake and its environs.

This study strives to mitigate the adverse impacts of tourism activities by advocating for sustainable practices, emphasizing alternative tourism, and promoting a harmonious relationship between human activities and the environment. Within this framework, ecotourism emerges as a specific form of alternative tourism that prioritizes environmental conservation, cultural respect, sustainability, and educational initiatives.

Lake Techirghiol, along with a significant portion of its surrounding area, obtained the designation of a RAMSAR site in March 2006, securing its place on the List of Wetlands of International Importance. Additionally, it is recognized as an important Bird Area in Dobrogea (ROSPA0061), covering an expansive area of 3035.3 hectares.

The protected natural area comprises the lake, arable land, crops, meadows, swamps that provide food, shelter, nesting, and living conditions for several species of migratory or sedentary birds, as well as other species of fauna such as benthal, amphibians, reptiles, mammals.

Table 1. The surfaces of the Natura 2000 site

Site of Community Importance ROSPA0061	
TECHIRGHIOL LAKE	44.20%
UNIRRIGATED ARABLE LAND	35.59%
MEADOWS	15.80%
SWAMPS	2.93%
INDUSTRIAL AND COMMERCIAL UNITS	0.89%
BUILT AREAS	0.56%
SPORTS AND LEISURE FACILITIES	0.03%

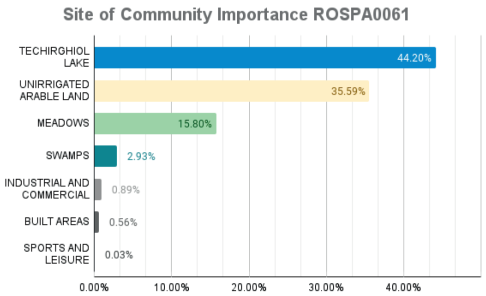

Figure 2. Illustrative graph for the surfaces of the site

MATERIALS AND METHODS

The methodological approach of this study commenced with a thorough exploration of theoretical foundations, involving an extensive review of existing literature. This encompassed a comprehensive consultation of documents, specialized literature, and case studies, aiming to establish a robust theoretical framework.

The practical phase involved a systematic approach to documentation, comprising three key components. Firstly, a meticulous examination of maps, general/zonal urban plans, and relevant documents was conducted to compile data about the site. Secondly, direct on-site visits were undertaken to glean firsthand insights into the specific characteristics and dynamics of the studied area. Lastly, on-site analyses included a detailed vegetation analysis to understand flora composition and ecological significance, a fauna analysis to assess biodiversity and ecological interactions, and a pollution analysis to gauge environmental stressors in both the lake and its adjacent areas. This integrated approach allowed for a holistic understanding of the Techirghiol Lake ecosystem, facilitating a nuanced exploration of its ecological intricacies and anthropogenic pressures.

The concluding phase of the study was dedicated to the planning and development of the Șincai Peninsula, an integrated area of the site, considering the principles of ecotourism to minimize the environmental impact of typical tourist activities. This approach aimed at mitigating the environmental ramifications associated with conventional tourist activities, thereby underscoring a commitment to sustainable and environmentally conscientious development practices.

RESULTS AND DISCUSSIONS

The establishment and enforcement of protected natural areas have resulted in a set of regulations and constraints governing the nature and conduct of human activities within these designated zones. In the vicinity of the lake, developmental initiatives adhere to the guidelines outlined in the area's management plan, strategically crafted to uphold and safeguard biodiversity (Băcănașu, 2020).

The conservation of biodiversity involves a multifaceted and dynamic approach, entailing efforts to preserve species and ecosystems in their most natural state feasible, shielding them from the detrimental impacts of human activities.
Although Lake Techirghiol is a protected natural area and is subject to certain rules, no significant action has been taken in recent years on human activities harmful to biodiversity.
The main analyses that supported this study and directed the best planning and development decisions are presented below.
The flora analysis was carried out on several vegetation categories as follows: algal flora, littoral sands flora, marsh flora, and calcareous hills flora. It includes species of high decorative value, rare, or even protected species presented in Figure 3.

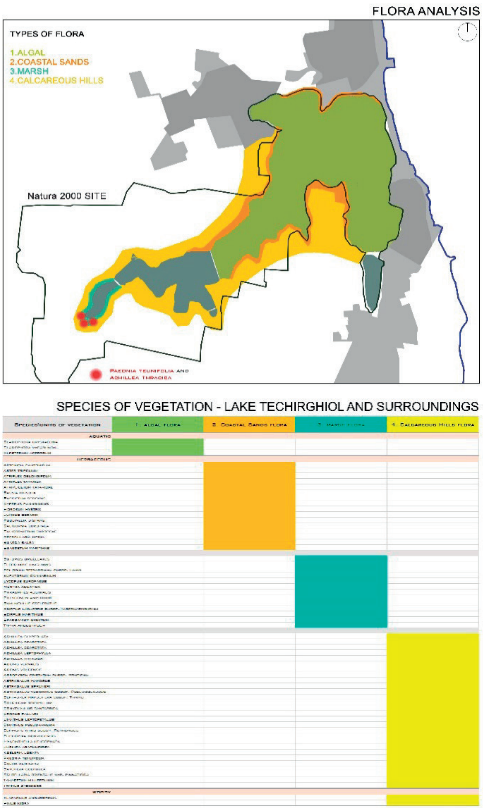

Figure 3. Flora analysis

Due to the high concentration of salts, only species with broad euryhaline limits can survive in the lake water. The algal flora is mainly represented by the green alga *Cladophora vagabunda*, one of the essential components of the sapropelic mud. The sapropelic mud, derived from organic-rich sediments, plays a significant role in balneotherapy, offering therapeutic benefits for various skin and musculoskeletal conditions. Known for its unique composition, sapropelic mud is rich in minerals, humic acids, and organic substances, making it a sought-after natural remedy in balneological practices.
Other species of macrophytic algae present in the lake are *Cladophora crystallina* and *Closterium acerosum*. These algae together with the crustacean *Artemia salina* are the main organisms involved in the formation of sapropelic slime. The flora of the littoral sands is made up of plant associations specific to marine salt marshes. The most common associations are *Suaedetum maritimae*, *Salicornietum europeae*, *Bassietum sedoidis*, *Atriplicetum tataricae*. Within these plant associations, halophilous species such as *Suaeda salsa*, *Salicornia europaea*, *Aster tripolium* subsp. *pannonicus*, *Spergularia media*, *Cyperus pannonicus*, *Bassia hirsuta*, *Artemisia santonicum*, *Atriplex oblongifolia*, *Atriplex tatarica*, *Hordeum hystrix*, *Juncus gerardi*.
The marsh flora is poorly represented and occurs mainly towards the tail of the lake where the water is fresh; it consists mainly of reeds (*Phragmites australis*), but also other hygrophilous species such as *Scirpus lacustris* subsp. *tabernaemontani*, *Scirpus maritimus*, *Eleocharis uniglumis*, *Butomus umbellatus*, *Sparganium erectum*, *Typha angustifolia*, *Eupatorium cannabinum*, *Lycopus europaeus*, *Mentha aquatica*, *Epilobium tetragonum* subsp. *lamyi*, *Polygonum amphibium*, *Ranunculus sceleratus* (Făgăraş et al., 2008).
Particularly interesting is the steppic flora and vegetation of the limestone hills in the south-western part of Lake Techirghiol, an area included within the boundaries of the ROSPA0061 area of importance for birds. The hills with a maximum altitude of 40-50 m, in places with sarmatic limestone on the surface, are known as the Peony Hills, due to the large populations of *Paeonia tenuifolia* (steppe peony), a European protected species, included in the annexes of the Bern Convention, together with another rarity, *Achillea thracica* (syn. *Achillea millefoliata*).

The number of floristic rarities in the south-western area of Lake Techirghiol is much higher, however, including *Achillea coarctata*, *Astragalus spruneri*, *Salvia aethiopis*, *Euphorbia dobrogensis*, *Dianthus leptopetalus*, *Crocus pallasii*, *Adonis vernalis*, *Adonis volgensis*, *Achillea clypeolata*, *Achillea coarctata*. To these species are added numerous rare elements of Pontic (in the broad sense) or southern (Balkan, sub-Mediterranean or Mediterranean) origin: *Achillea leptophylla*, *Agropyron cristatum* subsp. *ponticum*, *Dianthus pseudarmeria*, *Centaurea napulifera* subsp. *thirkei*, *Astragalus vesicarius* subsp. *pseudoglaucus*, *Astragalus hamosus*, *Convolvulus cantabrica*, *Hyacinthella leucophaea*, *Colchicum triphyllum*, *Jurinea arachnoidea*, *Echinops ritro* subsp. *ruthenicus*, *Satureja coerulea*, *Scutellaria orientalis* var. *pinnatifida*, *Tanacetum millefolium*, *Thymus zygioides*, *Koeleria lobata*, *Salvia aethiopis* (Făgăraș et al., 2008).

In addition to the floristic variation of the lake, it stands out, in particular, for the diversity of its avifauna, harbouring rare species in the context of Romania's avian fauna, including some that are endangered at global or European level. Of the over 400 species reported so far in Romania, 264 species of birds have been observed in the Techirghiol Lake area, meaning over 65% of the species present in Romania (SOR, 2017-2020), making it a unique ecosystem.

This remarkable diversity underscores the lake's significance as a vital habitat for a wide range of species, contributing significantly to the overall biodiversity in the region.

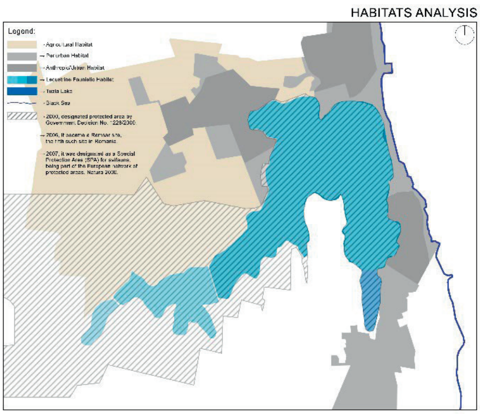

Figure 4. Habitats analysis

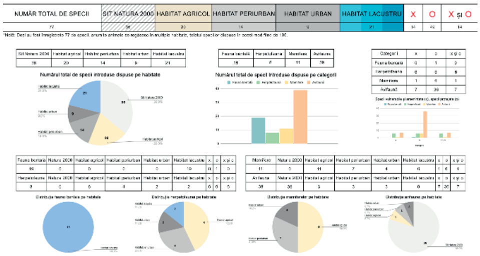

Figure 5. Illustrative graphs for the habitats analysis

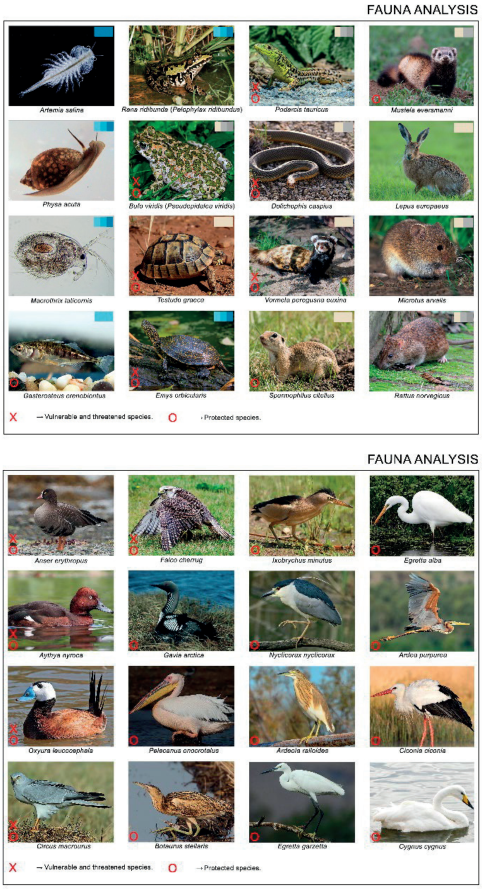

Figure 6. Fauna analysis

The lake in question is one of the main wintering sites in Europe for the red-breasted goose (*Branta ruficollis*). However, the surrounding region has been identified as a suitable habitat for more than 150 species of birds, both sedentary and migratory (Făgăraș et al., 2008). Among the vulnerable and threatened bird species in the Techirghiol Lake area (according to the Global IUCN Red List, 2008; Birds

Directive; Bern Convention, Bonn Convention), we mention: *Pelecanus crispus*, *Anser erythropus*, *Branta ruficollis*, *Aythya nyroca*, *Oxyura leucocephala*, *Circus macrourus*, *Falco cherrug* (Făgăraș et al., 2008).

Other strictly protected bird species in the Techirghiol Lake area (according to GEO 57/2007) are: *Gavia arctica*, *Pelecanus onocrotalus*, *Botaurus stellaris*, *Ixobrychus minutus*, *Nycticorax nycticorax*, *Ardeola ralloides*, *Egretta garzetta*, *Egretta alba*, *Ardea purpurea*, *Ciconia ciconia*, *Cygnus cygnus*, *Circus aeruginosus*, *Circus cyaneus*, *Buteo rufinus*, *Falco columbarius*, *Falco peregrinus*, *Charadrius alexandrinus*, *Pluvialis apricaria* , *Philomachus pugnax*, *Larus melanocephalus*, *Larus minutus*, *Sterna albifrons*, *Chlidonias hybridus*, *Chlidonias niger*, *Asio flammeus*, *Alcedo atthis*, *Lanius collurio*, *Lanius minor* (Făgăraș et al., 2008).

The impact of pollution on ecosystems is significant, affecting their health and functions. Various components of ecosystems, such as air, water and soil, are vulnerable to pollution, with adverse consequences for biodiversity and ecological balance.

This relationship extends beyond immediate environmental concerns, affecting human health, economies, and even contributing to global challenges like climate change. Recognizing the interconnected nature of these issues is crucial for developing holistic approaches that address the complex web of challenges posed by pollution. Therefore, adopting sustainable practices is essential for a comprehensive strategy to ecosystems.

The table presented bellow outlines the relationship between different types of ecosystems and pollution, with a focus on both the source and impact of pollution. Agricultural ecosystems may cause minor soil pollution, urbanized ecosystems pose risks, and lakes play a crucial role in biodiversity. Meadows and ecotones generally contribute positively to biodiversity with varying impacts.

Table 2. The relationship between ecosystems and pollution

SOURCE (LEFT) / IMPACT (RIGHT)	AGRICULTURAL ECOSYSTEM	LAKE ECOSYSTEM	URBANI ZED ECO SYSTEM	MEADOW ECOSYSTEM	ECOTONE (LAKE SHORE)	ECOTONE (BEACH)
AGRICULTURAL ECOSYSTEM		Food source. There are minor soil pollution factors.	Provides a food source for humans.	Food source for organisms, predominantly in the avifaunal zone.	The ecotone is very narrow due to the expansion of the agricultural area.	Does not interact directly.
LAKE ECOSYSTEM	Food source for birds, insects		Source of food and natural substances for treatment purposes.	Food source for organisms.	Increases biodiversity.	Increases biodiversity.
URBANIZED ECOSYSTEM	There may be a risk of built-up area expansion.	Water pollution source through discharges.		No direct impact.	Does not present major risk factors.	Human intervention has reduced the degree of biodiversity.
MEADOW ECOSYSTEM	Food source for birds, insects, rodents.	Increases biodiversity.	Does not present major risk factors.		Strongly biodiversified area in a good balance.	Does not interact directly.
ECOTONE (LAKE SHORE)	No strong impact interactions occur between zones.	Strongly biodiversified area in a good balance.	Does not present major risk factors over a short period.	Biodiverse environment.		Enhanced biodiversity.
ECOTONE (BEACH)	They do not interact directly.	Enhanced biodiversity.	Does not present major risk factors.	Does not interact directly.	Enhanced biodiversity.	

- NEGATIVE IMPACT
- POSITIVE IMPACT
- NO SIGNIFICANT IMPACT

For the second part of the study, after conducting the macro-analysis on Techirghiol Lake’s ecosystems, the case study examined in this paper is the Șincai Peninsula. From an administrative point of view, it is part of Eforie Nord and is located on the shore of Lake Techirghiol, constituting an essential part of the Techirghiol Natural Reserve. Its strategic position and its status as a protected area underline its importance in the conservation of biodiversity and ecosystems.

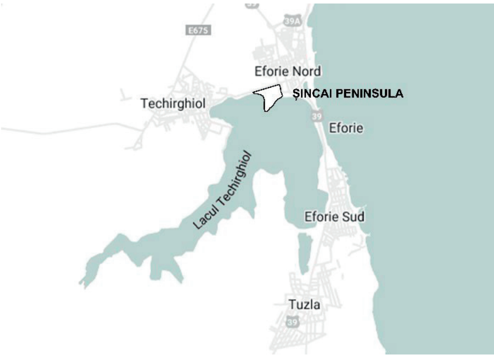

Figure 7. Map showing the location of the Șincai Peninsula in the context of Techirghiol Lake

According to the analyses carried out, it was found that the area of the Șincai Peninsula is under considerable pressure due to anthropic activities that have disturbed the ecological balance.

Upon closer local analysis, various abandoned structures and the concrete station can be observed on the peninsula, significantly impacting the fragile ecological balance of the lake. Within the peninsula, degradation of the vegetation landscape and the transformation of the recreational forest into an untended area can be observed. Additionally, due to construction along the shoreline, the location of the concrete station, and the uncontrolled development of green areas, the harmonious connection between human and nature has become impossible.

These disturbances not only affect the immediate environment but also have broader implications for the biodiversity and sustainability of the entire region. The repercussions extend beyond the Șincai Peninsula, impacting neighboring ecosystems and challenging the overall resilience of the local environment.

Increases in density are evident throughout Eforie Nord, either through new construction or the addition of extra floors to existing buildings. The rapid urbanization poses further challenges to maintaining a balance between human development and environmental conservation. Urgent measures and sustainable practices are necessary to address these issues and ensure the long-term health of the ecosystem in this region.

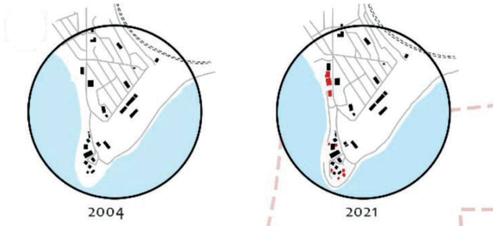

Figure 8. Urban growth trends of the Șincai Peninsula (Source: Guju, 2021)

However, increasing density at the end of the peninsula may have serious consequences for the fragile habitat of the bay and the natural landscape of the area. It should be noted that in Eforie's General Urban Plan and Local Planning Regulations, the Șincai peninsula, the landscape protection perimeter, is proposed for landscape rehabilitation.

Following the comprehensive analyses conducted in the area, incorporating a SWOT (which assesses the Strengths, Weaknesses, Opportunities, Threats) analysis can provide valuable insights into the overall strategies framework and potential future directions, as detailed below in Table 3.

Table 3. SWOT analysis

Strengths (S)
- Existence of natural resources: water with therapeutic benefits, sapropelic mud; - High biodiversity area; - Lake with great recreational, tourist and spa potential; - Existence of numerous types of landscapes.
Weaknesses (W)
- Hostile space, disadvantaged by concrete station; - Degradation over time of the typology and the way the station was originally designed; - Poorly diversified leisure facilities; - Chaotic buildings near the shore of Lake Techirghiol.
Opportunities (O)
- Growing the camping culture in Europe; - Lake Techirghiol is a RAMSAR site and can attract funding for research and protection projects;

- Increasing trend for alternative forms of tourism; - Possibility of scientific research on vegetation in areas of high biodiversity.
Threats (T)
- Decreasing interest of tourists in spa tourism; - Lack of vision to preserve natural heritage and promote cultural and spa tourism.

The potential of the site is represented by its high biodiversity and strategic location on the shore of Techirghiol Lake, a protected natural site known for its curative and treatment qualities. The dysfunctions of the site include: fracturing from the rest of the town due to the railway infrastructure, the location of the concrete station in the vicinity of a fragile habitat, the lack of connectivity between the peninsula and the town (i.e. the railway station), and the non-regulated growth of the built-up area near the shore.

Thus, the result of this synthesis demonstrates that the studied area is endowed with a high potential for landscape rehabilitation and for the valorisation of the curative properties of the lake, right on its shore.

Table 4. Diagnosis - Vision - Mission for the site

Diagnosis
Loss of camping culture, balneal dimension and environmental values.
Vision
Integration and regeneration of the balneal culture while preserving the natural environment.
Mission
To enhance the landscape value of the natural and balneal heritage. Promoting alternative tourism and its forms in the balneal context.

The development strategy for the Șincai Peninsula is grounded in four key aspects: preserving and regenerating the vegetation, addressing environmental problems related to the impact of the concrete station on the delicate habitat of Lake Techirghiol, restoring a balneal tourist function that aligns with current challenges, and enhancing connections between the peninsula and the town. Importantly, the strategy aims to achieve these objectives without significant intervention in the natural landscape.

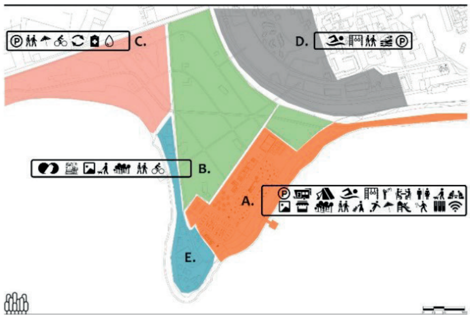

Figure 9. Șincai Peninsula's strategy
(Source: Guju, 2021)

The proposed project aims to address the following issues: conservation of vegetation, including the development of a long-term management plan, regulation of movements on the peninsula to accommodate various user types without compromising the habitat of the Techirghiol Lake, establishment of a hybrid campsite to meet the current demand of tourists while adhering to environmental requirements, refurbishment of disused structures near the concrete station to minimize de built footprint of the peninsula, serving as new campsite facilities, introduction of sports facilities catering to both campsite users and the local community, development of an ecological beach for campsite users, including naturists.

In recent years, the global tourism industry has witnessed a paradigm shift towards sustainable and eco-friendly practices. The integration of ecotourism principles into camping site development offers a blueprint for sustainable and ecological tourism.

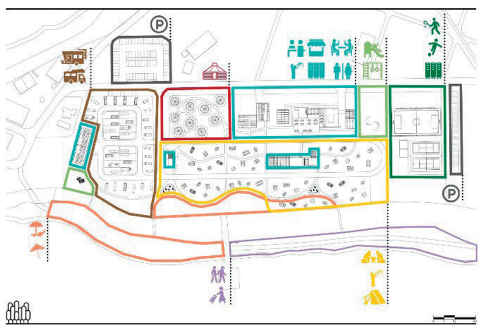
Figure 10. Proposed functions (Source: Guju, 2021)

By fostering a deep connection between campers and the natural environment, these sites become stewards of conservation, contributing to a more sustainable and harmonious relationship between humans and nature. As the demand for responsible tourism grows, camping sites embracing ecotourism solutions are poised to lead the way in shaping a more sustainable future for outdoor recreation.

Figure 11. Aeriel perspective on the Șincai Peninsula (Source: Guju, 2021)

CONCLUSIONS

In conclusion, this paper underscores the fragility of Lake Techirghiol's biodiversity and emphasizes the need of comprehensive measures to ensure its long-term preservation. Effective strategic planning is deemed essential for safeguarding the delicate balance of Lake Techirghiol and its surrounding environment. The recognition of human activities, particularly tourism, as primary threats to biodiversity underscores the urgency for sustainable practices.

Intervention in an area abounding in natural and cultural values must take into account a series of very well-established principles in order not to lead to their irreversible destruction. For this reason, all the activities in the studied site must be carried out in a controlled way, and the users of the space must take into account and educate themselves on how they can contribute to the protection and conservation of the fragile habitat of Lake Techirghiol.

In this way, camping, as a form of alternative tourism, responds to these requirements and is the most sustainable form of tourism. The use of endemic species or species that are already part of the ecosystem balance of the site is recommended in the planning project.

Ecotourism, identified as a specific form of alternative tourism, is positioned as a solution that prioritizes environmental conservation, cultural respect, sustainability and educational initiatives, providing a framework for a responsible and enriching engagement with Lake Techirghiol and its unique ecosystem. The paper draw attention to the imperative to protect the biodiversity of Lake Techirghiol through a multi-faceted approach that combines protection measures, strategic planning and a shift to sustainable and responsible tourism practices, with a specific focus on the merits of ecotourism.

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A CULTURAL LANDSCAPE OF CASTLE AND MANOR PARKS AND GARDENS NEAR AND AROUND BUCHAREST. CASE STUDY OF THE CATARGIU ESTATE IN MAIA

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Abstract

In between the 18th and early 20th century a large number of castles and manors with parks and gardens were built near and around Bucharest, particularly north of the city and near main roads and rivers. Together, these ensembles define a network and cultural landscape of peri-urban noble estates similar to other around big cities in Romania (eg. Cluj-Napoca, Târgu Mureș, Timișoara etc.) or along several historical and commercial routes such as the Prahova or Mureș river valleys or the road from Bucharest to Iași, and, to a certain extent, to other networks of castles and manors with parks and gardens such as those near Paris and the Loire valley, Florence, Rome etc. Either in communism, yet also after 1989, many of these sites were destroyed, their parks were fragmented into multiple properties, their buildings dismantled or left to decay etc. The following paper thus aims to address both the nowadays cultural landscape defined by the remnants of these ancient estates, as well as to emphasize on the importance of one of the oldest manors and its park at Maia.

***Key words**: Bucharest, Catargiu, cultural landscapes, garden history, historic monuments, Maia*

INTRODUCTION

This paper focuses on the commune of Maia, specifically the Catargiu Estate (now in ruins) and aims to emphasize the cultural and landscape values of the site and, moreover, on the importance of the preservation of its immovable, particularly landscape, elements.

Although only a handful of archival sources and more or less recent studies refer to Maia and its surroundings, its listed monuments and protected trees, as well as its (yet) not protected park and the village's surroundings represent valuable components of the cultural landscapes around the capital city of Bucharest.

The rapid development of the city of Bucharest, particularly since the 18th century, led to a similarly rapid development of a complex network of castles and manors surrounding the capital-city and/or built adjacent to some of the most important historic and commercial routes (see Monumente Uitate - https://monumenteuitate.org/ro/). All these ensembles were surrounded by parks and gardens of different styles and sizes, designed either by locally, regionally or internationally renowned gardeners or by the owners themselves. Among the noteworthy noble estates of this network (most of which are listed on the Historic Monuments' List - HML) - estates that are situated at varying distances from the capital - include: the Știrbei manor ensemble in Buftea, the Brâncoveanu palace complex in Mogoșoaia, the Ghica manor ensemble in Căciulați, the Lecca-Micșunești manor complex, the Hagianoff manor in Manasia, the Marghiloman manor in Hagiești, and, among others, the Oteteleșanu castle ensemble in Măgurele.

This network delineates a specific cultural landscape - that of the noble peri-urban estates and the leisure phenomena (*vilegiatura*) from different historical periods - and is similar to other networks and cultural landscapes around major cities (Bucharest, Cluj-Napoca, Craiova, Iași, Sibiu, Târgu Mureș, or Timișoara) and along various historical and commercial routes (e.g., the Prahova Valley, the Mureș Valley, or the road to Moldavia) (de Roo et al., 2021; Ion, 2011; Ion 2008; Lahovary, 1901; Răducan, 2004). Like the other similar networks, either in communism, yet also after 1989, many were destroyed, their parks were fragmented into multiple properties, their buildings dismantled or left to decay etc. Also, as in the case of all the other similar networks briefly referred to above, the cultural landscape of the peri-urban manors (with parks) around Bucharest is not leveraged and lacks a strategic and integrated approach for development.

The commune of Maia, through the presence of the Catargiu Estate (Figure 1), embodies a part of the expansive network of castles and manors (with parks) that surrounds Bucharest, which notably began to develop towards the end of the 18th century and saw more significant growth in the 19th century (Figures 2-4), but by the mid and late 20th century the estate and all its components were already severely damaged due to a vast number of reasons (not referred to in this paper).

Figure 1. The Catargiu manor’s church seen from the area where the manor’s ruins are located (source: authors, 2022)

Figure 2. The Ghica manor ensemble in Căciulați (source: authors, 2021)

Figure 3. The Marghiloman Manor in Hagiești (source: authors, 2020)

Figure 4. The Brâncoveanu palace complex in Mogoșoaia (source: authors, 2020)

MATERIALS AND METHODS

This study was conducted following the review of the documentation listed in the bibliography (consisting in only a handful of primary sources, as well as on few publications and recent surveys) and through several *in situ* study visits. Future investigations into additional documentary sources and/or field analyses may reveal new insights regarding the history and evolution of these respective sites, specifically concerning the elements where cultural, natural, or mixed values reside.

RESULTS AND DISCUSSIONS

On the territory of Maia’s Local Administrative Unit, there are several protected sites listed as historic monuments, as well as some legally protected trees.

Historic Monuments

The following listed historic monuments are situated in and around the village of Maia:

- The archaeological site - "settlement" (HML code: IL-I-s-B-14058, period Latène).
- The Church of the "Assumption of the Virgin Mary" with Barbu Catargiu's tomb (HML code: IL-II-m-A-14139, 1778, rebuilt in 1862) (Figure 5).
- The ruins of the Catargiu Manor (HML code: IL-II-m-B-20200, 1820) (Figure 1).

Figure 5. The Church "Assumption of the Virgin Mary" with Barbu Catargiu's tomb, LMI code: IL-II-m-A-14139 (source: authors, 2022)

Figure 6. Fragment of the Catargiu manor's park (source: authors, 2022)

Although not listed as historic monument, an important presence in the current and historical landscape of the village is the old park of the Catargiu Manor (Figure 6). It occupies a significant area at the northern-northeastern edge of the locality's built-up area and retains numerous plant components and landscape compositions that testify to past designs.

The most important archival document referring to the park is a mid-20th century map from the National Institute of Heritage's archive (NIH archive, Figure 7). It portraits a vast park surrounding the manor and the lake, a few walkways doubled by tree alignments and it divides the park into several functional areas such as the forest-park, the large plantations, the manor's park, the church grounds, the orchard, arable lands, the lake etc. Apart from this map, there are no other dedicated historic or contemporary plans of the park or of any of its functional areas.

Secondary documentary sources identify the author of the first landscaping as the German-origin landscape gardener Carl Friedrich Wilhelm Meyer (Kovacs et al., 2022) who designed several municipal public parks and castle/manor/palace gardens in the Principality of Wallachia, the most famous being the Kiseleff Public Garden (inaugurated 1847), Cișmigiu Garden (inaugurated 1852), the park of the Oteteleșanu manor in Măgurele (designed around 1850), and the park on the Cantacuzino domain in Florești (designed around 1850) (El-Shamali, 2012; Mexi and Zaharia, 2020; Mexi et al., 2018; Marcus, 1958 and other).

Although sufficient historical information (plans, images, descriptions, correspondence, etc.) has not been identified to deeply understand the project realized for the park in Maia and its execution in the field, the reviewed bibliography refers to a park of approximately 10 hectares, with stone-paved alleys brought from Germany, fountains built from white marble, a dance area, a "lake pavilion" (Figure 7), a small island, and numerous plant compositions including exotic trees. The image captured in a few period iconographic documents and the elements still preserved on site (e.g., some relief modelling, fragments of alignment plantations, groups of trees, or monumental specimens of exotic trees) can be attributed, by analogy with others similar from parks and gardens listed above, to the landscape gardener Carl Meyer, offering additional arguments regarding the identification of the author of this park and aiding in a better understanding of how the landscape arrangement was conceived. Among the elements mentioned in archival materials, a significant selection of build elements (e.g., the marble fountain, stone-paved alleys) were not identified in the few iconographic and cartographic documents discovered (Figures 8 and 9), nor today in the current situation *in situ*, requiring in-depth research, including archaeological investigations, for their identification.

Figure 7. The lake pavilion, undated
(source: private archive)

Some of those found on site are represented particularly by relief modifications, particularly around the lake and some plant compositions such as fragments of tree alignments (Figure 12) or small groups of ancient trees (particularly *Celtis australis, Pinus nigra* – (Figure 11) and *Quercurs cerris* (Figure 13), and even one ancient plane tree (*Platanus acerifolia* - Figure 10) near the lake (an exemplar protected by County Council Decision).

The park is preserved in a medium state of conservation and still retains enough valuable components, specifically plantations (including protected trees such as plane trees, relief modelling, the lake, traces of its former alleys and fragments of built vistas). It represents an inseparable component of the old noble ensemble from which only the church and the manor's ruins have been listed, being eligible - according to the national evaluation criteria - for listing as historic monument.

Figure 8. The boundaries of the park captured in the Josephine topographic survey, 19th century
(source: mapire.eu)

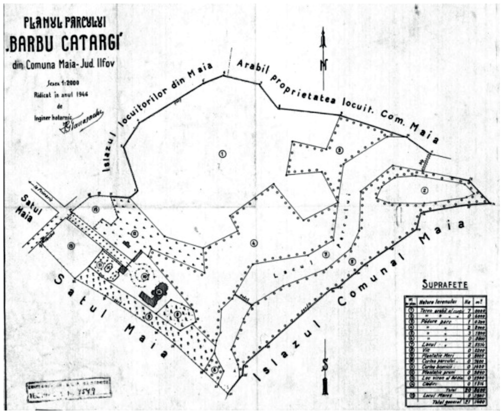

Figure 9. The plan of the Catargiu Manor's park, 1944 (source: NIH archive)

Figure 10. Century-old specimen of plane tree inside the manor's park (source: authors, 2022)


Figure 11. Group of ancient oaks inside the park (source: authors, 2022)


Figure 13. Group of ancient pine trees inside the park (source: authors, 2022)


Figure 12. Traces of tree alignments in the Catargiu Manor's park (source: authors, 2022)

CONCLUSIONS

The Catargiu manor ensemble is located in the northeast part of the locality of Maia and is today composed of two components easily visible on site: (1) the church (listed as historic monument) and the park (not listed) and (2) the ruins of the manor itself (listed as historic monument). It differentiates from the rest of the village through the size of the parcels that compose it, through topography and its relationship with the water surface, and through the density and diversity of the vegetation. Although in the past the ensemble was well connected with the fabric of the village, as it can be easily observed in historic maps of the area, today this connection is somewhat visible only in the area adjacent to the church and of the park (Figure 14). On the other hand, the manor's park represents a transitional element between the meadow landscape, the water bodies, and the agricultural area from the north-northeast of the locality.

The noble ensemble is identified as a distinct landscape unit in which mixed values reside, predominantly cultural: historical, architectural, urbanistic, landscape, and memorial.

Figure 14. Satellite image capturing the Catargiu manor ensemble (including the park - in the centre) and its relationship with the agricultural landscape, water bodies, and the built fabric of the village (source: google earth)

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The National Institute of Heritage archive (NIH archive)

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TOWARDS SUSTAINABLE URBAN GREEN AREAS: TREE INVENTORY IN GROZĂVEȘTI PARK (BUCHAREST, ROMANIA) FOR REVITALIZATION USING WATER-EFFICIENT APPROACHES

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Abstract

The Romanian law fails to give exact methodologies for the landscape study, planning, and management. Grozăvești Park is part of the strategy for increasing the amount and quality of green areas in District 6 in Bucharest, Romania. It also has the potential of an urban green pole, and will be integrated into the future green corridor of District 6. Considering the specific site conditions, as the metro under the park has water infiltrations, water scarcity and climate changes in Bucharest, there is a need for water-efficient approaches. To assess and protect the current park, comprehensive site analyses were carried out in spring 2024. The vegetation study identified 583 trees with 104 being dead or non-viable, leaving 479 viable trees. From the site analyses, topography and vegetation study, there was a clearer view for what set of recommendations to follow for the revitalization program of the park. The inventory of trees helped to identify young and mature trees that require irrigation and to select appropriate new plant species for efficient water usage.

Key words: *tree inventory, irrigation, green areas, water-efficient approaches, park revitalization.*

INTRODUCTION

At the level of the Municipality of Bucharest, the area occupied by green spaces must be expanded to reach the minimum limit imposed by the Ordinance of Emergency no. 195/2005 on environmental protection, respectively of 26 sqm/inhabitant. According to the Register of Green Spaces updated in 2011, the surface of green space per capita in Bucharest was 8.89 sqm/inhabitant (PIDU, 2021).

District 6 of Bucharest City Hall strategy is to increase the amount and quality of urban green areas in the District. Therefore, the revitalization of Grozăvești Park is under discussion.

This park was built around 1980 during the metro building. It is believed that most of the level difference comes from using ruins from the 1977 earthquake (Oanţă-Marghitu, 2012). Today, the Park is mainly crossed by users of the metro, these being forced to pass through a highly degraded landscape, partly resulting from some landslides, poor management, invasive species and vegetation not adapted to climate changes. Due to the Metro water infiltrations, water scarcity and climate changes in Bucharest, there are restrictions for using irrigation in this Park, therefore the concept for revitalization should follow water-efficient approaches and nature-based solutions. According to the Romanian Law (HG 907/2016), for an existing urban green area revitalization there is a minimum set of studies needed including: topography, geology and landscape. Unfortunately, the Romanian law fails to give exact methodologies for the landscape study, and for conducting a tree inventory. For the landscape study it is advisable to have all the information from: topography, geology, history of the site, tree inventories to visual analysis.

In recent years, there has been a higher interest regarding urban tree inventories, driven by challenges associated with pest and disease infestations in urban tree populations, alongside an increasing recognition among decision-makers of the diverse ecosystem services offered by trees within urban environments (Nielsen et al., 2014).

According to the Romanian Law 24/2007 on the regulation and management of green spaces, local public administration authorities are responsible for maintaining records of green spaces and vegetation inventories are required components of local green registries. The local green registries must be regularly updated to reflect any changes. However, Romanian local administrations often struggle to furnish accurate data regarding their managed green areas, resulting in inadequate planning and management of green spaces. Consequently, there is a notable decline in the ecological and aesthetic integrity of these areas (Morar et al., 2019; Morar et al., 2020).

Tree inventory systems serve as comprehensive tools for assessing the present and historical state of green areas, offering essential data for the development of planning and management strategies. Functioning as decision-making tools, these inventories compile vital parameters including: tree species, dimensions, health status, risk assessment, and spatial distribution (Ma et al., 2021).

In terms of dimensions, an easy to measure and commonly used is the diameter of trees at breast height - DBH (Cosovic, 2022); typically measured at 1.3 or 1.4 m above ground level, depending on regional professional standards. Beyond its inherent utility, tree diameter serves as a predictive metric for various other parameters, including such as tree growth, canopy spread, trunk flare, and tree height (Ma et al., 2021). Information on tree species condition and maintenance needs are also gathered (Ma et al., 2021).

Tree inventories are commonly conducted by arborists, urban foresters, landscape architects and other qualified experts. Additionally, community or citizen science initiatives, which involve the general public in tree data collection, have been observed with differing degrees of success (Ma et al., 2021; Roman et al., 2017). The precision of a tree inventory dictates the management decisions that can be derived from it. In comparison to data collected by professionals, the accuracy of information gathered by public volunteers may vary, potentially impacting its intended utility (Ma et al., 2021).

There are different strategies for efficient water usage in green spaces, including appropriate plant species (Rabbani KheirKhah and Kazemi, 2015).

MATERIALS AND METHODS

General descriptions of site

The study area is Grozăvești Park in the western part of Bucharest, District 6 (Figure 1). The climate in the southern region of Romania is a temperate climate with Mediterranean influence (Pârvu, 1980), but general mean temperatures are around 10.2-11.9oC; 400-500 mm annual rainfall (April and May with the highest amounts); 80-100 days with drought. During summer, the received water is 3-4 less than consumed water, resulting in aridity. To estimate climate trends, consideration was given to the projected changes for Europe regarding the Mediterranean climate impact drivers (Bednar-Friedl, 2022).

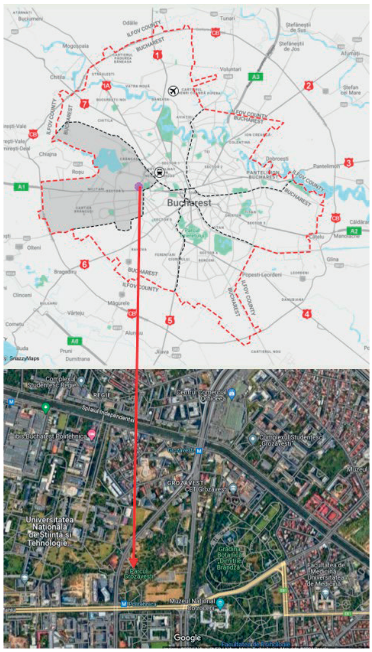

Figure 1. Grozăvești Park location - fitting into the context at the urban level of Bucharest (adapted from online maps)

The main destinations of the park will be recreation through quality landscaping and socialisation by creating a suitable setting with vegetation and urban amenities to facilitate sports, to encourage a healthy lifestyle, and other types of events that bring people together and create communities.

As it may be observed in Figure 2, Grozăvești Park is divided in 2 areas; the red dotted area is administered by the District 6 City Hall, while the blue area is privately owned. The total land surface has around 35,360 sqm.

Figure 2. Grozăvești Park location (red dotted is the actual studied area administrated by the District 6 City Hall, while the blue dotted is private)

Topographic study

During the planning phase of tree inventory process, GNSS RTK receivers were used to retrieve control points in the national coordinate system that were used for georeferencing, verification and constraint of determinations made with 3D laser scanning systems as well as for determinations made with the total station where there was no coverage from scanning. The 3D laser scanning systems contain state-of-the-art calculation algorithms and allow the creation of a model of the study area, with a relative accuracy of +/- 2 cm. The total station was used to fill the network of control points, where the environment was unfavourable for GNSS determinations, as well as to take over the detail points where, after the Lidar data post-processing, it was found that there was not enough coverage. Afterwards, the verification and constraint of the cloud was carried out on points obtained on control points and cloud generation end points, uncolored. The point clouds were imported into the CAD platform where the elements of interest were vectorized, the surfaces were calculated and the linear dimensions and reality were checked for compliance with the cadastral contours of the buildings of interest, these being extracted from the E-terra computer system. The topography CAD version was used for determining the location of trees and zoning (Figure 3).

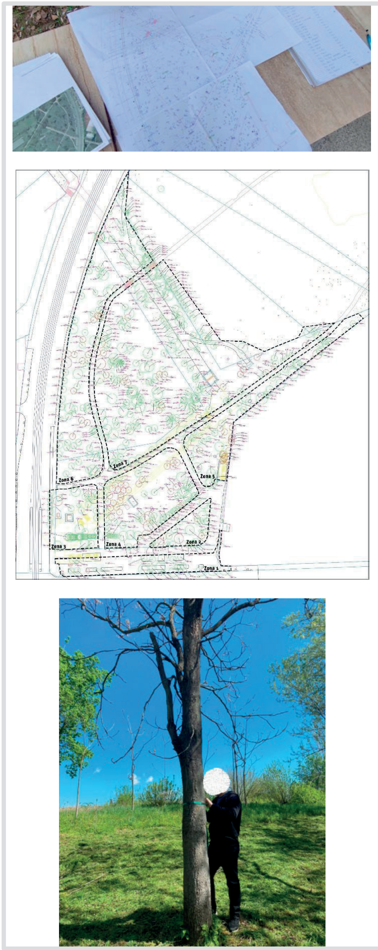

Figure 3. Tree field data location, zoning and collection by walking

Tree inventory

The study was conducted by three landscape architects with the help of five students from urbanism and landscape architecture for a total of four days of field study. Tree data was collected from the field during two periods: February 2024 (identification of tree genera) and April 2024 (inventory of tree species and other characteristics). In February due to winter conditions some species could not be determined, thus a new inventory to check was done during the vegetation period in April. Data collected for the tree inventory: species, DBH at 1.3, and viability. General visual and aesthetic analysis was conducted also in October 2023. All the data collected from the field was then added to the CAD file, and excel lists.

RESULTS AND DISCUSSIONS

The topographic analysis provided precise geospatial data for individual trees and detailed information about the site's terrain. While 3D imagery offered an overview of the site's general layout (Figure 4).

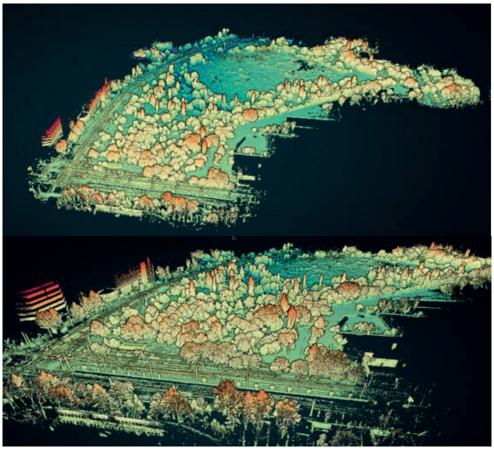

Figure 4. Location of existing woody vegetation

The tree inventory shows that in Grozăvești Park there are 583 trees, out of which 104 are death/non-viable, resulting in 479 viable trees.

There were 25 genus found during the inventory, out of which the most common Genus are *Populus*, followed by *Pinus, Thuja, Platanus, Pyrus and Tilia* (Table 1).

The assortment of trees belonging to the Genus presented in Table 1 are tolerant of dry soils, with the exception of *Thuja* (requiring moist to wet soil) and *Pyrus* (moist soil). There were 31 species found, out of which most common species representing around 50% of the total trees are: *Pinus nigra, Populus alba, Thuja* sp., *Platanus acerifolia, Tilia tomentosa* and *Pyrus* sp. (Table 2).

Table 1. Most common Genus

No	Genus	Frequency of counted cases	Dead Tree	Viable Tree	Percent of total viable cases (%)
1	*Populus*	77	5	72	15.03
2	*Pinus*	95	37	58	12.11
3	*Thuja*	53	6	47	9.81
4	*Platanus*	43	0	43	8.98
5	*Pyrus*	41	3	38	7.93
6	*Tilia*	40	2	38	7.93
7	*Ailanthus*	38	8	30	6.26
8	*Fraxinus*	35	7	28	5.85
9	*Acer*	34	7	27	5.64
10	*Prunus*	27	3	24	5.01
11	*Gleditsia*	25	2	23	4.80

Table 2. Most common species

No	Species	Frequency of counted cases	Dead tree	Viable tree	Percent of total viable cases (%)
1	*Pinus nigra*	94	37	57	11.90
2	*Populus alba*	56	5	51	10.65
3	*Thuja* sp.	53	6	47	9.81
4	*Platanus acerifolia*	43	0	43	8.98
5	*Tilia tomentosa*	40	2	38	7.93
6	*Pyrus* sp.	41	3	38	7.93
7	*Ailanthus altissima*	38	8	30	6.26
8	*Fraxinus* sp.	35	7	28	5.85
9	*Acer* sp.	30	5	25	5.22
10	*Gleditsia triacanthos*	25	2	23	4.80
11	*Populus nigra* Italica	21	0	21	4.38
12	*Prunus cerasifera*	23	2	21	4.38
13	*Carpinus betulus*	14	1	13	2.71
14	*Malus* sp.	17	4	13	2.71
15	*Aesculus hippocastanum*	4	0	4	0.84
16	*Ulmus minor*	4	0	4	0.84
17	*Juglans nigra*	5	1	4	0.84
18	*Prunus cerasifera Pissardii*	3	0	3	0.63
19	*Alnus* sp.	2	0	2	0.42
20	*Cydonia oblonga*	2	0	2	0.42
21	*Quercus robur*	2	0	2	0.42
22	*Salix matsudana* 'Tortuosa'	3	1	2	0.42
23	*Acer negundo*	4	2	2	0.42
24	*Betula pendula*	1	0	1	0.21
25	*Picea* sp.	1	0	1	0.21
26	*Pinus strobus*	1	0	1	0.21
27	*Ulmus pumila*	1	0	1	0.21
28	*Morus* sp.	2	1	1	0.21
29	*Salix babilonica*	3	2	1	0.21
30	*Robinia pseudoacacia*	11	11	0	0.00
31	*Prunus avium*	1	1	0	0.00
32	Unidentified	3	3	0	0.00
	Total	583	104	479	100

Pinus nigra species has the highest number of losses (39% of total *Pinus* specimens). According to European Atlas of Forest Tree Species, the Climate warming exacerbates water stress, which adversely affects the growth of this species. *P. nigra* is unusually tolerant of heat and summer drought needing watering planning within Grozăvești Park natural conditions. *P. nigra* 'Nana' is tolerant of drought, heat, and wind, being an alternative to be considered for park revitalization. An additional factor could be pest infestations or diseases, but this requires further examination by phytosanitary experts to confirm.

Fraxinus sp.: *F. excelsior* and cultivars require moist to wet soils; *F. pennsylvanica* and *F. velutina* require dry soil.

Acer negundo and cultivars need moist to wet soils. *A. negundo* tolerates flooding (a probable phenomenon during April and May, in the lowest parts of the park).

The most important result of the study was the extremely low frequency of *Quercus*, knowing that most species of this genus are adapted to dry soil conditions.

CONCLUSIONS

Grozăvești Park has the potential of an urban green pole due to existing mature vegetation, being possible to be integrated into the future green corridor of District 6. The vision of the project is to improve the quality of life of the inhabitants and increase urban resilience.

The existing vegetation was generally in good condition, with the exception of 104 dead specimens (mostly *Pinus* species) that were proposed for removal. The high number of dead trees was probably due to poor maintenance, possibly climate changes and orographic drought. For the vegetation that will be preserved, it is recommended to apply sanitary cuts to remove dry or broken branches.

The site's advantage of having mature indigenous species adapted to the climatic conditions was exploited for revitalization project proposal

In the concept for the revitalization of the Grozăvești Park, the existing vegetation was taken into account, keeping it mostly in the same form (but anticipating the dimensions after pruning).

The proposed species were mostly indigenous or naturalised species, both broadleaves and conifers to ensure a redundant decoration. Also, the proposed shrub species were indigenous species, adapted to the urban environment and drought soil site conditions in addition to their ornamental value.

After completion of the rehabilitation works, it is recommended to conduct a re-inventory of the young and mature trees and create a management plan for the park to track the effects of the investment over time.

Also, we recommend to use inventory of trees as a tool when tree intervention permits are issued. The results of this research will form the basis of the documentation necessary for the preparation of the Grozăvești Park Differentiated Management Plan, which will also show the design and maintenance priorities in the short-term, as well as the programming of interventions in the medium and long term. There is also the need for ongoing research to complete the database necessary for developing a tailored management plan for each category of elements, both vegetal and mineral, that constitute the landscape of Grozăvești Park.

In addition to the functions of relaxation and socialisation, Grozăvești Park can be transformed into a sustainable green space that can cope with environmental changes and help increase the area's attractiveness and economic development by valuing the existing vegetation and developing it with new tree planting that increase species and age diversity.

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INFLUENCE OF THE ULTRASOUND ON THE SOWING QUALITY OF SNAPDRAGON (*ANTIRRHINUM MAJUS* L.) SEEDS

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Abstract

The main goal of the present study was to establish the effect of ultrasound on the sowing parameters of snapdragon (Antirrhinum majus L.) seeds. Experiments were carried out with three varieties snapdragon. The seeds were sonicated with ultrasound for 2, 4, 6 and 8 minutes. The following parameters were investigated: germination energy, germinability, Mean germination time, Uniformity of germination, length of embryo root and hypocotyls, and fresh weight of the sprouts. The sowing parameters were improved from each duration of ultrasound treatment. The best results for germination and in most of the parameters were found about treatment with 6 minutes. The high correlation between the length of hypocotyls and embryo root and fresh weight was established. Polynomial regression with a high determination coefficient was registered.

Key words*: seeds, energy of germination, germination, mean germination time, seedlings.*

INTRODUCTION

Snapdragon is a flower crop widely used for landscaping open areas. It is applied often for small and courtyard gardens. The ornamental value of the species is determined by the specific flower structure and many different colors (Huxley & Griffiths, 1992). In addition, its flowering is long-lasting, throughout the growing season, which makes it extremely suitable for permanent landscaping. It is pointed out that in the Mediterranean region, it is even a perennial crop (Shafique et al., 2011). A positive side of snapdragon is the considerably long flowering period and the duration of preservation in good condition of the individual flowers. Although originally intended only for landscape use in parks and open public spaces as flower beds or borders, it has recently been grown for cut color (Sanderson & Martin, 1984). Various groups of cultivars have been developed and flowering can be regulated by temperature and light conditions (Ball, 1991; Rogers, 1992).

However, the species is characterized by relatively low seed viability and a long period of germination and emergence (Kabatliyska, 2005). In this regard, studies have been conducted to stimulate the vital processes of seeds by using various agents. Kang and Choi (2006) applied soaking of snapdragon seeds for two days in GA_3 solutions of different concentrations, pointing out that the application of 200 $ml.L^{-1}$ demonstrated the strongest stimulating effect. Similar experiments with GA_3 were conducted also by Bhargava et al. (2015). Additionally, these authors tested other chemical treatments with KNO_3 and KH_2PO_4 and bio-priming with *Trichoderma harzianum* and *Bacillus subtilis*. The greatest positive influence on the life processes of the seeds of this species is found in bio-priming, with an increase in germination by 20%. Contradictory results in the treatment of snapdragon seeds with chemical agents, and in particular with GA_3, were reported by Kepczynski (1979), indicating that a stimulatory effect was not reported in all cases.

The use of physical agents is another direction to improve the sowing qualities of the seeds of *Antirrhinum majus* L. The application of variable temperatures has a very high acceleration on the germination (Geo, 1991). Armitage (1991) also points out the influence of different temperature conditions on the improvement of seed viability. Research on the pre-sowing stimulation of snapdragon seeds is limited, and the application of some methods and agents is lacking. This determined the purpose of the study.

The main goal of the present study was to establish the effect of ultrasound on the sowing parameters of snapdragon (*Antirrhinum majus* L.) seeds.

MATERIALS AND METHODS

The experiments were carried out with three snapdragon varieties - as follows Apple Blossom, Sonnet Carmine and Orange Wonder. The seeds were treated with ultrasound in the Scientific laboratory of the Department of Horticulture at the Agricultural University - Plovdiv, Bulgaria. The tested periods of sonication were 2, 4, 6 and 8 minutes. As a control were used not treated seeds. Ultrasonic bath Nahita, model 620-1 of the company Auxilab, S.L. from Spain was applied for sound treatment. This aparate are characterized with the following parameters: frequency 40 KHz, volume 0.6 L.

After treatment, the seeds were placed in Petri dishes with a diameter of 10 cm. On the bottom of the Petri the filter paper Watman 1 moistened with 5 ml of water was set up. The 100 seeds in four replicates were put in Petri dishes for the establishment of their viability parameters. The germination energy (first count) and germination (final count) were determined, according to the requirements of ISTA (2013), as germinated seeds were counted daily. The time to germination of 50% of the seeds (T_{50}) was calculated by the equation of Farooq (2005). Mean germination time (MGT) and uniformity of germination (described by Panayotov, 2015) were determined. At the time of the germination establishment, the lengths of the embryo root and the hypocotyls were measured. These measurements were done on ten seedlings from the 4 replicates. The weight of the all developed sprouts in the four repetitions was established also in this moment and recalculated for one sprout.

The results were processed to ANOVA, and the correlation and regression analyses also were carried out (Fowel & Cohen, 1992).

RESULTS AND DISCUSSIONS

Germination energy, as an indicator that is taken into account in an earlier period of germination, provides information about the number of seeds that have higher vitality and in which the germination processes proceed more quickly (Panayotov, 2015). The data are shown in Table 1. The control seeds of the three tested cultivars had relatively similar germination energy. The application of ultrasound causes significant changes in this indicator, both depending on the variety and the duration of processing. The strongest effect of ultrasound treatment was found in the Apple Blossom variety, where even the weakest sonication of 2 minutes increased the germination energy by approximately 10%, while in the other two varieties, the changes in this variant were within about 3%. As the duration of the ultrasound processing increased to 6 minutes, the germination energy increased constantly, then decreased, but its values remained higher than the control, except for those of the Orange Wonder variety, where a stronger inhibition was observed. The highest germination energy, almost 21% above the control, was reported for Apple Blossom seeds and reached 71.33%. In the other two varieties, especially in Sonnet Carmine, the improvement of this parameter is weaker. The main indicator of the vital status of seeds, which most fully reflects their properties and standardizes quality, is germination (Black et al., 2006). It follows a similar trend as germination energy. As the sounding time increases, its values increase relatively evenly. The strongest influence, compared to the control, was reported for the varieties Orange Wonder and Apple Blossom, where even with the weakest treatment, germination increased by approximately 8.0%. Snapdragon seeds are initially characterized by a not particularly high germination rate (Bhargava et al., 2015).

With the help of ultrasound, seed viability was significantly improved, with the highest results obtained at the 6-minute variant for all three cultivars. The highest germination rate was observed in the Apple Blossom variety - 78.3%, followed by Orange Wonder with 74.6%. The data of germination energy are with statistical significance. At this duration of seed treatment with ultrasound, Panchev (2023) also obtained a high stimulatory effect in the seeds of decorative plants. There are different opinions about how ultrasound affects the seeds. One of the reasons for the increase in germination according to López-Ribera & Vicient (2017) and Aladjadjiyan (2007) is that when ultrasound is applied, the pores of the seed coat are open more. Close to this view is the conclusion of Nogueira et al. (2024) who pointed out that the effect of ultrasound treatment was due to the stimulation of the uptake of water and oxygen by the seeds and also of their metabolism. The authors emphasized that research on the influence of ultrasound at the biochemical and molecular level is limited, but one of the possible reasons for its effect is the degradation of starch and stimulation of the action of the enzyme α-amylases in the endosperm.

Table 1. Viability parameters of snapdragon seed after treatment with ultrasound

Vari-ants	Germination energy (%)			Germination (%)			$T_{50\%}$ germination (days)		
	Apple Blossom	Sonnet Carmine	Orange Wonder	Apple Blossom	Sonnet Carmine	Orange Wonder	Apple Blossom	Sonnet Carmine	Orange Wonder
Control	50.2	50.6	53.3	62.2	53.3	55.6	7.93	8.52	9.46
2 min	60.5	53.3	56.7	69.3	58.6	63.3	7.46	8.24	8.41
4 min	64.0	54.6	60.5	68.4	60.6	66.6	7.46	8.16	7.62
6 min	71.33	55.3	64.9	78.3	68.3	74.6	7.12	8.16	7.33
8 min	56.5	52.3	38.3	63.0	57.3	58.2	7.73	8.73	8.73
LSDp= 0.05%	6.5	4.0	5.8	4.8	4.4	6.1	1.1	1.4	1.3

The indicated changes in germination are also observed through the established regression relationships (Figure 1). For the three varieties, this dependence is polynomial. The coefficients of determination are high R^2=0.56 (Apple Blossom), R^2=0.67 (Sonnet Carmine), and R^2=0.72 (Orange Wonder). Using this coefficient, the influence of the factorial variable on the percentage of variance of the outcome variable can be assessed. This study shows that between 56% and 72% of cases of sonication, the observed changes in snapdragon seed germination will produce.

The influence of ultrasound on the germination time of 50% of the seeds, which is another important indicator for assessing their vital state, is weaker and a well-expressed varietal response is observed. In the Apple Blossom cultivar, it ranged from 7.93 days for the control to 7.12 days at 6 minutes. Within approximately 8 days, this indicator changes with Sonnet Carmine, and even at 8 minutes there is a slight increase. Better results were observed for Orange Wonder, especially for the 4 and 6-minute variants, with the required time for 50% of the seed germination decreasing by 1.84 and 2.13 days, respectively. The effect of snapdragon seed treatment on variation in 50% germination time was also reported by Bhargava et al. (2015).

Another important indicator for evaluating the sowing qualities of the seeds is the mean germination time (Table 2). It shows the average number of days for a seed to germinate (Trayanov, 2021). In summary, it can be stated that this time for the tested variants and varieties is more than 2 days. Sonication improved this trait except at 8 minutes for Sonnet Carmine and Orange Wonder cultivars. The least time for germination of one seed was obtained after treatment with 6 minutes of ultrasound - 1.98 days and 1.95 days, respectively, for Apple Blossom and Orange Wonder, the difference with the control being 0.43 and 0.97 days. Seed uniformity also reflects the impact of ultrasound on germination processes Its values are relatively low, which once again shows that snapdragon seeds have lower vital parameters. In untreated seeds, it is between 4.11% (Orange Wonder) to 5.29% (Sonnet Carmine). After the application of ultrasound, it increased uniformly in all variants and all varieties and reached maximum values at 6 minutes. The strongest improvement with 2.41% was registered with Orange Wonder, followed by Sonnet Carmine with 1.76%.

The morphological development of the sprouts gives additional insight into the vital status of the seeds.

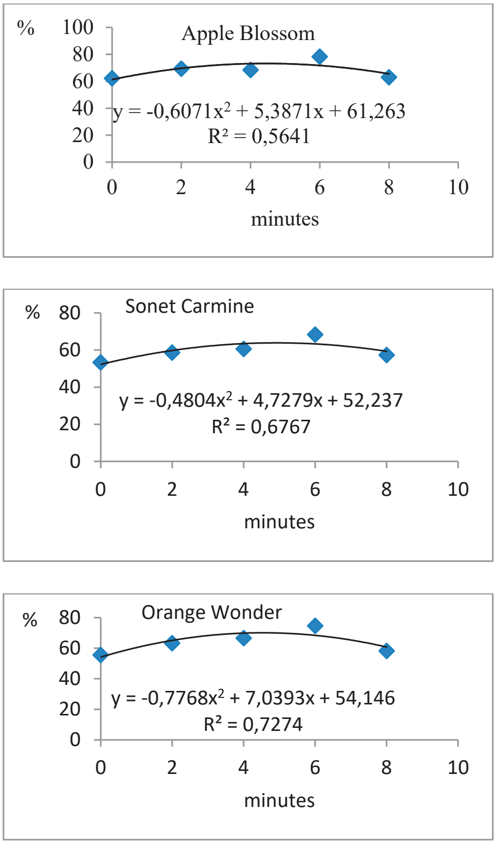

Figure 1. Regression dependence of germination and the duration of ultrasound treatment of snapdargon seeds

On the other hand, fresh mass is often used as an indicator of establishment the of seed vigor (Panayotov, 2015). The data are presented in Table 3. The length of the embryo root changes as a result of the action of ultrasound. For all variants tested, it increased to 6 minutes, with values ranging from 0.79 cm to 1.42 cm, respectively, for Apple Blossom and Sonnet Carmine. The differences compared to the control were 0.63 and 1.22 cm. At 8 min application, inhibition was reported, but the values were significantly higher than untreated seeds.

Table 2. Sowing indexes of snapdragon seed after treatment with ultrasound

Vari-ants	Mean germination time (days)			Uniformity of germination (%)		
	Apple Blossom	Sonnet Carmine	Orange Wonder	Apple Blossom	Sonnet Carmine	Orange Wonder
Control	2.41	2.71	2.32	5.10	5.29	4.11
2 min	2.36	2.48	2.40	5.79	5.38	5.47
4 min	2.27	2.40	2.53	6.24	5.71	5.69
6 min	1.98	2.21	1.95	6.66	7.05	6.52
8 min	2.32	2.84	2.72	5.81	6.48	4.75
LSDp= 0.05%	0.8	1.1	1.3	1.4	1.1	1.0

The hypocotyl is characterized by a much longer length and the stimulating effect is stronger. Under the influence of ultrasound, the sprouts develop long hypostyles. Growth is uniform up to 8 minutes of sonication. Sprouts from seeds treated for 8 minutes are characterized by the greatest length.

Table 3. Morphological characteristics of snapdragon seed after treatment with ultrasound

Variants	Length of embryo root (cm)			Length of hypocotyl (cm)			Fresh weight of one sprout (mg)		
	Apple Blossom	Sonnet Carmine	Orange Wonder	Apple Blossom	Sonnet Carmine	Orange Wonder	Apple Blossom	Sonnet Carmine	Orange Wonder
Control	0.76	0.55	0.56	2.96	3.00	3.33	0.10	0.08	0.09
2 min	0.99	0.73	0.68	3.23	3.20	3.53	0.11	0.10	0.11
4 min	1.09	1.15	1.98	3.46	3.43	4.26	0.12	0.11	0.11
6 min	1.39	1.74	1.82	3.70	3.66	5.33	0.13	0.14	0.13
8 min	0.79	1.42	1.15	4.40	4.26	5.46	0.12	0.12	0.07
r*							0.76	0.97	0.48
r**							0.77	0.69	0.34
LSDp= 0.05%	0.4	0.7	0.6	1.2	1.3	1.0	0.5	0.4	0.3

r* - correlation between fresh weight and length of embryo root; r** - correlation between fresh weight and length of hypocotyl.

The longest are those of the Orange Wonder variety - 5.46 cm or 2.13 cm more than the control, followed by that of Apple Blossom with a length of 4.40 cm. Fresh weight varies within narrower limits and differences between cultivars are insignificant. They range from 0.07 mg at 8 minutes for Orange Wonder to 0.14 mg at 6 minutes for Sonnet Carmine. A strong positive correlation was found between seedling fresh weight and embryo root and hypocotyl length with coefficients of r=0.76 and r=0.77 for Apple Blossom and r=0.97 and r=0.69 for Sonnet Carmine, respectively. In Orange Wonder, these correlations are also positive but weak.

CONCLUSIONS

Sonication has a significant effect on the sowing qualities of snapdragon seeds. A well-manifested cultivar response is observed. Treatments with 6 min of sonication increased the most germination energy and germination, with differences from the control reaching 20% in Apple Blossom variety. A polynomial regression, with high coefficients of determination, was found between the duration of the treatment and germination.

The germination time of 50% of the seeds, mean germination time and uniformity of germination are improved, with the highest effect observed in the 6-minute varinats. The morphological development of the sprouts was best when using 6 minutes of ultrasound, and positive correlations were established between their length and weight.

It is recommended to apply sonication for 6 minutes to improve the sowing qualities of snapdragon seeds.

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THE ROLE OF ORNAMENTAL HORTICULTURE IN PLANT INVASION: A CASE STUDY IN ROMANIA

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Abstract

Ornamental horticulture, a global practice, has significantly contributed to the proliferation of plant invasions on a worldwide scale. The European landscape, perpetually influenced by evolving horticultural trends, has witnessed the introduction of plants from diverse continents. The horticultural industry actively promotes ornamental species that exhibit characteristics traits conducive to their success, such as rapid growth, low maintenance requirements, resilience to local climatic conditions, and resistance to pests and pathogens. However, all these characteristics also ensure their success outside the gardens, so the number of alien plants escaping cultivation in native ecosystems increases from year to year. This study explores the evidence underscoring the fundamental importance of ornamental horticulture in introducing and promoting alien plant species, ultimately leading to their escape into natural ecosystems. In the context of Romania, a comprehensive analysis revealed that 264 ornamental taxa have escaped cultivation, and 30 of them have become invasive. Notably, six taxa, including Ailanthus altissima, Asclepias syriaca, Humulus scandens, Impatiens glandulifera, Ludwigia peploides, and Myriophyllum aquaticum, are of concern to the European Union, warranting special measures for population control and mitigation. This paper seeks to raise awareness about the urgent need for implementing international and European codes of conduct and codes of practice specifically addressing horticulture, ornamental plants, and the management of invasive alien species. The information provided highlights the crucial role of regulatory frameworks in mitigating the unintentional ecological impact of ornamental horticulture. It underscores the importance of collaborative efforts to preserve biodiversity and uphold ecological balance.

***Key words**: alien, non-native, decorative plants, floriculture, phytodiversity.*

INTRODUCTION

Plants with ornamental value have been cultivated and commercialized since ancient times. The Egyptians, Greeks and Romans were known to be plant lovers and cultivators of many ornamental plants (Huxley, 1978). Thus, over time, plant lovers and professional plant hunters from all over the globe have permanently introduced plants with ornamental characteristics into their personal collections, some even brought from faraway continents (Van Kleunen et al., 2018). But, these passion for collecting plants paved the way for more structured interventions; in particular, the horticultural industry has amplified this trend through the deliberate and mass introduction of a very large number of non-native ornamental species. These species have been selected for their cultivation in a wide range of landscapes from public and private gardens, to cemeteries, botanical gardens and dendrological parks (Bell et al., 2003, Dehnen-Schmutz & Touza, 2008; Drew et al., 2010; Humair et al., 2015; Mayer et al., 2017). However, certain introduced plants have crossed the borders of the gardens and successfully adapted to the wild landscape where they have begun to reproduce freely and uncontrolled. Through their uncontrolled spread, some species have become invasive, negatively affecting local biodiversity (Pysek et al., 2012).

It is found that ornamental horticulture has had a considerable impact on the introduction and spread of invasive plant species globally (Drew et al., 2010; Humair et al., 2015; Hulme et al., 2018; Bayón & Vilà, 2019). Specifically, in Europe, an estimated 80% of current alien plant species were originally introduced as ornamentals or for agriculture (Hulme, 2007). This trend has been amplified in recent decades by technological advances and marketing strategies that have revolutionized the way the horticultural industry produces and distributes ornamental plants. In this context, many of these plants are traded and distributed without proper assessment of their invasive potential, thus contributing to the growing problem of invasive species threatening native biodiversity (Drew et al., 2010).

The selection of plants for ornamental purposes is not a random process. Horticultural marketing strategies emphasize promoting plants with a visually appealing appearance to customers, but these kinds of plants often possess characteristics that predispose them to becoming invasive. These characteristics include large flowers, decorative fruits and seeds, an extended flowering period or the ability to flower several times a year, minimal care requirements, resistance to heat or drought conditions, high adaptability and increased stability in time (Anderson et al., 2006; Mack, 2005; Pysek et al., 2007; Van Kleunen et al., 2010; Van Kleunen et al., 2018). In addition, special attention is paid to the ease of propagation, the shortening of the early ripening period and the resistance to the stress associated with transport, as well as to a low sensitivity to harmful insects or pathogens (Drew et al., 2010; Hulme, 2011).

E-commerce is the latest and most accessible method for introducing ornamental plants, offering the possibility to order plants from different corners of the globe (Kowarik, 2005; Dehnen-Schmutz et al., 2010; Humair et al., 2015). More than fifty years after the advent of the Internet, it has evolved into the most convenient way to purchase products (Lenda et al., 2014). For these reasons, online trade is recognized as a major factor in promoting the introduction of invasive alien species and constitutes a significant biosecurity risk, especially by circumventing border controls and strict regulations on the plant trade (Ricciardi et al., 2007).

Even though some countries have implemented legal restrictions to limit e-commerce of invasive or potentially invasive alien plant species, online sales are increasing (Kikillus et al., 2012; Dehnen-Schmutz & Touza, 2008). Sellers find ways to get around the legislation by using outdated or popular names for invasive species to avoid detection and restrictions (Lenda et al., 2014).

Given the difficulty and high costs associated with the eradication of invasive species, it is essential to prioritize the prevention of the introduction, naturalization and invasion of new ornamental species. This approach is justified by a much more advantageous cost-benefit ratio, both from an ecological and economic perspective (Vanderhoeven et al., 2011).

Romania is particularly susceptible to invasions by alien plant species, largely due to its central geographical position in Europe and intensive trade with other countries (Anastasiu & Negrean, 2007; Sârbu et al., 2022). The first reports of invasive plant species in Romania date back to the beginning of the eighteenth century (Anastasiu et al., 2005; Dumitraşcu et al., 2010). However, systematic studies on alien ornamental plants commenced 18 years ago when Anastasiu et al. (2005) published the initial list of alien ornamental plants identified as escaped, naturalized, or invasive.

Years after this initial compilation, the situation in Romania evolved with additional ornamental plants being reported either in botanical gardens (Nagodă et al., 2014; Szatmari & Caprar, 2015; Negrean et al., 2017) or within urban spaces and private gardens (Sârbu, 2007; Anastasiu et al., 2011; Anastasiu et al., 2017). The most recent list of alien ornamental plants in Romania (Urziceanu et al., 2020) counts 264 alien species and subspecies of ornamental interest, including 199 occasional, 37 naturalized, and 28 invasive species. This signifies a nearly threefold increase in reported taxa over 15 years, accounting for approximately a quarter of the naturalized ornamental alien species in Europe (Pyšek et al., 2009; Urziceanu et al., 2020).

Starting from the recognition of the role that ornamental horticulture has on the introduction and spread of invasive alien plant species, as well as the urgent need to develop effective strategies for their prevention and management, our study aims to deepen the understanding of this complex phenomenon. In this context, the purpose of our study is to conduct a screening and analyse the presence of ornamental invasive plants in the online horticultural market.

MATERIALS AND METHODS

We used the updated lists of alien plant species in Romania, provided by the POIM project, as a starting point for the identification of ornamental species with invasive status (Anastasiu et al., 2019). A database containing synonyms, common name, ornamental properties, type of propagation, year of introduction, the life form was done.
To assess the presence of these species on the Romanian market, we studied internet offers from importers to producers, nurseries, wholesale suppliers, garden centers, DIY stores and individual offers. Google searches were conducted using relevant keywords as well as the scientific names, synonyms and common names of the plants. We experienced challenges with taxonomy and spelling, with some sites showing outdated and out-of-date names. We also identified cases where two different species were marketed under the same name. Google searches were conducted using keywords such as "garden plants", "ornamental plants", "honey plants", "nurseries", "forestry units (forest detours)", "plant shops", "ornamental perennial plants", "offer decorative plants", as well as the scientific names, synonyms and popular names of the plants.
We also found cases where two different species were marketed under a generic name, such as *Humulus* sp., *Reynoutria* sp., *Rudbecki*a sp., *Solidago* sp.

RESULTS AND DISCUSSIONS

Within the context of Romania's recognized list of 130 invasive and potentially invasive species (Anastasiu et al., 2019), we identified 30 invasive plant taxa that have been introduced for ornamental purposes within the national territory, as detailed in Table 1. These taxa comprise a mix of life forms, including 11 woody plants and a variety of herbaceous plants (5 annuals, 2 biennials, and 10 perennials). Such diversity indicates a wide array of establishment and spread strategies, underscoring the complexity of managing these species (Hodkinson and Thompson, 1997). These plants were introduced both for their ornamental value (flowers, leaves, overall foliage texture, fruit and aesthetic form) but in the same time for their high adaptability to environmental conditions and the ease of propagation.
Most of them were introduced between 1800 and 1900, 7 species being reported in the last 100 years (*Humulus scandens*, *Impatiens glandulifera*, *Oenothera glazioviana*, *Prunus serotina*, *Reynoutria japonica*, *Symphyotrichum lanceolatum*, *Myriophyllum aquaticum*) and only two in the last 20 years (*Reynoutria × bohemica* - 2004, *Ludwigia peploides* - 2020). This situation indicates that the invasiveness is determined by a long residence time, the propagules bank increasing over time and also the probability of spreading, establishment, and the founding of new populations (Rambuda & Johnson, 2004; Rejmánek et al., 2005; van Kleunen & Johnson, 2007). On the other hand, the fact that some species quite recently introduced became invasive very quickly can be explained by the fact that they were already adapted to local conditions when were introduced (Pyšek et al., 2009). It should also be mentioned that the last two species were automatically included on the list of invasive species in Romania, being present on the list of Union Concern species in Europe.
During our study, we examined 59 websites that sell ornamental plants to ascertain the market availability of invasive plant species. Regrettably, our study revealed that 57 of these (96.61%) included in their offers at least one invasive ornamental species. Many recognized invasive species are still offered daily on the internet to most countries in the world (Humair et al., 2015). This situation is not peculiar to Romania, the high number of websites that offer these types of plants may indicate that the problem of invasive plants is very little known in Romania or is known but ignored (Humair et al., 2015). This disregard is not necessarily determined for reasons of financial gain, although research has shown that horticulturists prefer exotic (non-native) species that attract customers having as a reason that native species do not have the features required by customers. The same study shows that horticulturists' familiarity with a non-native species and sometimes invasive might lead to a

cognitive conflict with the expert definition of the non-native origin of a species: a species that is characterized as ‘foreign’ by experts is ‘familiar’ to horticulturalists due to their daily work and they cannot perceive it as a plant that can have negative effects on the environment (Humair et al., 2014).

Also, another aspect is the deficit in the legislation regarding the management of alien invasive species. Although the accession to the Convention on Biological Diversity and the European Convention through Law no. 13/1993 imposes measures against invasive alien species, Ministerial Order no. 979/2009, the most direct regulation, has limited legal force. Emergency Ordinance no. 57/2007 recognizes the need to limit the introduction of alien invasive species, but does not provide the necessary tools for effective implementation. Weak control of trade in non-native species allows their import and trade in the absence of specific restrictions or prohibitions in other relevant sectors. On the contrary, in some situations the maintenance or introduction of these species is encouraged. For this reason, activities to prevent, control and eradicate invasive alien species do not benefit from legislative support in any activity sector (Bara et al., 2023).

Our analysis show the lack of information that the traders make available to the buyers taking regarding the invasive capacity of the sold species. Only a single website of the 59, provided additional information regarding the invasiveness of the species it sells. This highlights a significant gap in the disclosure of important information to consumers which could allow them to make informed decisions.

Among the 30 invasive ornamental plant taxa we investigated, 20 species could be bought in Romania's ornamental plant online market. The other 10 species (*Echinocystis lobata*, *Impatiens glandulifera*, *Oenothera glazioviana*, *Reynoutria × bohemica*, *Reynoutria japonica*, *Solidago canadensis*, *Sicyos angulatus*, *Symphyotrichum × salignum*, *Symphyotrichum lanceolatum*, *Ludwigia peploides*) are not currently for sale and gardeners usually got these through swapping propagules with each other.

For the 20 species present in Romanian trade, living plants (14 species), rare seeds (6 species) and vegetative organs (*Helianthus tuberosus* - tubers, *Rudbeckia laciniata* - rhizomes) were offered. Only two species were identified both offers as live plants and seeds (*Lycium barbarum*, *Humulus scandens*).

The rich offer of living plants as seedlings or saplings, with increased chances of survival, although these species have both types of reproduction can be an advantageous marketing strategy for both, producers and consumers. Thus, living plants, cost more than few seeds, but at the same time the consumer should invest the time and resources necessary to obtain quality plants like the ones offered by the horticultural industry.

The species most frequently listed in online catalogs are *Acer negundo* and *Lycium barbarum*. *Acer negundo* is particularly prevalent and has been widely planted in urban areas across much of Europe for centuries due to its horticultural and landscape appeal, including its rapid initial growth (CABI, 2024). Currently, ‘Flamingo’ variety, known for its variegated leaves, is very popular on the market. Although cultivars with variegated leaves appear to be less invasive, but those who own such plants must be aware of the wild rootstock on which they are grafted (Mędrzycki, 2011).

Lycium barbarum, first introduced as a decorative plant, has become nowadays very popular for its goji berries, which many people call 'Superfoods'. It's a tough plant that can handle pollution, drought, and cold weather well. It reproduces easily germinative by seeds and also vegetatively through suckers, creating dense bushes (Sîrbu & Oprea, 2011).

The online trade alarmingly lists five species that have raised concerns within the European Union: *Ailanthus altissima*, *Asclepias syriaca*, *Humulus scandens*, *Impatiens glandulifera*, and *Myriophyllum aquaticum*.

Ailanthus altissima was available in 4 offers as living plants. It was particularly surprising to find that the nursery of the Suceava Forestry Directorate, an entity comprised of forestry specialists, had listed *Ailanthus altissima* along with 3 other invasive species for sale as live plants *Lycium barbarum*, *Parthenocissus inserta*, and *Elaeagnus angustifo*lia. This oversight underscored a critical achievement: there is a pressing need for enhanced collaboration across different fields to ensure mutual understanding and address shared concerns regarding invasive species. This experience has highlighted the importance of interdisciplinary efforts in managing and preventing the spread of invasive species effectively. The first record of *Ailanthus altissima* in Romania dates back to 1866 in Transylvania, with further documentation in 1898 near Bucharest. Presently, it is considered among the most aggresive invasive species in Europe, encroaching upon disturbed habitats as well as dry grasslands, forest edges, and riverbanks in Romania. Its widespread planting in urban areas owes to its resilience to drought and air pollution. The species proliferates rapidly both through seeds and vegetatively via suckers, often starting with a few isolated individuals that excessively multiply.

Asclepias syriaca was found in the offers of 3 online stores as a living plant being present on the market, especially as a honey plant. Thus, against the backdrop of the pollinator crisis, the plant is grown for beekeeping by private garden owners as well as by small honey producers (Tokarska-Guzik & Pisarczyk, 2015). This species was initially mentioned in Moldova in 1836 and later in Transylvania in 1866. Previously cultivated mainly in private and botanical gardens as an ornamental plant, it is now prevalent in various habitats, including roadsides, river floodplains, meadows, orchards, and even within agricultural fields (Sîrbu & Oprea, 2011). The plant produces seeds that spread by wind to considerable distances and reaching different types of habitats (Csontos et al., 2009). As a clonal plant, it presents a large capacity for vegetative reproduction, with the help of buds on the rhizome and adventitious roots (Follak et al., 2021)

Humulus scandens was found in 2 online offers, only using the synonym name, *Humulus japonicus*. Being an annual species, both offerings were represented by seeds. The exact date of introduction in Romania of this plant is unknown, but its first mention as a spontaneous plant was made by Morariu in 1942. It is distinguished from *Humulus lupulus* by its 5-7 lobed leaves, longer petioles than the leaf blade, and smaller female flowers. This invasive climbing species invades flood plains, stream banks, roadsides, old fields, forest edges and waste areas where it can suffocate both herbaceous and woody plants (Georgescu et al., 2021).

Impatiens glandulifera was not found in any offerings on the market. This plant, known for its showy and scented flowers, was introduced to Romanian gardens as early as 1882 (Sârbu & Oprea, 2011). It is classified as one of the 100 worst alien species in Europe (DAISIE, 2009). It became naturalized and invasive in riparian and disturbed habitats. A single plant can produce (500) 800-1700 (2500) seeds (Hodgson et al., 2020). The seeds are explosively discharged from the capsule when ripe and also are dispersed by water when plants grow along streams and rivers (Helsen et al., 2021). The effects of this species' invasion are significant, studies suggesting that this species can alter ecosystem functioning and services such as erosion control, pollination and nutrient cycling (Martinez-Cillero et al., 2019).

Myriophyllum aquaticum was identified in 8 offers as a living plant. Among these, only one was a plant shop, with the rest being aquaristics websites, where the plant is mainly sold as an aquarium species. In Romania, this species has been cultivated since 1947 in the thermal waters of the thermal lake Pețea in Bihor County (the western part of Romania), currently without water (Sîrbu et al., 2021). Another location has not been mentioned since the date of introduction, its presence on the national list being due to the presence on the list of invasive alien species of Union Concern according to the Regulation (EU) No. 1143/2014 of the European Parliament and of the Council. However, the climate changes in recent years and its presence in Romanian trade increase the chances that the plant will establish itself in our conditions.

CONCLUSIONS

The high number of invasive planate species on the Romanian trade shows the need to implement management and control strategies to mitigate the impact of invasive ornamental plants. These must target educational campaigns for public awareness and also for as well as those involved in the horticultural industry, clear labelling of invasive plants, limiting sales of invasive plants and encouraging the use of native species (Peters et al., 2006; Burt et al., 2007; Gagliardi & Brand, 2007; Coats et al., 2011; Vanderhoeven et al., 2011; Yue et al., 2011; Humair et al., 2014). Also, implementing a complex monitoring system that includes all actors in the horticulture industry is also an effective approach to minimizing the introduction of invasive plant species (Humair et al., 2015). Furthermore, various voluntary codes of conduct have been proposed (Heywood and Brunel, 2009; Brundu et al., 2011; Verbrugge et al., 2014) to promote responsible practice among retailers, users and consumers.

In addition, it is essential that they are complemented by predictive tools capable of determining which species have the potential to acclimate and spread under current climate conditions, considering the impact of climate change. A relevant example is the Plant Risk Evaluation (PRE) tool for ornamental plants, developed in North America (Conser et al., 2015) what it can provides a promising basis for testing its applicability in Romania as well. The plant species that evaluated and accepted should be included in an national whitelist list and marked at points of sale with a "green" label, indicating a reduced likelihood of becoming invasive. On the other hand, species that are rejected and already spread outside the cultivation area should be given a "red" label, signaling a high risk of invasiveness, to allow consumers to make informed choices, according to the study by Hulme and collaborators in 2018.

Alarmingly, the online market features five species identified as concerning by the European Union, suggesting a disconnect between regulatory oversight and marketplace practices. The non-application of European legislation is reflected in the Romanian legislative gaps. However, in December 2022, Romania took a step forward by approving the National Action Plan to combat the introduction of invasive alien species, marking an improved commitment to managing these challenges (Bara et al., 2023).

All these measures will not materialize and will not be successful if improving of communication and collaboration among various experts and stakeholders in the field of ornamental plant management and characteristics won't happen.

Table 1. List of the 30 invasive plant taxa that have been introduced for ornamental purposes (Abbreviation: G - generatively, V - vegetatively)

No.	Taxon	Family	Common name (in romanian)	Ornamental features	Type og propagation	First report in Romania	Life form
1.	*Acer negundo* L.	Sapindaceae	Arțar american	leaves, aesthetic form	G, V	1856	Ph
2.	*Ailanthus altissima* (Mill.) Swingle	Simaroubaceae	Cenușer, Falsul oțetar	leaves, flowers, fruits, aesthetic form	G, V	1866	Ph
3.	*Amaranthus hypochondriacus* L. (syn: *Amaranthus hybridus* (Vell.)	Amaranthaceae	Moțul curcanului, Amaranth roșu	leaves, flowers, aesthetic form	G	1866	T
4.	*Amorpha fruticosa* L.	Fabaceae	Amorfa arbustivă, Salcâm pitic	flowers	G, V	1856	Ph
5.	*Asclepias syriaca* L.	Apocynaceae	Ceara albinei	flowers, fruits	G, V	1836	H
6.	*Bassia scoparia* (L.) A.J.Scott [syn: *Kochia scoparia* (L.) Schrad.]	Chenopodiaceae	Cipru de grădină	leaves, aesthetic form	G	1816	T
7.	*Echinocystis lobata* Torr. & A. Gray	Cucurbitaceae	Bostănaș spinos	fruits, aesthetic form	G	1904	T
8.	*Elaeagnus angustifolia* L.	Elaeagnaceae	Sălcioara mirositoare	leaves, flowers, aesthetic form	G, V	1792	Ph
9.	*Fraxinus pennsylvanica* Marshall	Oleaceae	Frasinul de Pennsylvania	leaves	G	1910	Ph
10.	*Helianthus tuberosus* L.	Asteraceae	Topinambur, Nap porcesc	flowers	V	1778	H
11.	*Humulus scandens* (Lour.) Merr. (*Humulus japonicus* Siebold & Zucc.)	Cannabaceae	Hamei japonez	aesthetic form	G	1937	T
12.	*Impatiens glandulifera* Royle	Balsaminaceae	Balsamina, Slăbănog	flowers	G	1925	T
13.	*Juniperus virginiana* L.	Cupressaceae	Ienupăr de Virginia	leaves, aesthetic form	G	1856	Ph
14.	*Lycium barbarum* L.	Solanaceae	Cătină de garduri, Goji	flowers, fruits	G	1842	Ph
15.	*Morus alba* L.	Moraceae	Dud decorativ	leaves, aesthetic form	G	<1600	Ph
16.	*Oenothera biennis* L.	Onagraceae	Lumina nopții, Lumânărică	flowers	G	1816	Th
17.	*Oenothera glazioviana* Micheli	Onagraceae	Primula de seară	flowers	G	1957	Th
18.	*Parthenocissus inserta* (A. Kerner) Fritsch	Vitaceae	Viță-de-vie ornamentală	leaves, fruits, aesthetic form	G, V	1898	PhLi
19.	*Prunus serotina* Ehrh.	Rosaceae	Cireș negru	flowers, fruits, aesthetic form	G	1967	Ph
20.	*Reynoutria* × *bohemica* Chrtek & Chrtková (*Fallopia* × *bohemica* [Chrtek & Chrtková) J.P.Bailey]	Polygonaceae	Troscot gigant	stem, leaves	G, V	2004	H
21.	*Reynoutria japonica* Houtt. [Fallopia japonica (Houtt.) Ronse Decr.]	Polygonaceae	Iulișcă, troscot japonez	stem, leaves	G, V	1952	G-H
22.	*Robinia pseudacacia* L.	Fabaceae	Salcâm	flowers	G, V	1750	Ph
23.	*Rudbeckia laciniata* L.	Asteraceae	Măriță-mă-mamă	flowers	G, V	1855	H
24.	*Sicyos angulatus* L.	Cucurbitaceae	Castravetele stea	fruits, aesthetic form	G	1816	T
25.	*Solidago canadensis* L.	Asteraceae	Sânziană de grădină	flowers	G, V	1866	H
26.	*Solidago gigantea* Aiton	Asteraceae	Vergea de aur gigant	flowers	G, V	1886	H
27.	*Symphyotrichum* × *salignum* (Willd.) G.L.Nesom (*Aster* × *salignus* Willd.)	Asteraceae	Steluțe, Aster	flowers	G, V	1866	H
28.	*Symphyotrichum lanceolatum* (Willd.) G.L.Nesom (*Aster lanceolatus* Willd.)	Asteraceae	Steluțe, Aster	flowers	G, V	1940	H
29.	*Myriophyllum aquaticum* (Vell.) Verdc.	Haloragaceae	Pene de papagal	aesthetic form	V	1947	HH
30.	*Ludwigia peploides* (Kunth) P.H. Raven	Onagraceae	Primulă de apă	flowers	G, V	2020	HH

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A GIS APPROACH TO GREEN REGISTER AND TREE MANAGEMENT IN BUCHAREST, ROMANIA - DISTRICT 1 CASE STUDY

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Abstract

This paper aims to analyse the concept of a green register, the component elements of a register, its utility and necessity, especially in the urban environment, using geospatial techniques and Geographic Information Systems (GIS). In the introduction phase, the international, national, and local situations were studied to determine the importance of trees and green areas. For the results and discussion phase, the trees located in the "Regina Maria" Park were identified, while the statistical data were centralised to establish the vegetation structure (species and their distribution, invasive potential, other characteristics). Last, but not least, recommendations were made to support the long-term maintenance of these trees. Thus, the phytosanitary issues were centralised to prevent human and material damage, while a management plan was drawn up with specific interventions and action points.

***Key words**: green register, green infrastructure, tree assessment and management, Bucharest, Geographic Information Systems.*

INTRODUCTION

A green register functions as a document outlining strategies and policy guidelines for the management and maintenance of vegetation in a study area or a city. It can provide valuable information on conservation, potential planting activities, tree removal, and maintenance work. Green spaces produce numerous benefits for people, especially in urban environments affected by pollution, real estate developments, and more. In the current context of climate change, ensuring a landscape management plan becomes a priority.

Monitoring green spaces, and implicitly, the trees, is essential for determining the index of green space per inhabitant, improving the urban microclimate, developing green-blue strategies, maintaining and developing green spaces. The optimization of territorial arrangements with ecological, aesthetic, and recreational purposes is also crucial.

At the international level, various institutions and organizations have initiated actions and activities aimed at drawing attention to the need to protect nature and encouraging the development of strategies and projects to improve the quality of urban life. National and local standards regarding the adequate area of public space differ significantly from one country to another.

For instance, the World Health Organization recommends a minimum surface of 9 m^2 of open green space per person (WHO, 2009).

Although there are inconsistencies regarding how green space is defined and perceived, many cities are striving to achieve this minimum recommendation, while others aspire to a much more generous green surface, such as the Italian law that mandates 18 m^2 of green area per person in new urban developments (EAA, Urban Agenda for the EU, 2022).

On average, around 40% of the area of European cities is comprised of urban green infrastructure, with approximately 18.2 m^2 of publicly accessible green space per inhabitant, while 44% of Europe's urban population lives within 300 m of a public park (Maes et al., 2018; Corbane et al., 2020).

However, the presence of green areas, both public and private, in cities varies greatly. While some city centers, such as Vienna (Austria) and Freiburg (Germany), even have forested areas in their midst, others lack any green spaces (Maes et al., 2018; Corbane et al., 2020). This disparity is particularly noticeable in Mediterranean regions, as illustrated in Figure 1.

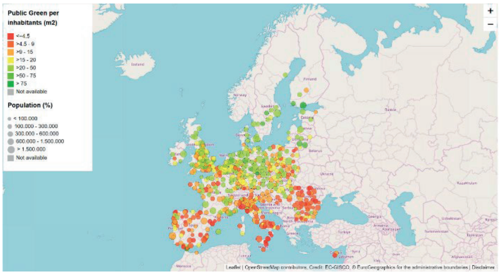


Figure 1. Public green space available per inhabitant in European cities. Source: CE JRC, Maes et al., 2019

The importance of trees in the urban environment is also recognized in the European Union Biodiversity Strategy for 2030, which mandates that cities with more than 20,000 inhabitants develop urban greening plans. These plans should encompass measures to create diverse and accessible urban green spaces, including parks, forests, and street alignments.
At the national level, specific regulations regarding green spaces and intervention on trees have been established through laws and emergency ordinances, as well as local council decisions.
Green spaces are defined by Law no. 24/2007 on the regulation and administration of green spaces in urban areas, with subsequent amendments and additions.
According to O.U.G no. 114/2007 for the amendment and completion of the Government Emergency Ordinance no. 195/2005 on environmental protection, Art. II, para. (1), local public administration authorities are obligated to ensure a green space area of at least 20 m^2/inhabitant until December 31, 2010, and at least 26 m^2/inhabitant until December 31, 2013. In this sense, the local public administration has the obligation to keep track of green spaces within the territorial administrative unit by creating the register of green spaces.
The Greenpeace Romania organization analysed the establishment of the green register at the level of the Municipality of Bucharest and affirms the fact that in 1989 each inhabitant of Bucharest had at their disposal, on average, an area of 16.79 m^2 of green spaces.
In 2006, a report by the Bucharest City Hall showed that the area of green space per inhabitant was reduced to 9.67 m^2. At the end of 2011, the public administration completed the Register of Green Spaces.
According to Greenpeace Romania, the measurements of the Bucharest City Hall revealed in 2011, 23.21 m^2 per inhabitant, more than double the figures previously presented. Comparatively, Vienna had, in 2012, an area of 120 m^2 of green space per inhabitant, followed by Helsinki (100) and Stockholm (86). Capitals much larger than Bucharest had more green space, among them London - 27 m^2, Berlin - 38 m^2 and Rome - 45 m^2 (Greenpeace, 2020).
The Court of Accounts showed, however, that this massive increase in 2011 in the area of green spaces per inhabitant was done superficially. The data used also contain the surfaces of the green space related to the private domain. According to the Court of Accounts, in 2014 Bucharest had only 9.86 m^2 of green space per inhabitant, not 23.21, as announced by the authorities (Greenpeace, 2020).
Although by 2013, the area of green space per inhabitant was supposed to reach 26 m^2, according to the national legislation in force and the rules of the European Union, this ideal is still unfulfilled. From 2011 until now, despite the publication of the Green Register in 2013, no updates of its data have been made.

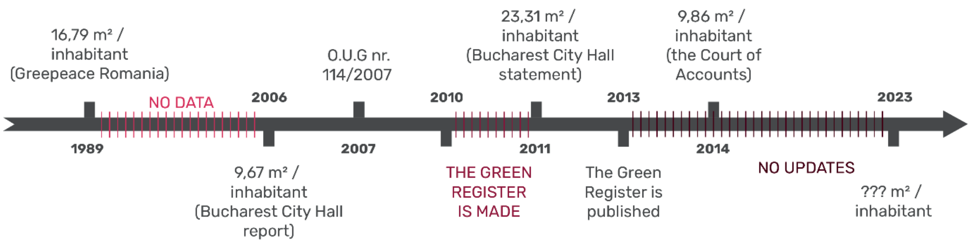


Figure 2. Illustrative scheme regarding the Green Register's situation in Bucharest

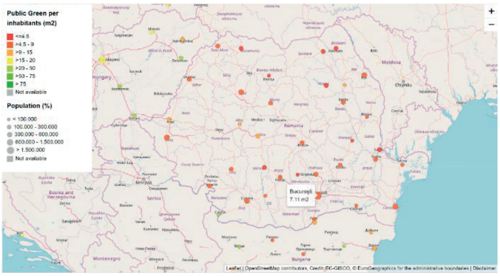


Figure 3. Public green space available/inhabitant in Bucharest (7.11 m^2) (Source: CE JRC, Maes et al., 2019)

In 2022, a research report on the state of the environment in Bucharest was published based on the Green Register made in 2011, detailing the components of the city's green infrastructure, as shown in Figure 4.

The report notes that the distribution of the existing green space is insufficient and uneven. According to the report, the largest area of green space is available in District 1, which will be analysed during this study, representing 77 m^2 of green space and 2.44 trees for each inhabitant. At the opposite pole is District 2, with the least green space and the fewest trees (Buletin de Bucureşti, 2022).

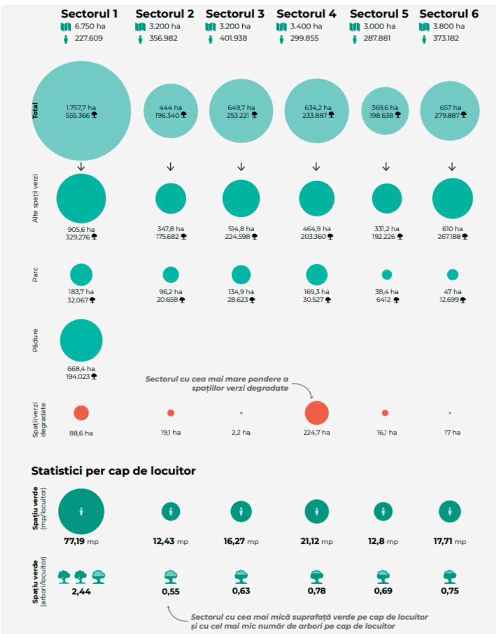


Figure 4. The green spaces related to each district (Source: Raport de cercetare privind starea mediului în Bucureşti, 2022)

MATERIALS AND METHODS

To begin with, a macro-level processing was carried out for the entire District 1, using satellite images, to determine the normalized difference vegetation index (NDVI) and the land surface temperature (LST). This processing was prepared as a complement to the situation presented in the introduction, in order to observe the degree of vegetation cover and the way in which parks and tree vegetation contribute to lowering temperatures.

For this stage, Landsat 8 images were used, taken from the www.earthexplorer.usgs.gov platform, from two different moments, 10.06.2013 and 06.06.2023, also representing a comparative analysis.

At the micro-level, "Regina Maria" Park was chosen in order to create a green register. Opting for a park over an alignment or a boulevard enhances ecological diversity.

The process involved identifying and inventorying the trees of the studied site, while their placement was carried out using the QField application.

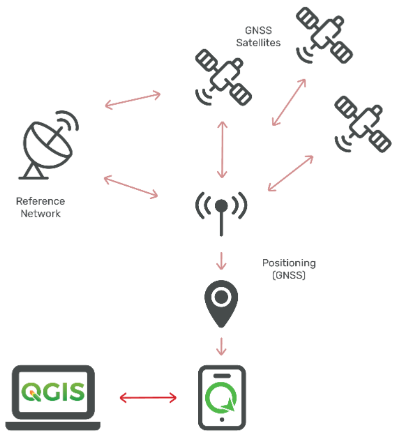


Figure 5. Workflow for synchronizing data collected in the field through QField for QGIS

Taxonomic data and phytosanitary status characteristic of each specimen were recorded. This data includes: species and variety (where applicable), health status (excellent, good, reasonable, deficient, dry/dead, stump), age category (young, mature, declining specimens), biological origin of the species (native or non-native), and invasive potential.

The purpose of this information is to understand the existing tree composition and identify any problems or risks. The collected data can act as an archive for each tree, helping ensure compliance with legal requirements and serving as a reference for monitoring and implementing specific commands in the field.
For this study, both vector and raster geospatial data were utilised to create the thematic maps, graphics, and their interpretation.

Table 1. Types and sources of data used

Type of data extracted	Data sources
Vector data: built space, street network, UAT limits, county limits, park limits, cemeteries limits, forest limits, hydrography	https://geoportal.ancpi.ro/portal/home/ www.openstreetmap.org
Satellite images: Landsat 8, Sentinel-2, Google Maps / Google Earth	www.earthexplorer.usgs.gov https://scihub.copernicus.eu/ Google Earth Pro, Google Satellite
Topographic map "Regina Maria" Park (1978, 2022)	Administrația Domeniului Public Sector 1
Data on green spaces	Primăria Municipiului București - https://www2.pmb.ro/ Administrația Domeniului Public Sector 1 - https://adp-sector1.ro/
Software for field data extraction	QField
Data processing software	ArcMap, QGIS
Map styling software	Adobe Illustrator, Adobe Photoshop, Autodesk AutoCAD
Software for creating graphics	Microsoft Excel

RESULTS AND DISCUSSIONS

The first step for the results and discussions phase was to centralise the green and blue infrastructure of the District 1.
According to the Law no. 24/2007, Art. IV, a), for the purposes of this law, the term "park" has the following meaning: a park is defined as a green space with an area of at least one hectare, consisting of a specific plant framework and built-up areas, including facilities and equipment intended for cultural-educational, sports, or recreational activities for the population.

Table 2. Green infrastructure - parks of District 1

GREEN INFRASTRUCTURE - PARKS OF DISTRICT 1		
Name	Surface	Administrator
"Regele Mihai I al României" Park (Herăstrău)	141.8 ha	Bucharest City Hall (PMB)
Cișmigiu Park	15.7 ha	PMB
Kiseleff Park	14 ha	District 1
Bazilescu Park	13.1 ha	District 1
Bordei Park	8.4 ha	PMB
Floreasca Park	7.8 ha	PMB
Operei Park	4.7 ha	District 1
"Regina Maria" Park	1.6 ha	District 1
"Elisabeta Rizea" Park	1.5 ha	District 1
Gării de Nord Park	1.5 ha	District 1

Table 3. Blue infrastructure - lakes of District 1

BLUE INFRASTRUCTURE - LAKES OF DISTRICT 1		
Name	Surface	Administrator
Herăstrău Lake	77 ha	PMB
Grivița Lake	75.85 ha	PMB
Floreasca Lake	70 ha	PMB
Străulești Lake	44 ha	PMB
Băneasa Lake	40 ha	PMB
Cișmigiu Lake	2.65 ha	PMB

Despite the considerable green space areas of District 1 compared to the rest of the districts of the Municipality of Bucharest, their distribution is disproportionately skewed, as displayed in Figures 6 and 7.

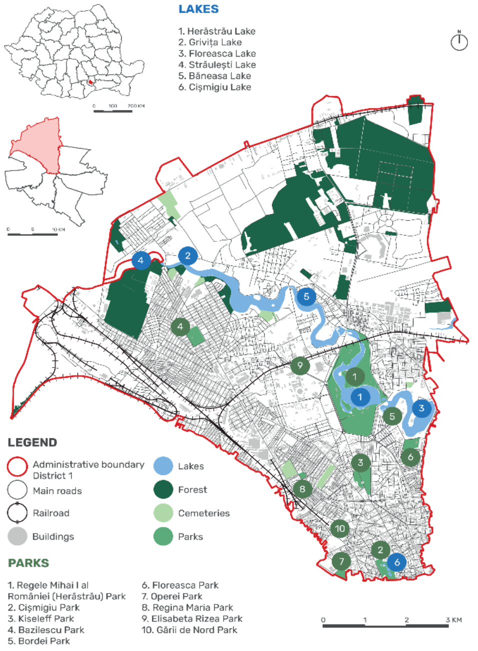


Figure 6. Blue-green infrastructure in District 1

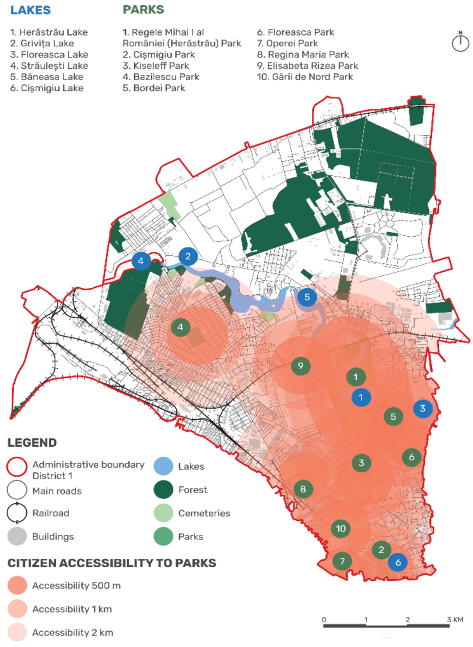


Figure 7. Citizen accessibility to parks (500 m, 1 km, 2 km)

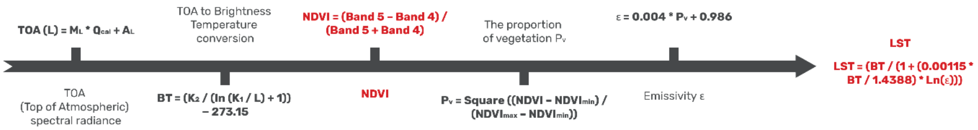


Figure 8. Workflow for processing NDVI and LST using ArcMap

Two analyses, NDVI (Normalized Difference Vegetation Index), and LST (Landscape Surface Temperature), were conducted for the month of June in 2013 and 2023, respectively, using the formulas shown in Figure 8 above.

NDVI, the Normalized Difference Vegetation Index, indicates the health of vegetation based on leaf chlorophyll content, reflecting the amount of plant biomass. NDVI values range from -1 to 1. Thus, the higher the NDVI, the healthier the vegetation (Agrivi App, 2022).

LST, land surface temperature, is an index that quantifies the thermal radiation emitted by the Earth's surface. It describes processes such as the exchange of energy and water between the land surface and the atmosphere, influencing the rate and timing of vegetation growth.

LST data helps understand and monitor heat patterns in urban areas, analyse land-atmosphere interactions, and study the thermal behaviour of different types of land cover.

It is found that, for the NDVI analysis (Figures 9 and 11), the differences in values are not significant over the two years analysed, except for the central area, where the situation improves in 2023 compared to 2013. However, this area is the most affected in terms of thermal stress.

The lowest NDVI values are around lakes, as water areas absorb both red and near-infrared light, resulting in negative NDVI values.

The highest values are distinguished in the parks and Băneasa Forest in the north of the district, as well as in the northwest corner, along the railway infrastructure, representing unbuilt land.

LST values are influenced by various factors, including solar radiation, cloud cover, vegetation cover, soil moisture, and local climatic conditions. It is important to note that LST represents the temperature of the land surface itself, which may differ from air temperatures measured at different heights in the atmosphere. For the two years analysed, a considerable improvement is noted in 2023 due to the development of the dendrological vegetation. The lowest temperatures are observed on the shores of the lakes, in the parks, in the Băneasa Forest area, as bodies of water and tree vegetation regulate the temperature and contribute to the reduction of thermal stress.

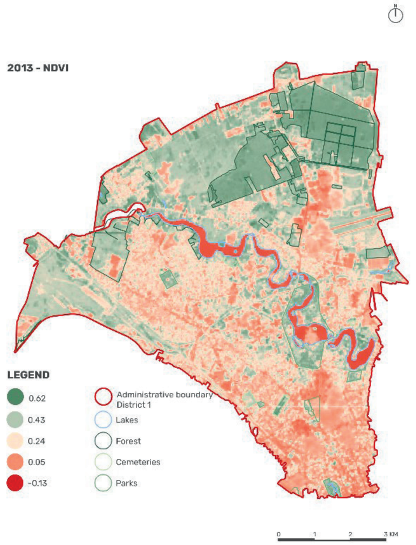


Figure 9. NDVI for 10.06.2013

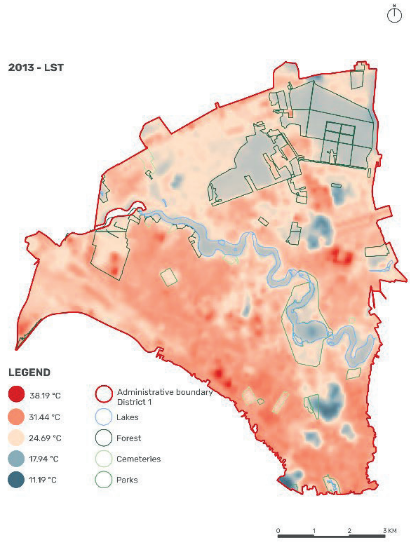


Figure 10. LST for 10.06.2013

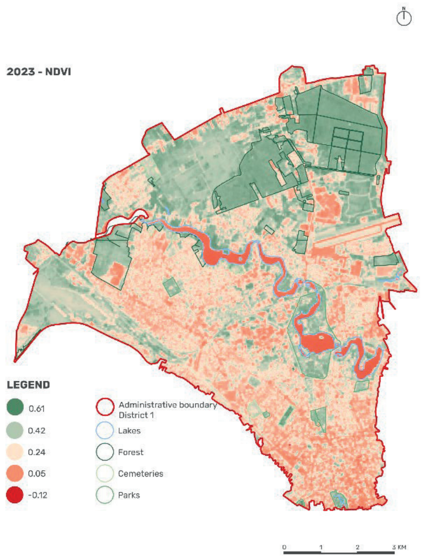


Figure 11. NDVI for 06.06.2023

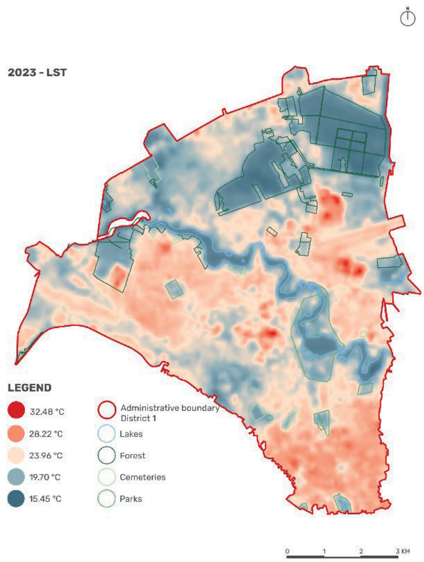
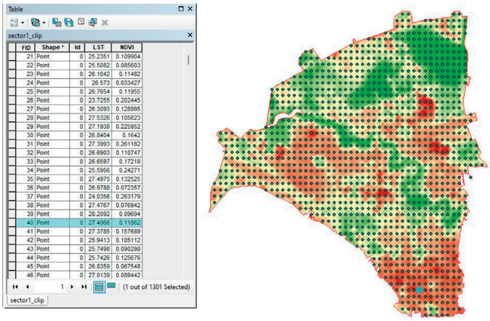


Figure 12. LST for 06.06.2023

Following the analyses of NDVI and LST, there was a desire to correlate the two indices, which are used on a large scale to study the relationship between vegetation dynamics and temperature patterns. A negative correlation may provide information about the chilling effect or the impact of drought stress on plant health. These indices find application in environmental monitoring, ecological studies, agricultural and

biodiversity management, as well as climate change research.
The correlation was executed through the ArcMap program and involved creating a Fishnet (located in Arc Tool Box under Data Management Tool, Sampling, Create Fishnet) and extracting the values recorded for NDVI and LST (found in Spatial Analyst Tools, Extraction, Extract Multi Values to Point).



FID	Shape *	Id	LST	NDVI
21	Point	0	25.2351	0.109904
22	Point	0	25.5082	0.085603
23	Point	0	26.1042	0.11482
24	Point	0	26.573	0.033427
25	Point	0	26.7654	0.11955
26	Point	0	23.7255	0.202445
27	Point	0	26.3093	0.128995
28	Point	0	27.5326	0.105623
29	Point	0	27.1938	0.225952
30	Point	0	26.8404	0.1642
31	Point	0	27.3993	0.261182
32	Point	0	26.6903	0.110747
33	Point	0	26.6597	0.17218
34	Point	0	25.5956	0.24271
35	Point	0	27.4975	0.132525
36	Point	0	26.9788	0.072357
37	Point	0	24.0356	0.263179
38	Point	0	27.4767	0.076942
39	Point	0	28.2092	0.09694
40	Point	0	27.4066	0.11062
41	Point	0	27.3785	0.157689
42	Point	0	25.9413	0.105112
43	Point	0	25.7498	0.090298
44	Point	0	25.7426	0.125676
45	Point	0	26.8359	0.067548
46	Point	0	27.0139	0.089442

Figure 13. Screenshot from ArcMap illustrating correlated LST and NDVI values

In the attribute table, after removing the null values (Analysis Tools, Extract, Clip), the correlated values could be observed, and a graph could be created for illustrative representation, generated in Excel. It is noticed that the high values (+50-60) recorded for NDVI are situated in areas with lower temperatures, ranging between 20 and 25°C.

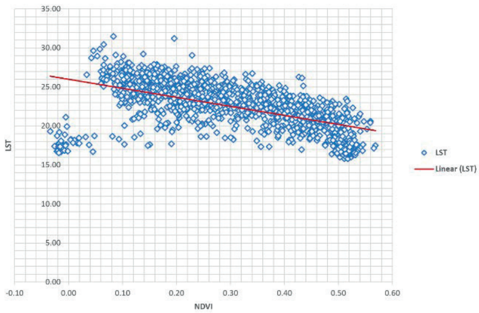


Figure 14. Illustrative graph regarding LST-NDVI correlation

For the micro-level analysis, the “Regina Maria” Park was examined, and a green register was established. The park is situated in the southwest area of District 1, near the border with District 6.

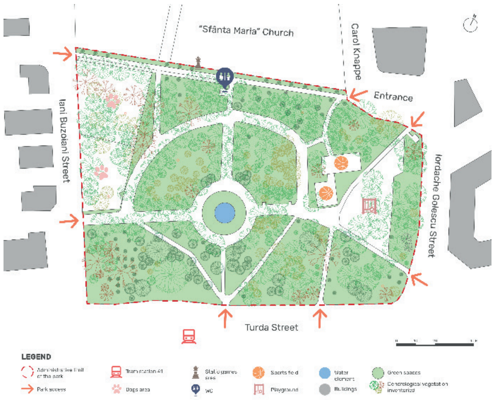


Figure 15. Map of the “Regina Maria” Park

In the “Regina Maria” Park, 410 tree specimens belonging to 29 species were identified and inventoried. Among these species, only two, *Acer negundo* and *Ailanthus altissima*, have invasive potential.

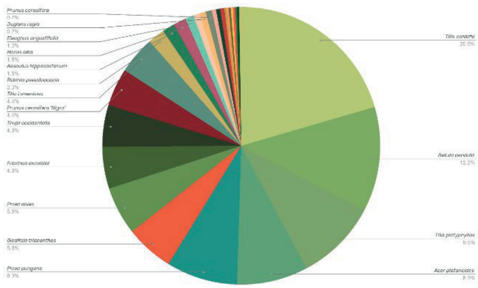


Figure 16. Illustrative graph for the registered trees

It is observed that the species representing more than 20% of the tree composition in the park is *Tilia cordata* (20.5%), followed by *Betula pendula* with 12.2% and *Tilia platyphyllos* with 9.5%. In between, there are *Acer platanoides* and *Picea pungens*, each accounting for 8.3%, and *Gleditsia triacanthos* and *Picea abies*, each with 5.6%. The least represented species include *Abies* spp., *Acer negundo*, *Ailanthus altissima*, *Catalpa bignonioides*, *Celtis occidentalis*, *Laburnum anagyroides*, *Malus domestica*, *Pinus sylvestris*, with only one specimen identified, representing 0.2% each.

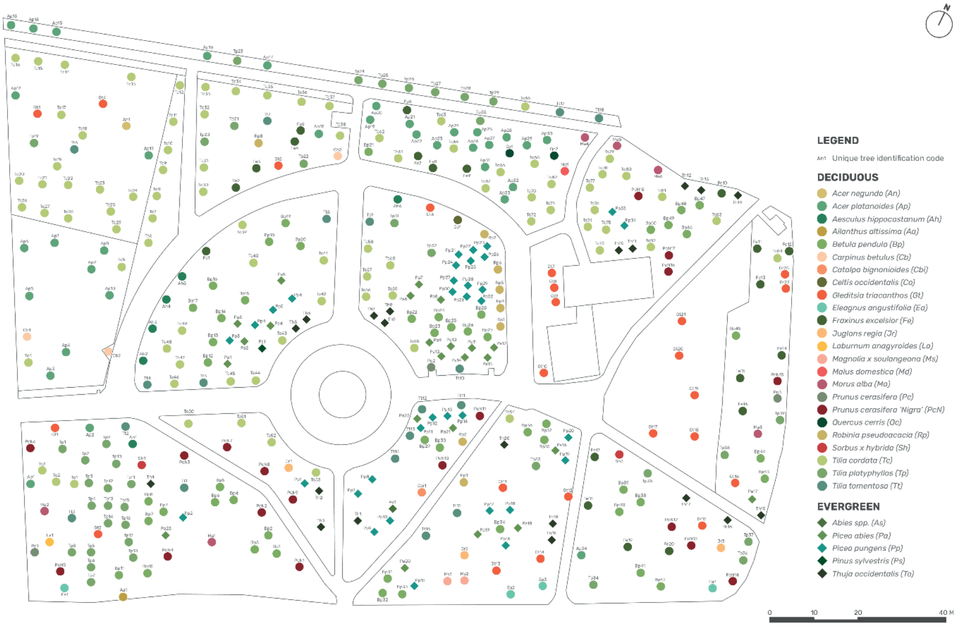


Figure 17. Map of identified and inventoried trees

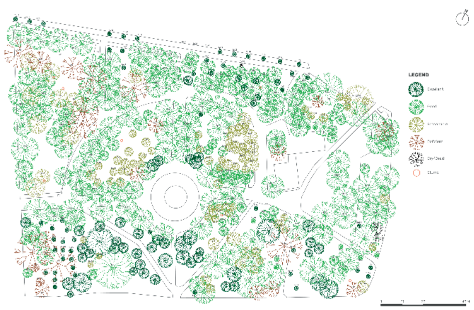


Figure 18. The phytosanitary status of the inventoried trees

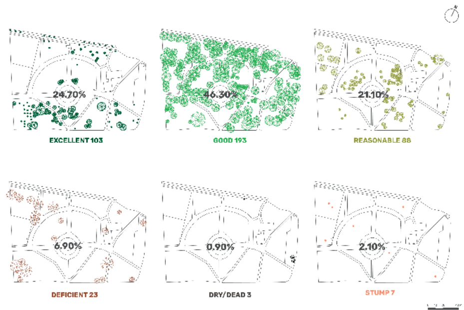


Figure 19. The phytosanitary status of the inventoried trees on categories

The data was also presented to illustrate the phytosanitary state, divided into six main categories (excellent, good, reasonable, deficient, dry/dead, stump).

Out of the 410 trees identified, 103 are in excellent condition, representing 24.70%, 193 are in good condition, accounting for the highest value of 46.30%. The reasonable is attributed to 88 trees, illustrating 21.10% of the composition, while the deficient state is observed in 23 trees, making up 6.90%. Additionally, 3 trees were identified as dry/dead, representing 0.90%. Furthermore, 7 stumps were observed, constituting 2.10% of the total 417 registrations.

Figure 20. Distribution of deciduous specimens vs. evergreen

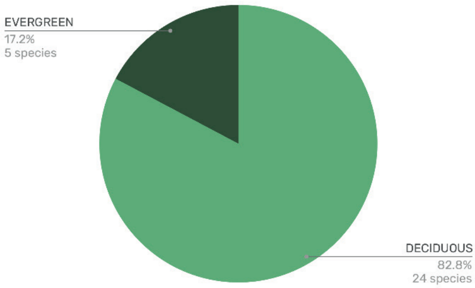


Figure 21. Distribution of the species

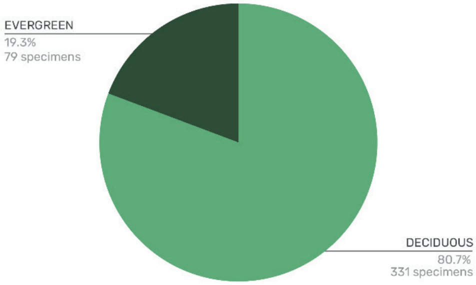


Figure 22. Distribution of the specimens

At the park level, 24 species of deciduous trees and 5 species of evergreen trees were identified. Regarding their distribution, there are a total of 331 deciduous specimens and 79 evergreen specimens.

Although the balance between native species (16) and non-native species (13) is approximately equal in terms of their presence in the park, native species predominate when considering the number of specimens. Specifically, 323 specimens of native species were recorded, while the total number of non-native specimens is 87.

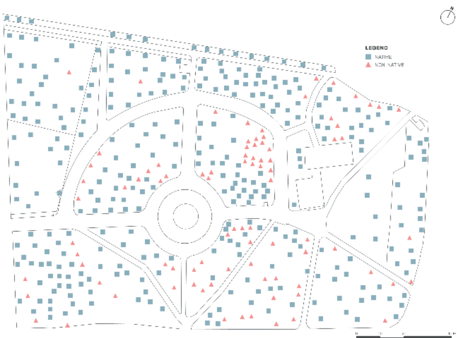


Figure 23. Distribution of native vs. non-native

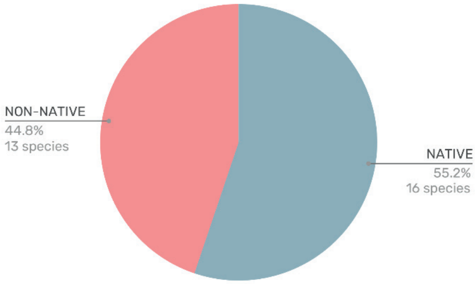


Figure 24. Distribution of the species

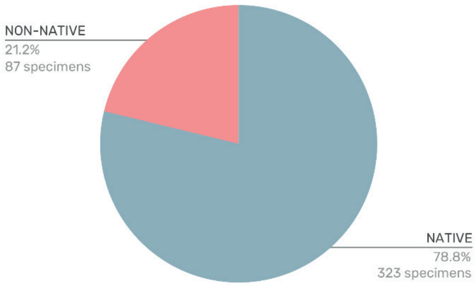


Figure 25. Distribution of the specimens

Concerning the age categories, 26 specimens in biological decline (experiencing at least one phytosanitary problem endangering the viability of the tree), 179 young specimens, and 205 mature specimens were recorded. While it is gratifying that numerous trees have been planted in the last 10 years (approximately the age of young specimens), not all of them were planted correctly, as some are situated beneath the already formed crown of mature specimens. This improper planting could impact the healthy growth and development of both the newly planted trees and the existing mature ones.

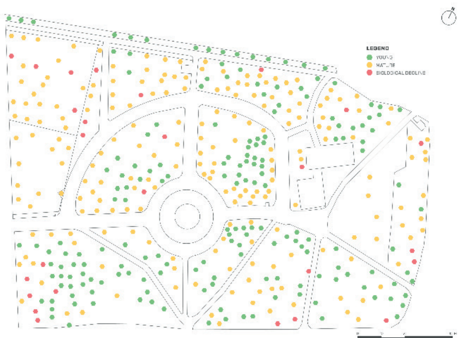


Figure 26. Distribution of age categories

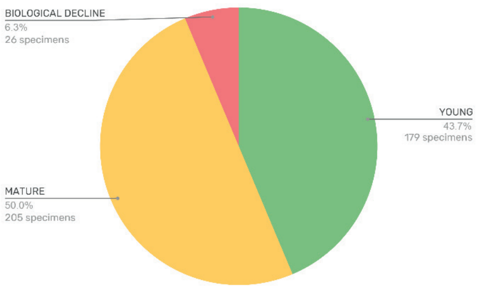


Figure 27. Illustrative graph for age categories

Among the phytosanitary issues, the following were identified on site: poor grip, broken, and dry branches, mechanical damage, rots on the parcel, trunk, and stems, damaged central cylinder, bent trunk, bacterial cancer, fungi saprophytes, and attack of caries or insects.

Figure 28. Phytosanitary issues identified on site

At the "Regina Maria" Park level, at least 7 out of the total of 29 identified species in the field exhibit bacterial cancer. Another common phytosanitary problem in the park is mechanical damage, resulting from improper pruning and trimming carried out in the past, affecting 9 out of the 29 species.

The primary causes of these symptoms include natural phenomena, such as wind, drought, and frost, pollution factors in air and soil, improperly executed maintenance works, such as cutting and trunk watering, transmission of pests from other planted specimens, and more.

Table 4. Phytosanitary issues identified for each species

Scientific name	Phytosanitary issues identified										
	poor grip branches	broken branches	dry branches	affected bark	mechanical damage	rots on the parcel/trunk/ stems	damaged central cylinder	bent truck	attack by diseases and pests: bacterial cancer	attack by diseases and pests: fungi saprophytes	attack by diseases and pests: caries / insects
Abies spp.											
Acer negundo								■	■		■
Acer platanoides					■						
Aesculus hippocastanum			■		■				■		■
Ailanthus altissima											
Betula pendula			■					■	■		
Carpinus betulus					■						
Catalpa bignonioides					■						
Celtis occidentalis											
Gleditsia triacanthos									■	■	■
Eleagnus angustifolia	■	■	■	■		■		■			■
Fraxinus excelsior						■	■		■		
Juglans regia											
Laburnum anagyroides				■		■					■
Magnolia x soulangeana											
Malus domestica											
Morus alba				■	■	■	■		■		
Picea abies			■								
Picea pungens			■								
Pinus sylvestris									■		
Prunus cerasifera					■						■
Prunus cerasifera 'Nigra'			■	■		■					
Quercus cerris											
Robinia pseudoacacia					■						
Sorbus x hybrida											
Tilia cordata				■	■	■	■				■
Tilia platyphyllos											
Tilia tomentosa					■						
Thuja occidentalis			■								
Scientific name	poor grip branches	broken branches	dry branches	affected bark	mechanical damage	rots on the parcel/trunk/ stems	damaged central cylinder	bent truck	bacterial cancer	fungi saprophytes	caries / insects
									attack by diseases and pests		
	Phytosanitary issues identified										

CONCLUSIONS

Dead/dry specimens (3) and those showing severe biological decline (7) are proposed for deforestation. For the remaining tree specimens that display phytosanitary problems or a deficient condition (16), it is suggested to use a tomographic device to accurately assess the viability of the tree and determine whether it represents an imminent danger. This comprehensive approach aims to ensure a through evaluation and appropriate action for trees with compromised conditions in the park.

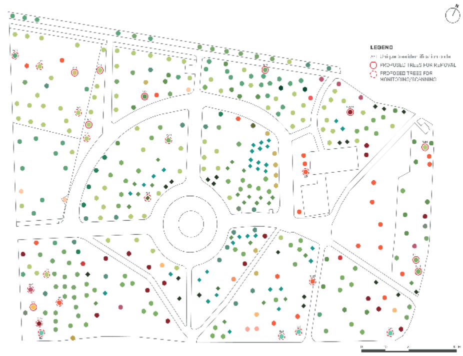


Figure 29. Proposed trees for removal and monitoring/scanning at the park level

The tomographic device can detect cavities and density differences present in the wood structure, as illustrated below in Figure 30.

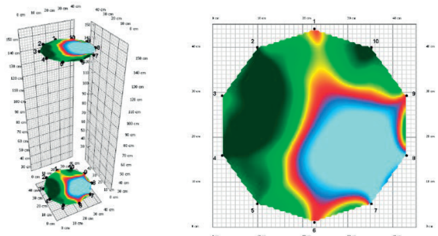


Figure 30. Example of using tree tomography (using ArborSonic 3D). Source: personal archive

Simultaneously, to comply with the provisions of Art. 7 of H.C.G.M.B 304/2009, it is assumed that for each viable deforested tree, six specimens from the same category of the decommissioned tree are planted as compensation. According to Art. 8 of the same decision, point 4) specifies the obligation to plant in compensation in a ratio of 1:1 for completely dry trees. Thus, at the park level, 7 stumps were identified that must be extracted and replaced according to the legal provisions with new dendrological material.
The implementation of a green register could facilitate monitoring compensatory tree planting, particularly in limited spaces that require prompt planting action.

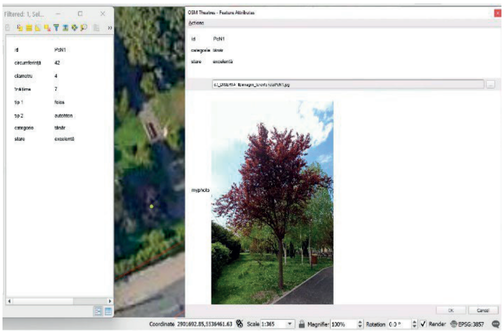


Figure 31. Screenshot from QGIS illustrating features via Open Attribute Table and publishing the image using Feature Attributes

To protect the trees from additional mechanical damage, a graphic was created for the pruning and trimming season for identified tree species. This could help making well-informed decisions on how to proceed.

Table 5. Pruning and trimming season for each species

Scientific name	Pruning and trimming trees											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Abies spp.												
Acer negundo	■	■						■				■
Acer platanoides	■	■						■				■
Aesculus hippocastanum	■										■	■
Ailanthus altissima		■	■									
Betula pendula	■											■
Carpinus betulus	■											■
Catalpa bignonioides	■	■									■	■
Celtis occidentalis	■	■										■
Gleditsia triacanthos	■	■										
Eleagnus angustifolia	■											■
Fraxinus excelsior	■	■						■				■
Juglans regia	■						■	■	■	■	■	■
Laburnum anagyroides							■					
Magnolia x soulangeana							■					
Malus domestica	■	■										■
Morus alba											■	■
Picea abies												
Picea pungens												
Pinus sylvestris												
Prunus cerasifera							■					
Prunus cerasifera 'Nigra'							■					
Quercus cerris	■	■	■									
Robinia pseudoacacia							■	■				
Sorbus x hybrida			■									
Tilia cordata	■						■	■	■	■	■	■
Tilia platyphyllos	■						■	■	■	■	■	■
Tilia tomentosa	■						■	■	■	■	■	■
Thuja occidentalis			■				■	■				

The heat island phenomenon raises the average annual temperatures in Bucharest by 0.9-1.2°C compared to the surrounding areas, with frequent instantaneous differences of 4-6°C higher. In the summer, the average annual temperature in Bucharest is 3-4°C higher than in the surroundings areas. The central area of the city is the most exposed to thermal stress (Raport de cercetare privind starea mediului în București, 2022). Therefore, ensuring proper tree management and planning any tree interventions according to a plan is mandatory.
Currently, Bucharest lacks a strategy and plan for adapting to climate change, a General Urban Plan, an Integrated Urban Development Strategy, or other instruments intended to support the fight against climate change, as well as the development of a green-blue strategy.
The primary purpose of a green register is to provide a database supporting decision-making, planning, and monitoring of environmental initiatives. A green register makes it easy to track progress towards sustainability goals.
A green register can also provide information on the degree of tree cover, resulting biomass level, the amount of carbon absorbed, and the quantity of trees and green space per inhabitant.

Geographic information systems play a significant role in landscape analysis, planning, and management. GIS allows the collection and analysis of various spatial data relevant to the landscape and beyond. By integrating and analysing datasets, geographic information systems can offer insights into landscape relationships and dynamics. GIS tools make it easier to identify sensitive areas and help minimize negative impacts by enabling informed decisions.

GIS assists in monitoring and mapping the distribution of plant species, identifying invasive species, and assessing vegetation health and condition. It can also be used to plan and implement habitat restoration or conservation projects by analysing suitable spaces and monitoring the effectiveness of interventions.

The implementation of a green register is crucial for the effective management of dendrological vegetation in the urban environment, establishing tree health, reducing risks, maximizing environmental benefits, as well as efficiently allocating resources.

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INFLUENCE OF COLD TREATMENTS ON FLOWER QUALITY OF BULBOUS ORNAMENTALS

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Abstract

Ornamental plants are among the most extensively grown plants worldwide. They main purpose is for different occasions, like Valentine Day, International women and Mother's Day and bulbous ornamental plants are one of the most important parts of these special moments. The aim of the present research was to find out for how long should be kept under cold conditions in order to produce cut and potted flowers. For this study it was selected the Tulipa gesneriana, Hyacinthus orientalis, and Muscari armeniacum. Different cold treatment types and times were applied to the bulbous plants. The results indicate that the bulbous plants under the influence of 7 weeks cold treatment under controlled temperature proved to be the best and suitable for cut flowers production at all three selected bulbous ornamental plants. In conclusion, the present work could be usefully for cultivation of different ornamental bulbous plants.

Key words*: cold treatment, grape-hyacinth, hyacinth, tulip.*

INTRODUCTION

A lot of flower bulbs are purchased each year for their importance in outdoor and indoor decoration, moreover their flowering in periods when few other ornamental plants can do it (Cantor & Gheorghita, 2011). Bulbous ornamental plants are also called ornamental geophytes (Kamenetsky & Okubo, 2012) or are often referred as bulbs which means underground storage organs like rhizomes, corms, bulbs or tubers (Li et al., 2023). The ornamental geophytes include more than 800 different genera (Bryan, 1995), although the ornamental industry is mostly dominated by 7 genera's as: *Tulipa*, *Narcissus*, *Gladiolus*, *Iris*, *Crocus*, and *Hyacinthus* (Benschop et al., 2010). Their main utilization is commercial bulb production, growing as potted plants, forced fresh cut and potted flowers, and in landscaping (De Hertogh et al., 2012; Chawla et al., 2022). According to Gul et al. (2020) ornamental bulbous plants dominate the global flower market, and one of the main reasons is that they follow perfectly timed phenological period. The life cycle of the ornamental bulbous plants is actually connected with the temperature warm-cold-warm cycle which is a key factor leading to flower initiation (Khodorova & Boitel-Conti, 2013).

In the recent years the Romanian flower industry produced cut flowers and potted plants from a variety of different bulbous plants (Székely-Varga et al., 2019).

Hyacinthus orientalis L. beside is an excellent ornamental garden plant it could be also easily forced as cut or potted plant (Nazari et al., 2011). According to Sabo et al. (2018) in Europe firstly was introduced in the 16th century, and it was cultivated first by the Dutch. The hyacinth is a native species from southern Turkey to northern Israel, it is a tuberous earth plant that grows primarily in temperate biomes (POWO, 2023). Tulips are outstanding potted, cut or bedding flowers and can be grown in open or protected conditions (Nayeem & Qayoom, 2015). *Tulipa gesneriana* L. is an endemic plant to Turkey, it is mostly found in the temperate biome and is a bulbous geophyte (POWO, 2023b). The *Muscari armeniacum* Leichtlin species is endemic to Southeast Europe and the Caucasus, it is mostly found in the temperate biome and is a bulbous geophyte (POWO, 2023c). The grape-hyacinth is a perennial bulbous plant with simple basal leaves and short flower stalks (Bokov, 2019).

The aim of the present paper was to determine how long should *Hyacinthus orientalis*, *Muscari armeniacum*, and *Tulipa gesneriana* bulbs kept under cold treatments, as forced culture to produce cut or potted flowers. Also, an important aspect was to manage the flowering to be ready for 8th of March.

MATERIALS AND METHODS

The experiment was conducted at Sapientia Hungarian University of Transylvania, in Târgu Mureș, between December and March. As plant material were selected three bulbous ornamental plants as followed: *Hyacinthus orientalis* L. `Miss Saigon` (circumference 18/19 cm) - very compact hyacinth, leaves are light green, flowers are blue and one of its characteristics is that it can produce several flowers per bulb; *Tulipa gesneriana* L. `Yellow Baby` (circumference 10/11 cm) - strong, rigid tulip, characterized by grey-green leaves and wide yellow flowers; *Muscari armeniacum* Leichtlin `Grandioso` (circumference 10/11 cm) - with bright blue flowers. The bulbs were obtained from Agrosel (Câmpia Turzii, Romania) and were planted in 7×8 cm pots in a mixture of peat and sand 50:50 ratio. Before planting, each bulb was treated with fungicide (Captan 80 WDG), and after the planting the bulbs were irrigated. Two types of cold treatments were applied one in natural conditions, where the bulbs were kept under an unheated greenhouse covered with soil, the second when the bulbs were placed in artificial environment in a refrigerator (Kühlschrank 2 Türen aus Edelstahl). At the natural condition the temperature was between -4 and 6°C and the humidity 80-100%. In the case of the artificial conditions the temperature and the humidity were regulated with 5°C and 90%. For the experiment 180 *Hyacinthus orientalis*, 300 *Muscari armeniacum* and 300 *Tulipa gesneriana* bulbs were selected (Table 1), which were treated with three different cold treatments times 7, 8, and 9 weeks for both environmental conditions. Each cold cycle was repeated three times.

After the mentioned cold treatments, the bulbs were brought into an indoor place where the temperature was above 10°C, and were covered with a black foil for three days, to prevent the bulbs from a sudden strong light.

Table 1. Experimental schema

Time	Natural cold treatment			Artificial cold treatment		
	Hyacinthus orientalis	*Muscari armeniacum*	*Tulipa gesneriana*	*Hyacinthus orientalis*	*Muscari armeniacum*	*Tulipa gesneriana*
7 weeks	30 pcs	50 pcs	50 pcs	30 pcs	50 pcs	50 pcs
8 weeks	30 pcs	50 pcs	50 pcs	30 pcs	50 pcs	50 pcs
9 weeks	30 pcs	50 pcs	50 pcs	30 pcs	50 pcs	50 pcs

At the end of the cold treatments for each bulb the flowering stem of plant height was measured each third day until 8th of March.

The significance of the differences between the treatments was tested by applying two-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

Considering the *Hyacinthus orientalis* could be clearly observed that the different cold treatments and cycles significantly influenced the stem length (Figure 1).

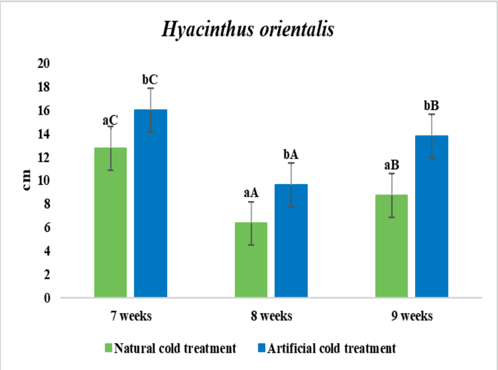

Figure 1. Stem length of *Hyacinthus orientalis* under the effect of different cold treatment and cycle. Bars represent the means ± SE ($n = 30$). Different lowercase letters above the bars indicate significant differences between cold treatments, and different uppercase letters indicate the significant differences between the cold cycle ($p < 0.05$)

Comparing the 7 weeks cold treatment under the effect of artificial cold the stem length reported a 16 cm significantly higher than the other treatment. In the case of 8 and 9 weeks the same significant differences were observed. Furthermore, when comparing the cold treatment time, distinctively the bulbs kept for 7 weeks treatment reported the highest stem length, which was significantly different compared to the other two treatments. Moreover, the smallest stem length was reported at the 8 weeks treatment.

Regarding the *Muscari armeniacum* in the case of the 7 weeks treatment the stem length of the bulbs kept in artificial conditionswas significantly greater compared to the other treatment (Figure 2). At the 8 and 9 weeks no significant differences were reported. The 8 and 9 weeks treatments recorded longer stems compared to the 7 weeks treatment, from which could be concluded that the 7 weeks treatment is more suitable for *Muscari armeniacum* cultivation.

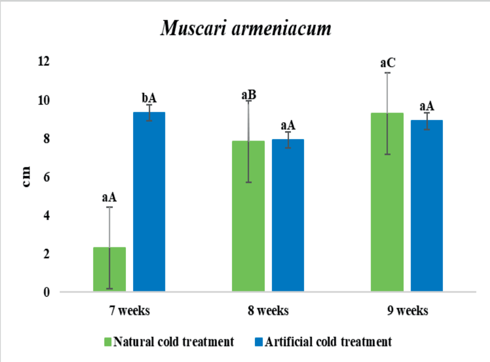

Figure 2. Stem length of *Muscari armeniacum* under the effect of different cold treatment and cycle. Bars represent the means ± SE ($n = 50$). Different lowercase letters above the bars indicate significant differences between cold treatments, and different uppercase letters indicate the significant differences between the cold cycle ($p < 0.05$)

At *Tulipa gesneriana* the longest stem length was determined at the 7 weeks cycle and natural cold treatment, approximately ~ 31 cm (Figure 3). In the case of 7 and 8 weeks cold treatment the bulbs under the natural conditions reported significant differences compared to the other treatment, however at 9 weeks it was contrary. When comparing the cold cycle, a significant higher change was recorded at 7 weeks treatment compared to the other two.

Regarding the growth dynamics of the plants at 7 weeks could be clearly observed that the tulips recorded the fastest growth, and at both type of cold treatments the growth dynamic was almost similar, however at the start of the growth the bulbs cultivated under the natural cold effects have had a slower growth compared to the other even so at the end of the experiment the stem length was longer (Figure 4). At hyacinth a stronger growth was recorded at the bulbs cultivated under artificial conditions. Also, the artificial conditions highly influenced the growth of the grape-hyacinth, in which case the growth have recorded a more pronounced start, and this advantage remained until the end of the experiment.

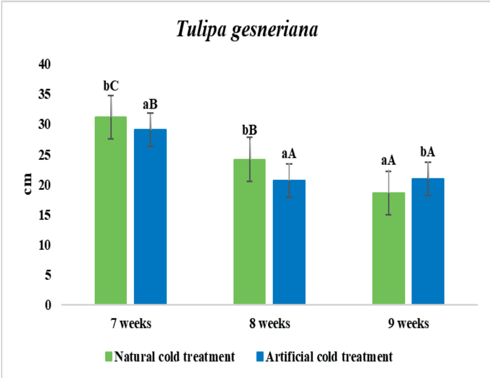

Figure 3. Stem length of *Tulipa gesneriana* under the effect of different cold treatment and cycle. Bars represent the means ± SE ($n = 50$). Different lowercase letters above the bars indicate significant differences between cold treatments, and different uppercase letters indicate the significant differences between the cold cycle ($p < 0.05$)

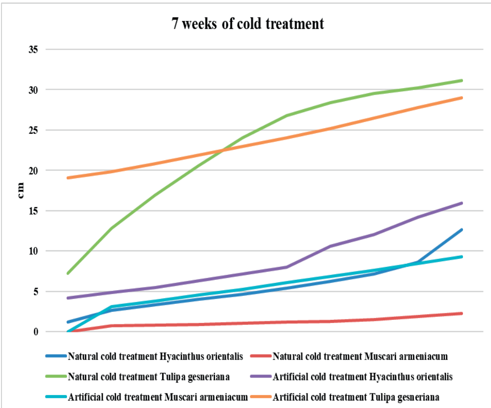

Figure 4. Growth dynamics of the plants under the effect of 7 weeks of cold treatments and cold cycles

As expected at the 8 weeks cold treatment similarly the highest growth was recorded at tulips (Figure 5). The tulip bulbs under natural conditions reached a higher growth. At the *M. armeniacum* the growth dynamics was similar. And again at *H. orientalis* the artificial conditions recorded the faster growth.

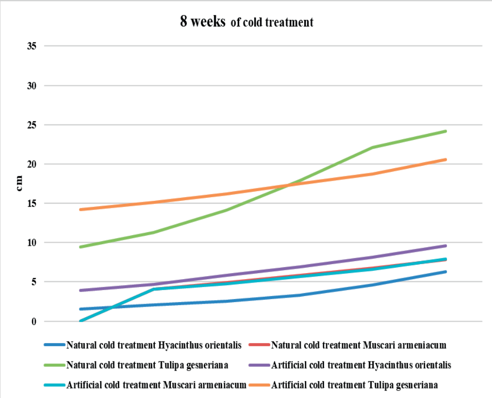

Figure 5. Growth dynamics of the plants under the effect of 8 weeks of cold treatments and cold cycles

Here again, at *T. gesneriana* a faster growth was observed at the bulbs under artificial conditions (Figure 6). Once more hyacinth bulbs under the influence of artificial conditions reported a faster growth dynamic, compared to the other. In the case of *Muscari armeniacum* in natural conditions bulbs achieved a better growth than the bulbs under the artificial conditions, however the difference is small.

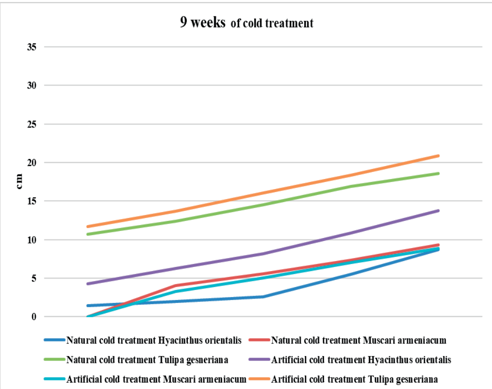

Figure 6. Growth dynamics of the plants under the effect of 9 weeks of cold treatments and cold cycles

According to Khodorova & Boitel-Conti, (2013) a several environmental factors that can affect bulb development (light, humidity, temperature), temperature has been shown to play a crucial role in controlling bulb growth and flowering The ideal cooling period would be 10 to 15 weeks, maybe even longer, during this period, plant roots form and the stem length increases (Graine & Scoggins, 2014).

In a study it was highlighted that at *Tulipa gesneriana* hybrids 'Royal Virgin' and 'Ad Rem' the low temperature and time treatment influenced positively the plants flowering (Carillo et al., 2022), moreover in another experiment it is also mentioned that the flowering time is mostly a genotype-specific response (Wang et al., 2019). According to Koçak & Çığ (2019), the 1st of December and 16th of December planting times are more suitable for hyacinths cultivation and also the planting substrate significantly influenced the bulbs. Additionally, is also determined that biostimulators can have a positive effect on the growth, floral characteristics and the yield of the volatile oil at hyacinths (Altaee, 2021). Furthermore, cold cycle can also have a positive effect on the seed's germination of *Muscari neglectum*, where the greatest results were obtained at the seeds under the treatment of mid-winter and natural conditions (Labbaf et al., 2023). In another study it was demonstrated that along cold cycle also the light colour could have influence on the bulbs, as it was determined that the yellow light colour recorded the greatest results (Smigielska et al., 2014).

CONCLUSIONS

From the present experiment could be concluded that the 7-week cold treatment proved to have the best growth dynamics, which means that the plants under this treatment are also suitable for cut flowers, because the stem length increased very quickly. The 8-week treatments were almost similar with 7-week but they did not show an equal or close trend in stem length growth. The results of the 8-week treatments indicated more pot use. The 9-week treatments did not achieve adequate stem by the time when their mostly sold on the market (8th of March). From this point of view, the 7-week treatments proved to be the most suitable, which could be also explain by the fact that this treatment had the most time to develop after the treatments.

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VARIABILITY OF SOME LEAF PARAMETERS IN *LIQUIDAMBAR STYRACIFLUA* L. IN CONDITIONS OF LEAVES CHLOROSIS

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Abstract

The study analyzed the variability of some leaf parameters in the species Liquidambar styraciflua L. under conditions of leaf chlorosis. Leaf samples (normal and chlorotic) were taken from trees in the urban area, Timisoara Municipality. A differentiated variation of the values of the determined leaf parameters was recorded: leaf length, L = 6.15 – 15.00±0.42 cm; scanned leaf area, SLA = 23.68 – 132.48±5.27 cm^2; photosynthetic pigments, Chl = 1.77 – 50.36±3.23, Car = 1.76 – 10.16±0.52; fresh weight, Fw = 0.327 – 3.127±0.127 g; dry weight, Dw = 0.058 – 1.120±0.052 g; specific fresh weight, SFw = 0.0138 – 0.0236±0.0005 g cm^{-2}; specific dry weight, SDw = 0.0024 – 0.0087±0.0003 g cm^{-2}; fresh weight to dry weight ratio, Fw/Dw = 2.6012 – 5.6379±0.1755; specific dry weight to specific fresh weight ratio, SDw/SFw = 0.1774 – 0.3844±0.0119. Correlation of different levels of intensity was recorded between the analyzed parameters, and the regression analysis led to models in the form of equations and graphic form, under conditions of statistical safety (p<0.001). According to PCA, distribution diagrams were generated in relation to categories of leaf parameters, in which the main components (PC1, PC2) explained the presence of variance.

Key words: *Liquidambar, leaf chlorosis; models, PCA, photosynthetic pigments, specific dry weight.*

INTRODUCTION

Leaf chlorosis represents a quality of life problem for plants, as a result of some interferences and imbalances in the synthesis of chlorophyll. Low levels of chlorophyll are the main factors of plant nutrition imbalances, abiotic stress or inadequate water quality (Abbasi et al., 2023).

Chlorophyll deficiency in leaves affects the photosynthetic process, plant growth and development, plant productivity, harvest quality. In the case of ornamental plants, their ornamental appearance is affected.

Chlorosis is associated, most frequently, with the deficient absorption of iron and the deficient supply of the plant organism this nutrient (Li et al., 2021; Yoneyama, 2021).

Chlorosis has been studied in different plants from the spontaneous flora or plants of economic interest, herbaceous or arboreal, such as grasses (barley, rice), palm species, plane trees, petunias, aromatic plants, and many other species (Šrámek and Dubský, 2009; Aalipour et al., 2019; Li et al., 2021; Yoneyama, 2021; Mehrotra et al., 2022).

The manifestation of chlorosis is varied, from a few leaves, to branches, parts of the crown of trees or even to entire trees, depending on the growing conditions associated with the soil, but also the favorable factors (Koenig and Kuhns, 2002). The authors communicated that under the conditions of maintaining chlorosis for long periods (sometimes for several years), individual elements of the crowns of the trees can dry out, and in some cases the entire tree plant can die.

In experimental conditions, with different types of substrate, fertilization and pH of the substrate, the intensity of petunia chlorosis was evaluated (Šrámek and Dubský, 2009). The authors of the study reported the presence and variation of the intensity of chlorosis in plants with the increase in the pH value associated with the addition of limestone, but also associated with some substrate components (pH = 4.4, optimal value for petunias, under the study conditions). The reduction of chlorosis was registered under the conditions of the application of nutrient solutions with different microelements (including Fe, EDTA chelated form).

The phenomenon of leaf chlorosis in some ornamental plants was studied in relation to the application of some hormonal treatments (s-ABA) (Waterland et al., 2010). The authors of the study recorded the variation of chlorosis intensity in relation to the plant species, the time and the concentrations of s-ABA applied.

The manifestation of chlorosis was studied in *Oxalis regnellii* (ornamental potted plant) in relation to viral infections, nutritional deficiencies with Fe and Mn (analyzed independently and in association), or with growth conditions, e.g. temperature (Miller et al., 2012). Through the comparative analysis of the considered factors, the authors elucidated the initial hypotheses, and communicated the favorable response of the plants to the treatments with foliar products with Fe (EDDHA).

Symptoms that expressed plant deficiencies, including chlorosis, were studied by PCR tests on different ornamental plants (Khasa et al., 2016). The prevention and correction of chlorosis was studied in the pea crop under the conditions of application of different fertilizing products, at various times of vegetation, with the formulation of effective technological recipes (Kabir et al., 2016). The phenomenon of chlorosis (severe chlorosis) was studied in tomatoes in relation to the spectrum and light intensity (Pham et al., 2019). The authors of the study recorded the variation in the level of chlorotic intensity of the leaves depending on the light conditions of the plants (blue or red - monochromatic; combined). High intensity of chlorosis was recorded under the conditions of plant growth in blue, monochromatic light.

Chlorosis was analyzed in accordance with the state of nutrition and certain physiological characteristics of the plane tree, under experimental conditions under the influence of some variants of organic, mineral and mycorrhization fertilization (Aalipour et al., 2019). The authors of the study recorded a high level of chlorosis in the control variant, and reduced values in the fertilized and mycorrhized variants.

Genetic analyzes were done to understand the genetic mechanisms that intervene in the regulation of poplar (*Populus deltoides*) leaf color, with the variation and degradation of chlorophyll content (Zhang et al., 2019). Vaid et al. (2020) used a genetic approach to study genes involved in hybrid chlorosis in *Arabidopsis thaliana*.

Morphological and physiological changes (e.g. reduction of chlorophyll content, reduction of fresh weight) associated with iron deficiency and chlorosis, was communicated by Li et al. (2021) in palm (*A. catechu*) seedlings. The authors of the study identified the sensitivity of the species to iron and communicated possible mechanisms of plant adaptation to iron deficiency, through the synthesis and accumulation of flavonoids and organic acids.

The phenomenon of chlorosis was studied in rice in relation to fixed and variable doses of N (Kharim et al., 2020). The authors reported the lowest level of chlorosis in the case of variable doses of nitrogen, compared to fixed doses.

Chlorosis, associated with Fe deficiency in alkaline soil conditions, has been studied in different species of aromatic plants (Mehrotra et al., 2022). Such crops often occupy marginal land, affected by various limitations, including soil reaction, nutrient content and the availability of mineral elements for plant nutrition. The study presented by Mehrotra et al. (2022) shows interest from the perspective of formulating technological recipes (foliar fertilizers, chelated products, nanoparticles) for the efficiency of cultivating these categories of plants. Biocompatible Fe nanoparticles have complex effects on plant metabolism, in addition to direct iron supplementation (Sala, 1999; Gracheva et al., 2023).

Associated with the spring and autumn seasons, chlorosis (different levels of intensity, from normal to severe chlorosis) was recorded and studied in citrus species (Xiong et al., 2023). The authors reported different metabolic and physiological disorders associated with chlorosis. Different methods and techniques are used to evaluate chorosis, based on visual inspection, foliar diagnosis (leaf samples and laboratory determinations), imaging analyzes (Abbasi et al., 2023).

This study analyzed the phenomenon of chlorosis in the leaves of *Liquidambar styraciflua* L. in relation to the content of photosynthetic pigments and leaf parameters and physiological indices.

MATERIALS AND METHODS

In relation to the purpose of the study, leaf samples were taken from *Liquidambar styraciflua* L. trees, which showed symptoms of chlorosis. For comparison, normal leaves were also taken. The leaf samples were taken in July 2022, from ornamental trees, public domain, Timisoara Municipality. Examples from the set of leaf samples are shown in Figure 1.

Figure 1. Examples of chlorotic and normal leaf samples, *Liquidambar styraciflua* L.

In order to characterize the leaves in relation to the chlorotic state, several determinations were made. Dimensional parameters of the leaves were determined (length – L, scanned leaf surface – SLA) (Rasband, 1997).
The photosynthetic pigments were determined, namely the chlorophyll content (Chl) and the carotenoid content (Car). The determinations were made with SPAD-502Plus devices (KONICA MINOLTA), and respectively with ACM-200 Plus (OPTI-SCIENCES).
Fresh weight (Fw, g) was determined by weighing each fresh leaf, and dry weight (Dw, g) was determined by weighing each dry leaf. Drying and weighing of the leaf samples was done with an AXIS thermal balance (ATS 60 model, Gdańsk, Poland). Parameters Fw and Dw were determined for each leaf.
Starting from the value of the leaf area (SLA) and the Fw, respectively Dw values, related to each leaf, SFw (Specific fresh weight, g cm^{-2}) and SDw (Specific Dry weight, g cm^{-2}) parameters were calculated. Based on the determined parameter values, the ratios Chl/Car, Fw/Dw and SDw/SFw were calculated.
The recorded data were analyzed and interpreted mathematically and statistically appropriately, in relation to the purpose of the study (Hammer et al., 2001; JASP, 2022).

RESULTS AND DISCUSSIONS

Leaf samples (*Liquidambar styraciflua* L.) taken from trees that showed symptoms of chlorosis and from trees with normal vegetation were analyzed to determine some biometric parameters (L, SLA), the content of photosynthetic pigments (chlorophyll - Chl, carotenoids - Car), of the weight of the fresh substance (Fw) and of the dry substance (Dw). Based on the recorded values, the ratios between chlorophyll and carotenoids (Chl/Car), between fresh substance and dry substance (Fw/Dw) and between Specific dry weight and Specific fresh weight (SDw/SFw) were calculated.
The recorded experimental data were analyzed statistically, and the resulting values are presented in Table 1. The recorded results showed statistical certainty, according to ANOVA Test, Table 2.
Regarding the chlorophyll (Chl) content, six leaf samples (leaf sample Ls1 to Ls6) were included in the 1st quartile (lower quartile), which presented the lowest chlorophyll content (Chl < 7.800 SPAD). These samples showed a very strong chlorosis.
In the 2nd quartile (same as the median zone), 12 leaf samples (Ls7 to Ls18) were included, which presented the chlorophyll content with values close to the median (Median = 23.48), (Table 1), respectively Chl = 7.800-34.998.
Six leaf samples (Ls19 to Ls24) were included in the 3rd quartile (upper quartile), which showed high values of chlorophyll content, Chl > 34.998. The graphic distribution of the chlorophyll content on the quartiles, is shown in Figure 2 (a).
In the case of the carotenoid content, six leaf samples (Ls1 to Ls6) were included in the 1st quartile, with the carotenoid content Car <

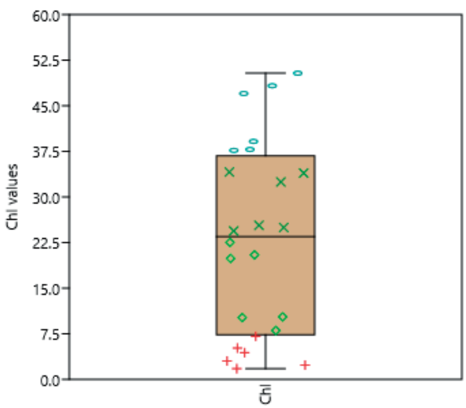
2.188. In the 2nd quartile, 12 leaf samples (Ls7 to Ls18) were included with the content of carotenoids in the median area (Median = 3.34) (Table 1), respectively Car = 2.188-4.532. Six leaf samples were included in the 3rd quartile, with carotenoid content Car > 4.532. The graphic distribution of the carotenoid content is presented in Figure 2 (b).

Table 1. The values of statistical parameters in *Liquidambar styraciflua* L. leaves

Statistical parameters	L	SLA	Chl	Car	Fw	Dw	SFw	SDw	Chl/Car	Fw/Dw	SDw/SFw
N	24	24	24	24	24	24	24	24	24	24	24
Min	6.15	23.68	1.77	1.76	0.327	0.058	0.0138	0.0024	0.98	2.6012	0.1774
Max	15.00	132.48	50.36	10.16	3.127	1.120	0.0236	0.0087	8.66	5.6379	0.3844
Sum	252.95	1700.41	550.75	96.44	29.102	7.869	0.3962	0.1010	126.40	101.5229	5.9400
Mean	10.54	70.85	22.95	4.02	1.213	0.328	0.017	0.004	5.27	4.2301	0.2475
Std. error	0.42	5.27	3.23	0.52	0.127	0.052	0.0005	0.0003	0.47	0.1755	0.0119
Variance	4.24174	665.64590	250.68030	6.53096	0.38495	0.06378	0.00001	0.00000	5.32621	0.73936	0.00339
Stand. dev	2.0595	25.8001	15.8329	2.5556	0.6204	0.2526	0.0025	0.0017	2.3079	0.8599	0.0582
Median	10.65	68.25	23.48	3.34	1.082	0.252	0.0159	0.0037	5.5300	4.1209	0.2427
25 prcntil	9.475	56.698	7.800	2.188	0.872	0.175	0.015	0.003	3.680	3.906	0.199
50 prcntil	10.65	68.245	23.480	3.340	1.082	0.252	0.016	0.004	5.530	4.121	0.243
75 prcntil	11.413	83.915	34.998	4.532	1.296	0.337	0.017	0.004	7.255	5.021	0.257
Skewness	-0.1039	0.3255	0.1856	1.6204	1.4418	1.9218	1.9314	1.6820	-0.4555	-0.4116	1.0558
Kurtosis	0.6556	0.4039	-1.2433	1.8763	2.9316	3.7081	3.3721	2.1566	-1.0109	-0.7892	0.1526
Geom. mean	10.3332	65.8508	15.7903	3.4449	1.0768	0.2602	0.0164	0.0040	4.5828	4.1388	0.2416
Coeff. var	19.5411	36.4149	68.9949	63.5979	51.1668	77.0271	14.8475	40.6953	43.8201	20.3271	23.5271

Table 2. ANOVA Test

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	256312.8	8	32039.1	2943.579	5.24E-78	3.833807
Within Groups	685.7175	63	10.88441			
Total	256998.6	71				



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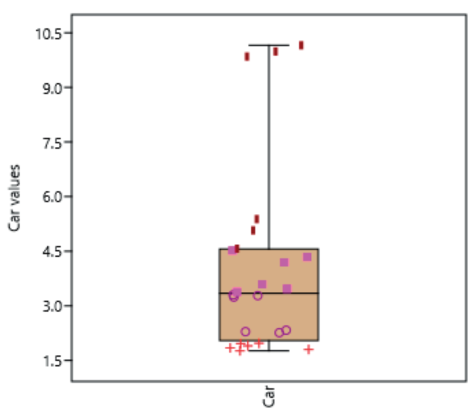

B

Figure 2. Photosynthetic pigments distributions in boxplot format, (a - Chl; b - Car), *Liquidambar styraciflua* L. leaves

Considering the determined parameters as factors in the description of the *Liquidambar styraciflua* L. leaf samples, PCA analysis was used to evaluate the importance and mode of action of the factors. Under the conditions of the data analysis, the grouping of factors resulted, according to Table 3. In the Factor 1 category, parameters were grouped (descending order of the action): SDw/SFw ratio (r = 0.893), Fw/Dw ratio (r = -0.884) , SDw (r = 0.804), Chl (r = 0.802) and Car (r = 0.761). In the Factor 2 category, parameters were grouped (in descending order of action): SAL (r = 0.900), Fw (r = 0.887), leaf length, L (r = 0.840) and dry matter, Dw (r = 0.777). The characteristics of the factors per group (Factor 1, Factor 2) are presented in Table 4. The distribution diagrams of the factors are presented in Figure 3 and figure 4.

Table 3. Factor Loadings

Parameters	Factor 1	Factor 2	Uniqueness
SDw/SFw	0.893		0.005
Fw/Dw	-0.884		0.048
SDw	0.804		0.036
Chl	0.802		0.167
Car	0.761		0.104
SLA		0.900	0.092
Fw		0.887	0.004
L		0.840	0.157
Dw		0.777	0.023
SFw			0.119
Chl/Car			0.802

Table 4. Factor Characteristics

	Unrotated solution			Rotated solution		
	SumSq. Loadings	Proportion var.	Cumulative	SumSq. Loadings	Proportion var.	Cumulative
Factor 1	8.711	0.792	0.792	4.894	0.445	0.445
Factor 2	0.738	0.067	0.859	4.549	0.414	0.858

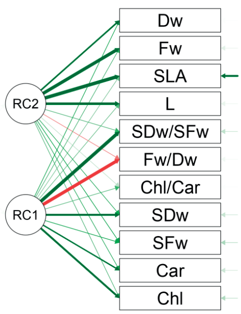

Figure 3. Path Diagram

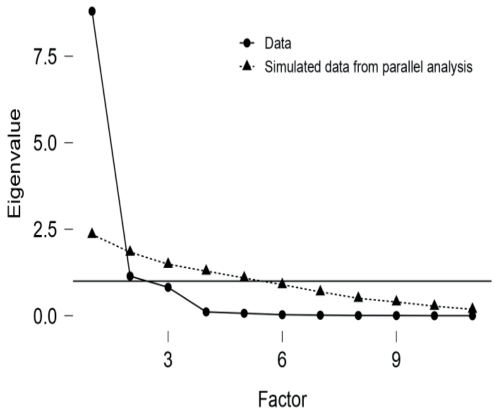

Figure 4. Scree plot

According to the value in Table 3 and the distribution in figure 2 (r = -0.884; red line), the Fw/Dw ratio has a negative effect, and the other factors have a positive effect. Specific fresh weight (SFw) and the Chl/Car ratio remained outside the two groups of factors.

Starting from the position of the parameters in relation to Factor 1 and Factor 2 (Table 3), the distribution of leaf samples (Ls1 to Ls24) was analyzed, according to the first two parameters from each group of factors.

Depending on the SDw/SFw ratio (r =0.893) and the Fw/Dw ratio (r =-0.884), the first ones ranked in Factor 1, Table 3, respectively in RC1, Figure 3, the PCA diagram in Figure 5 resulted, in which the variants were distributed in relation to the two ratios, as biplot.

PC1 explained 98.936% of variance, and PC2 explained 1.064% of variance.

According to SLA (r =0.900) and Fw (r =0.887), the first ranked in Factor 2, Table 3, respectively in RC2, Figure 3, the PCA diagram in Figure 6 resulted, in which the variants were distributed in relation with the two parameters, as biplot.

PC1 explained 97.417% of variance, and PC2 explained 2.5828% of variance.

From the analysis of the determined parameters, in the characterization of *Liquidambar styraciflua* L. leaf samples, it was found that the parameters, considered as influential factors, had a different contribution in the analysis and description of the leaf samples.

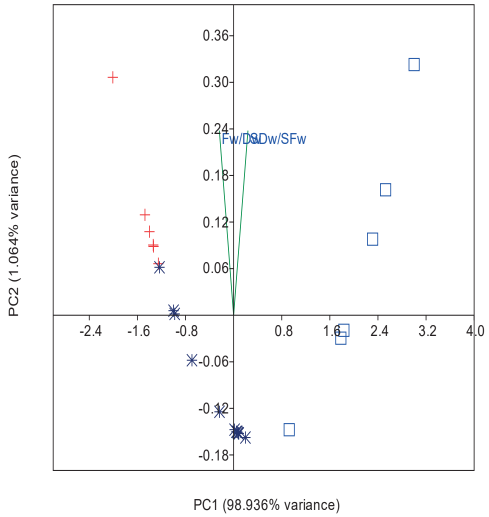

Figure 5. PCA diagram in relation to parameters SDw/SFw, and Fw/Dw, as byplot; Ls in 1st quartile - cross symbol; Ls in 2nd quartile - Star symbol; Ls in 3nd quartile - Square symbol

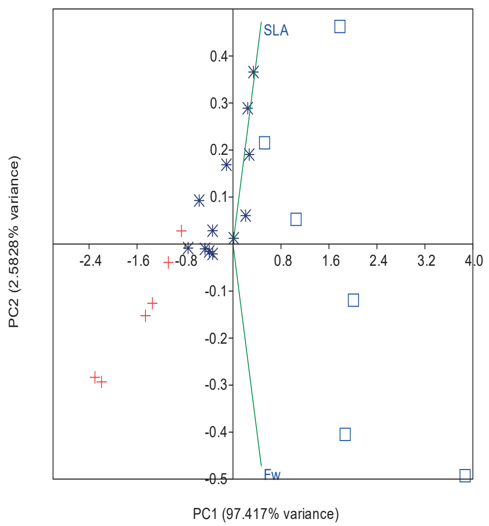

Figure 6. PCA diagram in relation to SLA parameters, and Fw, as byplot; Ls in 1st quartile - cross symbol; Ls in 2nd quartile - Star symbol; Ls in 3nd quartile - Square symbol

The length of the leaves (L) was positioned in the Factor 2 category, with positive action, of moderate intensity (r = 0.840).

The scanned leaf surface (SLA) was positioned in the Factor 2 category, with a positive, very strong action (r = 0.900). In relation to the dimensional parameters L and SLA of the leaves, it was found that the leaves with small to medium sizes, the young leaves (among the leaf samples), showed very strong and strong chlorosis.

The content of photosynthetic pigments directly expressed the level of chlorosis of the leaves. Both parameters Chl, Car) were positioned in the Factor 1 category, with positive, strong and moderate action (r = 0.802 in the case of Chl; r = 0.761 in the case of Car). The lowest values of Chl and Car were recorded in young, small leaves.

Parameters Fw and Dw were positioned in the Factor 2 category, with positive action, strong in the case of Fw (r = 0.887), and moderate in the case of Dw (r = 0.777). The Specific dry weight (SDw) parameter was positioned in the Factor 1 group, with a positive, strong action (r = 0.804).

The Specific fresh weight (SFw) parameter was not placed in the two categories of factors, similar to the Chl/Car ratio. The calculated Fw/Dw and SDw/SFw ratios were placed in the Factor 1 category, with strong positive action in the case of the SDw/SFw ratio (r = 0.893) and strong negative action in the case of the Fw/Dw ratio (r = -0.884).

Aalipour et al. (2019) reported the improvement of the photosynthetic rate by up to 60% in plane trees under the influence of fertilizer treatments and mycorrhization, compared to the control variant. Without interventions to correct chlorosis in tree species, after a period of several years of stress, Koenig and Kuhns (2002) reported the drying of some portions of the crown with negative visual/aesthetic ornamental effects, and even the drying of some trees. In the case of the present study, *Liquidambar styraciflua* L. trees were found to exhibit chlorosis in the first year, but even then corrective interventions would be recommended.

Although the primary source of chlorosis is most often the soil, foliar treatments with appropriate products generate rapid corrections (Šrámek and Dubský, 2009; Kabir et al., 2016; Mehrotra et al., 2022). In parallel with the foliar treatments, soil interventions are recommended to balance the agrochemical properties, with the aim of long-term balances for arboreal plants (ornamental trees and shrubs).

Abbasi et al. (2023) used imaging analysis to characterize chlorosis and reported an accuracy of 95% for the obtained models. The Computer

Vision models and the proposed application (based on the cloud) by the authors find practical applicability for the efficient management of crops.

CONCLUSIONS

From the analysis of the parameters determined in the leaf samples of the species *Liquidambar styraciflua* L., and their inclusion in the two categories of factors (Factor 1, and Factor 2), it was found that they presented different action in sense and intensity in relation to the chlorosis phenomenon of the leaves.

The Factor 1 category included five parameters, among which one with negative action (Fw/Dw) and four with positive action (SDw/SFw, SDw, Chl and Car). The Factor 2 category included four factors with positive action (SLA, Fw, L, Dw).

Depending on the parameters with the highest weight in Factor 1 (the ratio SDw/SFw, r=0.893, and the ratio Fw/Dw, r =-0.884), PC1 explained 98.936% of variance, and PC2 explained 1.064% of variance.

According to the first two positions in Factor 2 (SLA, r = 0.900 and Fw, r = 0.887), PC1 explained 97.417% of variance, and PC2 explained 2.5828% of variance.

The analysis of the data set showed that the smaller leaves (young leaves) showed the highest intensity of the chlorosis phenomenon, expressed by the lowest chlorophyll content of carotenoids (palsed leaf samples in the 1st quartile).

Foliar treatments associated with balanced soil interventions are necessary for the correction of chlorosis and the medium-long-term improvement of the vegetation of the studied *Liquidambar styraciflua* L. trees.

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WHAT IS THE DIFFERENCE BETWEEN THE INTERNATIONAL/ NATIONAL AND LOCAL METEO STATIONS FOR ONE SPECIFIC ORCHARD? ALMOST 100 YEARS OF RECORDS DATABASE ANALYSIS

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Abstract

Temperature is one of the most controversial parameters studied in fruit-growing technologies, considering the direct influence on each growing stage of the plant and not only. Climate change has led to strategies and specific measures for managing extreme events in recent years. More international meteo stations have continuous data specific to a larger or delimited area. However, modern technologies require more accurate records that are as close as possible to the crop, giving a trend to have in each specific point local meteo stations. This study compares the parameters recorded by the international/national and local meteo stations for a specific orchard in Bucharest, Romania. Dynamics of chilling hours, chilling portions, and Growing degree hours are also presented.

Key words: *phenology, temperature, GDH, CP, CH.*

INTRODUCTION

Temperature is one of the most controversial parameters studied in fruit-growing technologies, considering the direct influence on each growing stage of the plant and not only. Climate change has led to strategies and specific measures for managing extreme events (Benmoussa et al., 2020; Fernandez et al., 2021; 2023). More international meteo stations have continuous data specific to a larger or delimited area (Ecad.Eu, 2024; Luedeling et al., 2009). However, modern technologies require more accurate records that are as close as possible to the crop, giving a trend to have in each specific point local meteo stations. Modeling the correlation between tree-growing stages (Meier, 2018) and climate factors, especially temperatures, have been studied since the early XX century (Luedeling, 2012). Still, more accurate models have been elaborated and implemented in the last years due to technological and artificial intelligence advancements. Most of them focused on the accumulation of chilling hours by the trees, more of the traditional fruit-growing areas being threatened in the following years to change the cultivar's assortment with those with low chilling requirements (South Spain, Mediterranean area, etc.) (Drogoudi et al., 2023). Most of the algorithms include different analyses of chilling hours accumulation, widely used being the chilling hours (CH) model, the Dynamic model, and the heat accumulation Growing Degree Hours (GDH) model (Al Suwaid et al., 2023).

Worldwide, there is a network of meteo stations where historical data are available online and can be used in models (Ecad.Eu, 2024, R linked databases). Local stations are required for more precise analysis, but there are many cases when they are unavailable, or the recordings have gaps.

This study compared the parameter temperature recorded by international/national and local meteo stations for a specific orchard in Bucharest, Romania. It also presents the dynamics of chilling hours, chilling portions, and Growing degree hours for the studied periods.

MATERIALS AND METHODS

The international/national Bucharest Băneasa meteo station data for 1929-2023 were downloaded and analyzed (Ecad.Eu, 2024). Three local stations, coded Pessle, Enten, and Pinova, were compared for temperature data for the last four years.

Bucharest Băneasa, placed at the extremity of the city, at 4.3 km from the University campus, recorded only daily values for minimum, maximum, and average temperatures. Filling day gaps and transforming daily hourly data have been done with the chillR v. 0.75 package from R software. In the Experimental fields of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Pinova meteo station is situated in a region surrounded very closely by buildings, while Enten and Pessle stations, at 1 km distance, in a plainer field. All of them are in the Agronomie - Herastrau University campus, in the North-West part of Bucharest city (44º28'12", 26º03'51", 86 m altitude).

Daily and hourly temperatures were used for the local stations, and the chillR v. 0.75 package from R software was used to fill in gaps in days or hours.

Chilling Hours (CH), Chilling Portions (CP), and Growing Degree Hours (GDH) were determined using the same R package functions based on the hourly temperatures for all stations. Statistical analysis was performed with the R program (with RStudio 2024.04.2+764), and ANOVA with Tuckey posthoc tests were used for p<0.5 significance.

RESULTS AND DISCUSSIONS

Trends and dynamics in temperature at Bucharest-Băneasa international/national station

Annual mean temperature dynamics for maximum (Tmax), minimum (Tmin), and average (Tavg) values were compared statistically from 1929 to 2023 (Figure 1).

Annual average temperature (Tavg) fluctuated, ascending from 10 to 13ºC in the last years. Maximum yearly temperature (Tmax) ranged between 14-18ºC and ascended from 16 to 21ºC in the previous twenty years. Annual minimum temperature (Tmin) ranged between 3 to 7ºC, with a similar ascending trend in the last years.

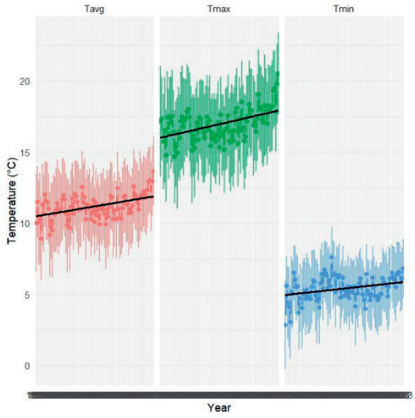

Figure 1. Average, maximum, and minimum temperatures in the 1929-2023 period

The analyzed data show that Bucharest's average temperature has increased over the last 23 years, with four years having values between 10-11ºC, 11 years with annual temperatures between 11 and 12ºC, six years between 12 and 13ºC, and the last 2 years between 13 and 14ºC. Compared to the previous periods, annual temperatures of 11-12ºC were present in all intervals, 12-13ºC one year from twenty, and none for 13-14ºC (Figure 2).

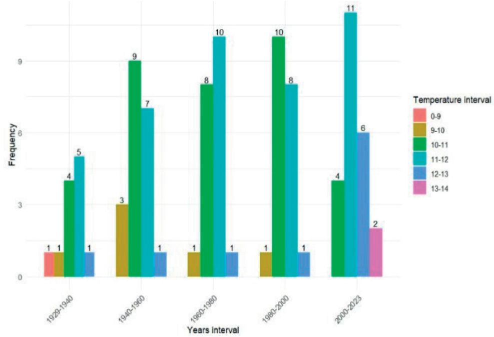

Figure 2. Frequencies of occurrences of average annual Tavg by temperature intervals and years

For monthly average temperature distributions, July and August had similar values for Tavg, Tmax, and Tmin. April and October also presented similar values. Multiannual monthly values are presented in Figure 3.

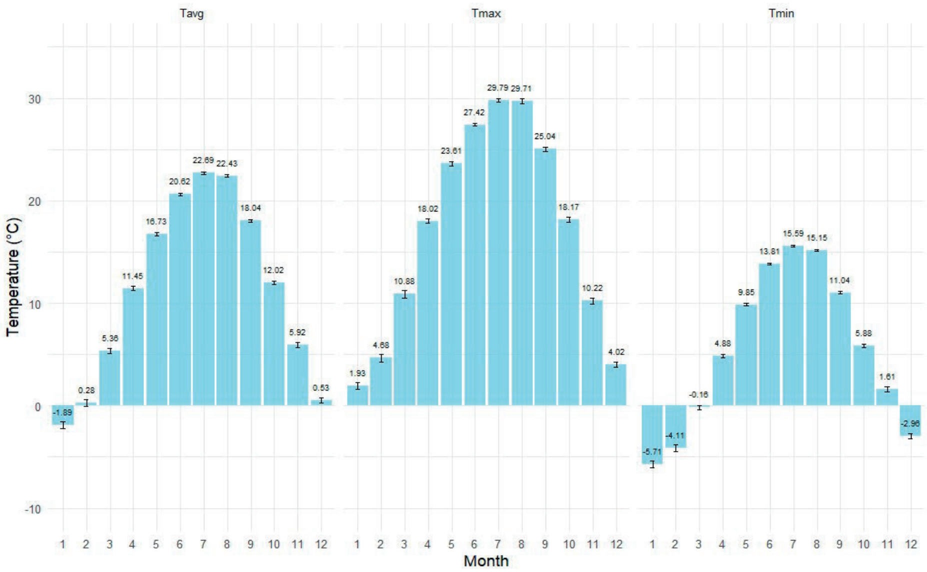

Figure 3. Monthly mean temperatures

Analyzing monthly temperature dynamics (Figure 4) from 1929 to 2023, it can be observed that some months, such as April, May, June, July, August, September, and October, presented a constant trend. However, January, February, and March had a distinct ascending trend, showing higher temperatures. In the last period, November and December had a slightly ascending trend, presenting more warmed months. The minimum (Tmin) and maximum (Tmax) followed the same pattern (Figure 5).

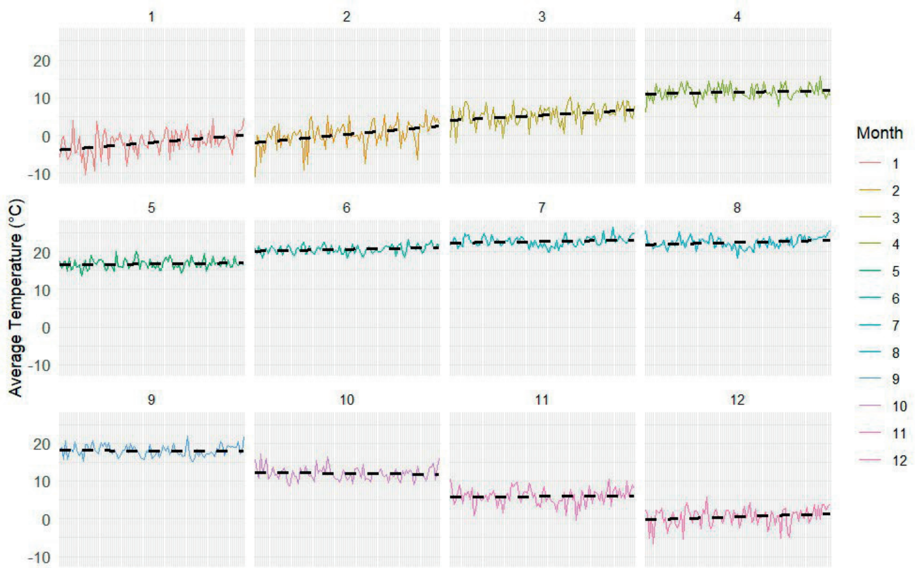

Figure 4. Monthly average temperature trends by year (1929-2023)

Figure 5. Evolution of extreme temperatures each month and year

An indicator directly influencing tree phenology is the days under 0ºC, between 0ºC and 7.2ºC, and above 7.2ºC (Figure 6). At the beginning of the interval, it can be observed that there were more than 50 days under 0ºC per year as usual; in the last 23 years, under 50 days were usual, and in the previous five years, 11-28 days were recorded under 0ºC. The interval of 0-7.2ºC of temperatures directly influences the dormancy break in trees, being relatively in a constant trend in the period. More than 7.2°C days were recorded in the last part of the period.

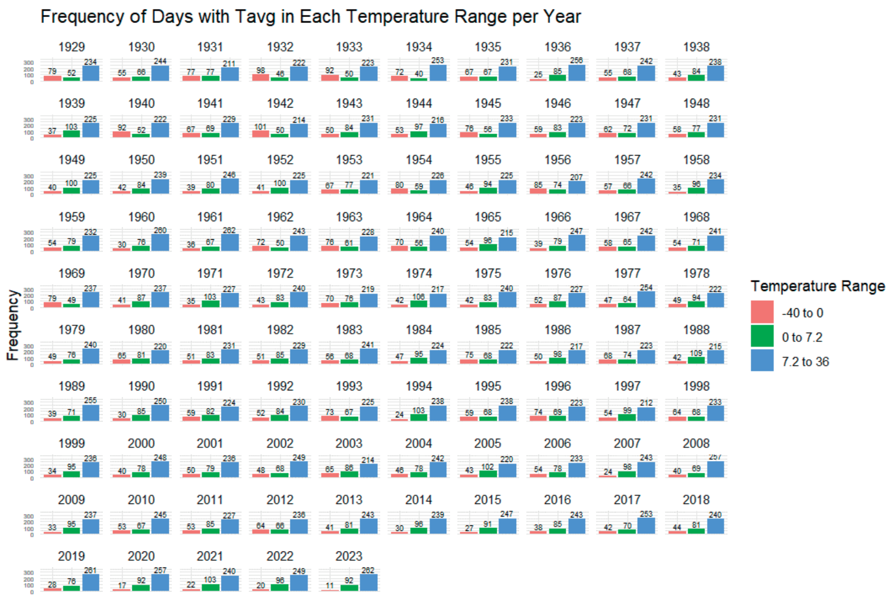

Figure 6. Frequency of Days with Tavg in each temperature range per year.

Temperature comparison between Bucharest-Băneasa international/national and local stations

When comparing annual temperatures between stations in the last four years, Tmax values were similar. Still, Tmin and Tavg presented differences between Băneasa and local stations (Figure 7), confirming the place's protective nature inside Bucharest. Annual monthly temperature followed the same pattern.

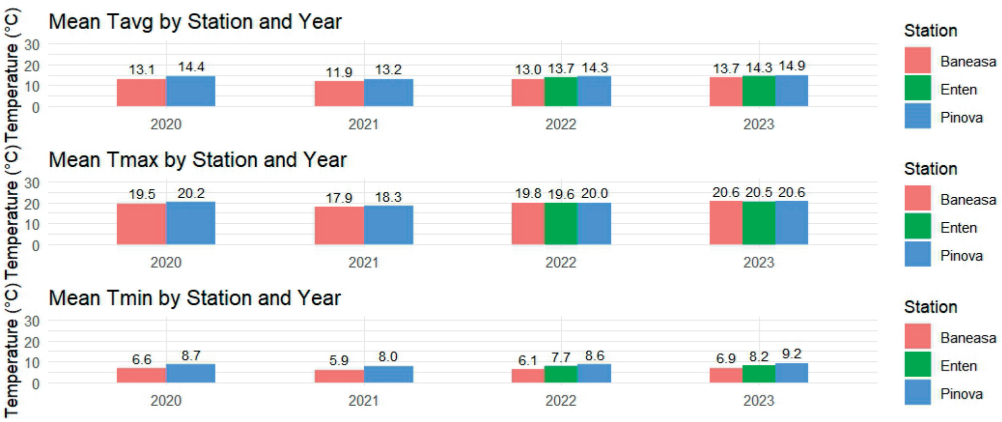

Figure 7. Annual Tavg comparison between stations

Hourly intervals in the day when minimum and maximum temperatures appear

Literature and most of the models take into consideration down intervals (4-6 am) for minimum temperature occurrence and 2 pm for maximum temperature of the day. In the analyzed data for the local stations, it was observed that Enten station presented the minimum in the day at 11 pm, 3 and 2 am, and maximum to 1 pm. Pessle station had a minimum at 5 and 6 am and a maximum at 3 pm. Pinova station recorded the minimum at 5 am, 4, and 6 am and the maximum at 3 and 2 pm (Figures 8-9).

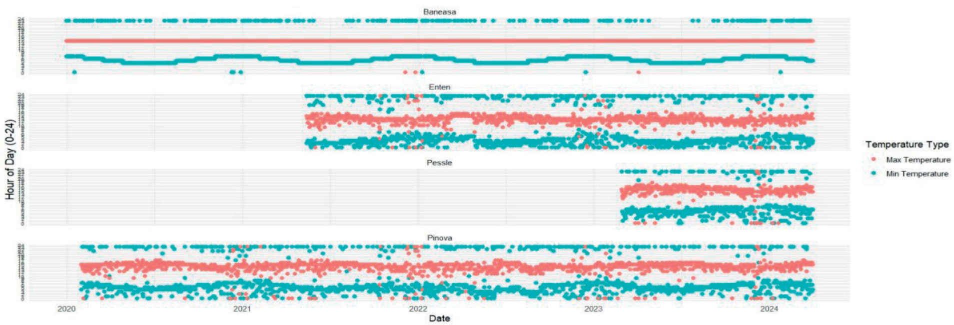

Figure 8. Times of minimum and maximum temperatures by stations

Figure 9. Times of minimum and maximum temperature frequency by stations

Chilling and Heat accumulation comparison

Analyzing the entire interval at the Băneasa station, the accumulation of Chilling Portions (CP) and Chilling Hours (CH) was within a range that assured sufficient chilling for the local temperate fruit species (Figures 10 and 11) correlated to (Fernandez et al., 2023). The cumulative growing degree hours (GDH) are presented in dynamics in Figure 12.

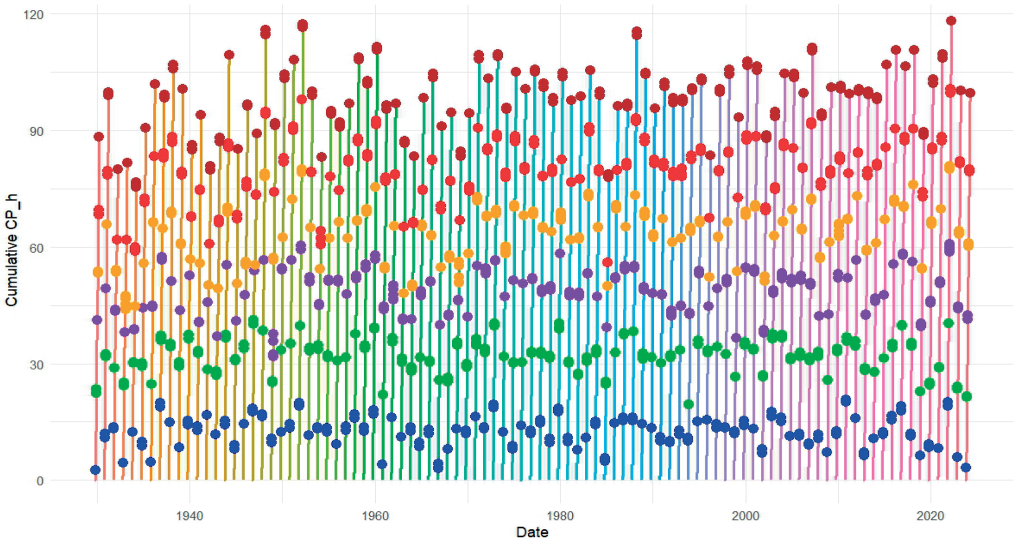

Figure 10. Cumulative CP (October-March) across years 1929-2024

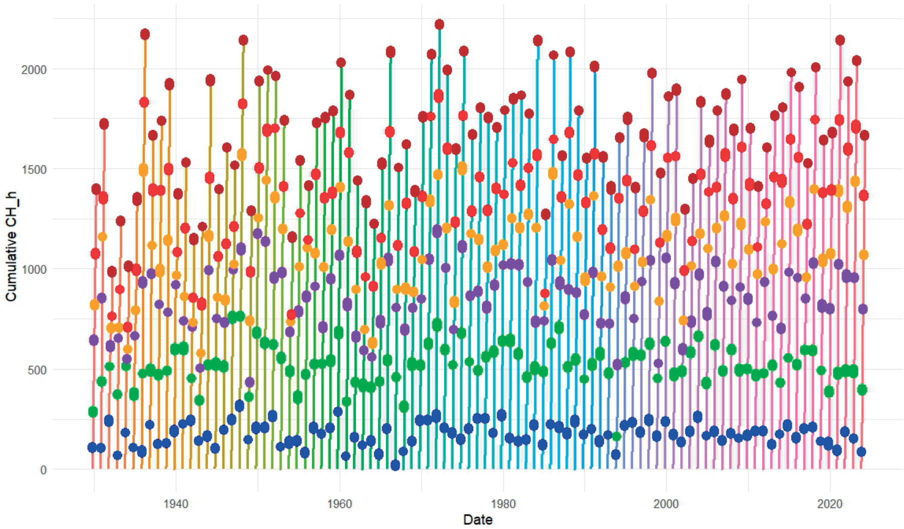

Figure 11. Cumulative CH (October-March) across years 1929-2024

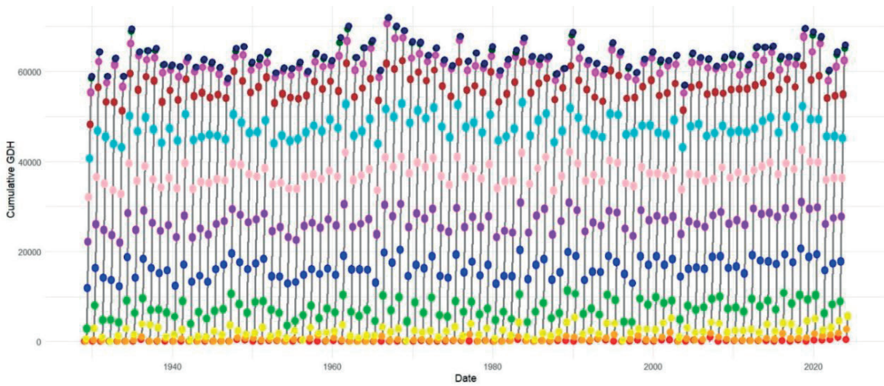

Figure 12. Cumulative GDH (January - December) across years 1929-2023

When comparing CP and CH between stations, all stations had similar values in the last four years (Figures 13 and 14).

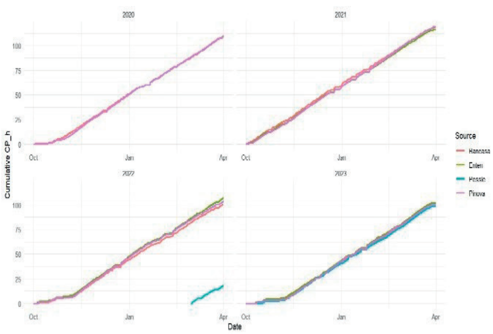

Figure 13. Cumulative CP (October-March) 2020-2023

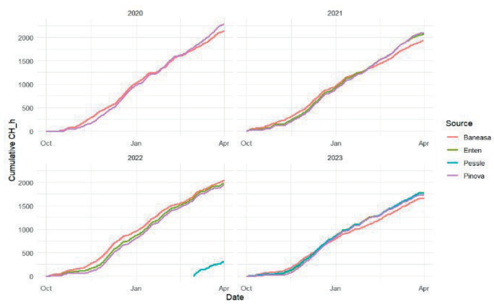

Figure 14. Cumulative CH (October-March) 2020-2023

For heat accumulation, expressed with the GDH parameter, there were differences between local stations and Băneasa stations, with higher values at the first ones (Figure 15).

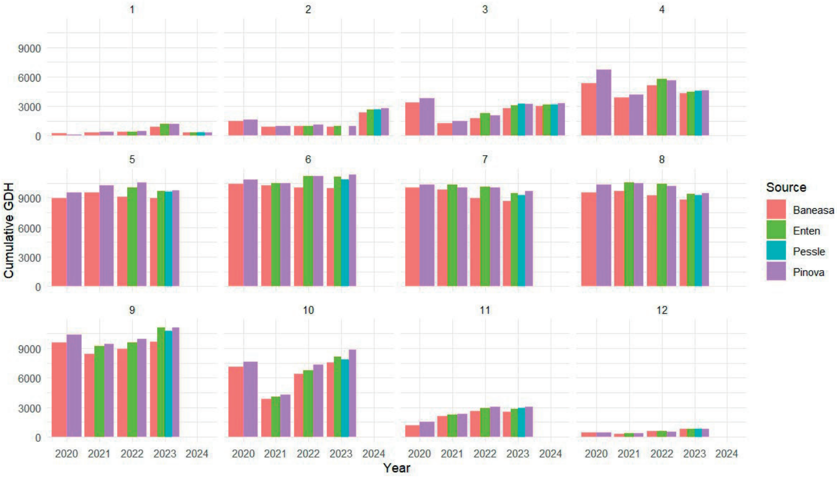

Figure 15. Comparing monthly cumulative GDH by year across stations

The number of tropical nights (with temperatures below 20 ºC, between 8 pm and 6 am) also differed between stations (Figure 16).

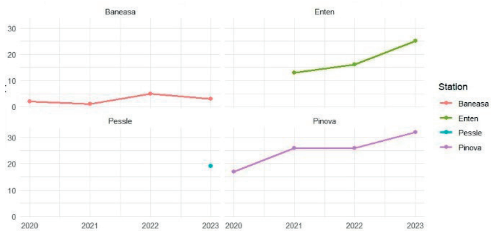

Figure 16. Tropical nights comparison between stations.

CONCLUSIONS

Climate change, including horticulture, has become a top priority on almost every agenda. The present study analyzed the similarities and differences between orchard proximity and local meteo stations in the temperature parameter, considering the influence on tree chilling and heat accumulation. The international meteo station, with almost 100 years of records, gave an image of the temperature changes. Until the 2000 year, the minimum, maximum, and, respectively, average annual temperature ranged in a specific interval. Since then, there has been an ascending trend, and there is a need to consider for the Bucharest area an average temperature of 11-12ºC and even 12-13ºC instead of 10-11ºC. Analyzing the monthly average values, an interesting finding was in the warm months, from April to October, where the temperature in time had a constant trend. January to March became warmer, and, with lower trends, November and December.

When temperature trends were compared between the international meteo station, placed 4 km from the orchard, and the local ones, maximum temperatures were similar, but minimum temperatures and the average were higher due to the city-protected effect.

Chilling accumulation quantity is essential in breeding programs, but not only. An overview of almost 100 years in the Bucharest region showed that there were no challenges with chilling hours/ portions necessary for the local species.

For the last years, all the stations showed similar chilling accumulation, but the local ones presented significantly higher values for heat accumulation (16%), which is essential for phenology models. Analyzing the monthly heat accumulation, February and March presented higher values in the last year, influencing earlier vegetation seasons.

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RESEARCH ON THE REALIZATION AND TESTING OF THE FERMENTATION CAPACITY OF NATURAL SOURDOUGH FORTIFIED WITH *SPIRULINA* POWDER

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Abstract

Bread is one of the most important product of cereal origin. It was, from ancient times, a worldwide basic food, and undoubtedly of great value to both human nutrition and the economy. Natural sourdough is a leavening agent that has many benefits, both in terms of bread quality (pleasant taste and aroma, high bioavailability of minerals, etc.) and consumer health. This paper presents the results of the research undertaken for the realization and testing of the fermentation capacity of natural sourdough fortified with Spirulina powder. For the fortification of natural sourdough with Spirulina powder, two levels of fortification were used (3% and 5%). Natural sourdough fortified with Spirulina (fortification level 5%) has the highest protein content (7.65%), total ash (0.74%), total polyphenols (124.95 mg GAE/100g), vitamin C (3.40 mg/100 g), mineral elements and the highest antioxidant capacity. The fermentation capacity of this sourdough was tested in the preparation of white bread, with very good results in terms of sensory and physico-chemical qualities.

***Key words**: sourdough, Spirulina, fortification, fermentation, bread*

INTRODUCTION

Bread is one of the most consumed fermented foods in the world, being a basic element in the people diet in many countries (Graça et al., 2021). One of the oldest ways of leavening bread is sourdough fermentation, transforming cereal flour into attractive, tastier and more digestible products (Graça et al., 2021; Fraberger et al., 2023).

Sourdough is a key element in traditional and artisan bread making (Hernández-Figueroa et al., 2024). Sourdough bread is obtained by spontaneous fermentation or inoculation of a mixed microbial culture, called sourdough starter. Sourdough starter is a mixture of water and flour fermented by yeasts and bacteria (usually lactic acid bacteria) which is traditionally used to produce bread, panettone, pancakes, pizza, cakes, cookies, buns and waffles (Albagli et al., 2021).

Sourdough fermentation is a technique that can use several types of flour, such as wheat, rye or other grains, and water. The oldest process of sourdough preparation is spontaneous fermentation and acidification due to the local microbiota, in a complex process of interaction, mainly between lactic acid bacteria and yeasts (Arora et al., 2021; De Vuyst and Neysens, 2005; Gänzle, 2014). Using sourdough to ferment the dough ensures a higher quality bread with health benefits. During fermentation, through the chemical reactions that take place, metabolites and flavor compounds result, which give the bread a superior sensory quality. At the same time, longer fermentation allows adjusting the gluten level, delaying starch digestibility and increasing the bioavailability of vitamins and minerals in the human body (Akamine et al., 2023).

During the fermentation of sourdough dough, various compounds such as organic acids, peptide compounds and exopolysaccharides are formed, which ensure the increase of the shelf life of the bread and the reduction of the growth rate of molds on its surface, preserving the quality characteristics of the product

(Chavan et al., 2011; Park et al., 2006; Corsetti et al., 2012; Luz et al., 2019). International studies have shown that aqueous extracts from fermented sourdoughs inhibit the growth of the main molds that can affect the quality of bread, and can be considered natural antimicrobials for bread and bakery products (Luz et al., 2019; Ryan et al., 2011; Samapundo et al., 2017; Hernández-Figueroa et al., 2023). Fermentation of sourdough dough results in bread with a lower glycemic index compared to traditional bread (Maioli et al., 2008; Fekri et al., 2018).
Also, the fermentation of the dough with sourdoughs lead to other advantages of the final product: the increase in the content of soluble fibers (Coda et al., 2014; Coda et al., 2017), the increase in the content of soluble phenolic compounds and the antioxidant capacity (Gobeti et al., 2019; Wang et al., 2019); phytate content decrease (Coda et al., 2015). Chavan et al. (2011) and Zou et al (2016) mention the fact that the use of sourdough as a leavening agent of the dough increases the bioavailability of minerals, the production of peptides with antioxidant activity and the increase of the shelf life of the bread.
This paper presents the results of the research undertaken for the realization and testing of the fermentation capacity of natural sourdogh fortified with *Spirulina* powder.

MATERIALS AND METHODS

Materials

To obtain the natural sourdough fortified with *Spirulina* powder the following materials were used: *Spirulina*, white wheat flour, whole wheat flour, rye flour and "Bucovina" still water.

Natural sourdough fortified with Spirulina powder-making

Natural sourdough fortified with *Spirulina* powder was obtained and tested at the Human Nutrition Laboratory in IBA Bucharest.
Firstly, experiments followed the achievement of control natural sourdough (C) by fermenting a mixture of white wheat flour type 650, whole wheat flour, rye flour and "Bucovina" still water. The experiments were carried out at room temperature, over 12 days. The control natural sourdough (C) was kept under refrigeration conditions (4-8°C) and fed in a ratio of 1:3:3 = natural sourdough: "Bucovina" still water: mix of white wheat flour and whole wheat flour, once every three days.
Secondly, experiments followed the achievement of natural sourdough, fortified with *Spirulina* powder (fortification levels were 3% and 5%, respectively). Thus, 3% and 5%, respectively, of the amount of wheat flour used to feed the control natural sourdough (C) was substituted with *Spirulina* powder. The fermentation of the culture obtained from the control natural sourdough (C), white wheat flour, whole wheat flour, *Spirulina* powder and "Bucovina" still water was carried out under refrigeration conditions (3-8°C), for 30 days. The culture was fed in a ratio of 1:3:3 = natural sourdough fortified with *Spirulina* powder: "Bucovina" still water: mix of white wheat flour whole wheat flour and *Spirulina* powder, once every three days, for a period of 30 days.

In Figure 1 are presented "Control Natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder" (V1-fortification level 3%; V2-fortification level 5%).

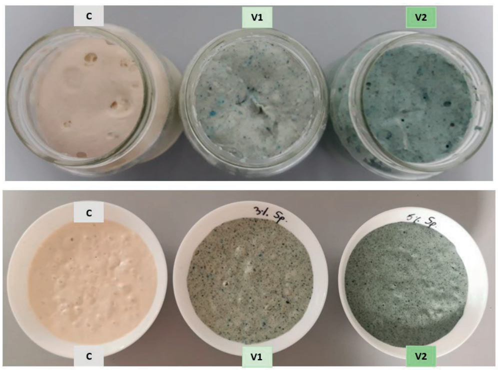

Figure 1. "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder" (V1 - fortification level 3%; V2 - fortification level 5%)

Testing the fermentation capacity of natural sourdough

The fermentation capacity of "Natural sourdough fortified with *Spirulina* powder" compared to that of "Control natural sourdough" (C) was tested by making the "White bread" product, using the biphasic process. For this purpose, the following technological operations were carried out: preparation of preferment, dough kneading, dough fermentation, dough division and intermediate shaping, final shaping, final leavening, baking, cooling, packaging, marking.

Figure 2. "White bread" (general aspect and section), prepared with "Control natural sourdough" (C)

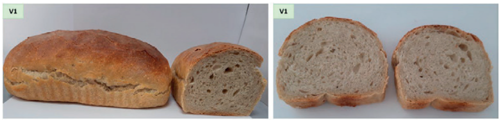

Figure 3. "White bread" (general aspect and section), prepared with "Natural sourdough fortified with *Spirulina* powder" (V1 - fortification level 3%)

Figure 4. "White bread" (general aspect and section), prepared with "Natural sourdough fortified with *Spirulina* powder" (V2- fortification level 5%)

Methods

Statistical Analysis

The samples were analyzed in triplicate, the results are presented as arithmetic mean and standard deviation.

Sensory analysis

Sensory analysis (appearance, taste, smell) was performed using the descriptive method.

The determination of instrumental color parameters (L*, a* and b*) was carried out using CM-5 colorimeter (Konica Minolta, Japan) with SpectraMagic NX software.

The determination of the texture parameters (firmness, elasticity) was carried out using the Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA) and Bluehill 3.13 software.

Physico-chemical analysis

Physico-chemical analysis was performed using the following methods: AOAC 979.09 (protein content), AOAC 923.03 (ash content), AOAC 985.29 (total dietary fiber), AOAC 963.15 (fat content), ACC 44-15A (moisture content).

Physical indicators (volume, porosity, elasticity) and acidity of the bread made with natural sourdough were determined with SR 91:2007 "Bread and fresh pastry products. Methods of analysis". Total carbohydrate content was analytically determined using the following formula: Total carbohydrate (%) = 100 – moisture (%) – ash (%) – protein (%) – fat (%).

Energy analyses (expressed in kcal/100 g and kJ/100 g) was carried out according to the provisions of Commission Regulation no. 1169/2011 (European Commission, 2011).

Bioactive compounds content

Total polyphenol content was performed by extracting the sample in a methanol:water = 1:1 mixture, applying the Folin-Ciocalteau spectrophotometric method, using UV-VIS Jasco V 550 spectrophotometer (Horszwald and Andlauer, 2011). Determination of the absorbance of the extracts was performed at wavelength λ = 755 nm and a gallic acid calibration curve was used, in the concentration range of 0-0.20 mg/mL. β-carotene content was determined using a chromatographic method (Catană et al., 2020). Vitamin C content was determined using a chromatographic method (Asănică et al., 2019).

Antioxidant capacity

The antioxidant capacity was determined by applying the DPPH spectrophotometric method Horszwald and Andlauer (2011). The method is based on measuring the ability of antioxidants to scavenge stable radicals. The free radical DPPH (1,1diphenyl-2-picryl hydrazyl) is reduced to the corresponding hydrazine, when reacting with hydrogen donors, and this stability is visible by the discoloring test, which evaluates the decrease in absorbance at 517 nm produced by the addition of the antioxidant to the solution of DPPH in methanol. UV-VIS Jasco V 550 spectrophotometer and calibration curve of Trolox (0-0.4375 mmol/L) were used.

Microbiological analysis

The microbiological parameters were determined by using the following methods: SR ISO 21527-1:2009 (Yeasts and molds), SR EN ISO 21528-1:2017 (*Enterobacteriaceae*), SR ISO 15214/2001 (Lactic acid bacteria).

RESULTS AND DISCUSSIONS

Sensory analysis

Following the sensory analysis, it was found that the "Control Natural Sourdough" (C) is like a fermented acid dough, with an aerated appearance, white-yellowish and has a pleasant taste and smell, specific to wild yeasts and lactic bacteria. At the same time, the sensory analysis of "Natural sourdough fortified with *Spirulina* powder" revealed that has the form of a fermented acid dough, with an aerated appearance, gray-green to light green colour and a pleasant, specific taste and smell.

Following the sensory analysis of the breads made with "Control natural sourdough" (C), respectively with "Natural sourdough fortified with *Spirulina* powder" it was found that they are well leavened, have an elastic, dense core and present a pleasant taste and smell/aroma, characteristic of well-baked bread. It is noteworthy that the addition of *Spirulina* to natural sourdought did not affect the taste and smell/aroma of bread made with this natural leavening agent.

Following the instrumental analysis of the color (Figure 5), it was found that the addition of powder *Spirulina* to the composition of the natural leaven caused a slight darkening of the color of the core of the breads made with this natural fermentation agent, a fact that is reflected in the decrease of the luminance value L*. Thus, the bread made with "Control natural sourdough" (C), had the luminance L* = 75.37, and the bread made with "Natural sourdough fortified with *Spirulina* powder" (fortification level 5%) had the luminance L* = 74 ,44.

At the same time, the addition of powder *Spirulina* in the composition of natural sourdough caused a decrease in the values recorded by the parameters a* (red-green color coordinate) and b* (yellow-blue color coordinate).

The luminance L* of the breads made with "Control natural sourdough" (C), respectively with "Natural sourdough fortified with *Spirulina* powder" is lower compared to that reported by Illueca et al. (2023) in the case of bread made with sourdough (L* = 86.30).

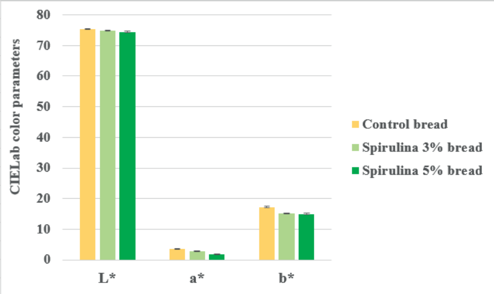

Figure 5. Color parameters of the breads prepared with "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder" (V1 - fortification level 3%; V2 - fortification level 5%)

The textural properties of the breads made with "Control natural sourdough" (C), respectively with "Natural sourdough fortified with *Spirulina* powder" packed in a polypropylene bag, for 7 days from the date of manufacture, are presented in Table 1.

Table 1. The textural properties of breads made with “Control natural sourdough” (C) and “Natural sourdough fortified with *Spirulina* powder”

Bread	Period (days)	Firmness (N)	Elasticity
Control bread	1	5.40±0.16	1.00±0.01
	2	5.83±0.14	1.00±0.01
	3	6.59±0.16	1.15±0.01
	6	6.89±0.19	1.17 ±0.03
	7	7.09±0.21	1.18 ±0.03
Spirulină 3% bread	1	5.29±0.08	0.99±0.01
	2	5.42±0.09	0.99±0.01
	3	5.50±0.08	1.07±0.06
	6	5.56±0.09	1.08±0.05
	7	5.67±0.09	1.10±0.07
Spirulină 5% bread	1	5.37±0.05	0.98±0.00
	2	5.51±0.06	1.00±0.00
	3	5.67±0.06	1.03±0.01
	6	5.78±0.07	1.06±0.03
	7	5.84±0.09	1.09±0.03

According to the results, the addition of *Spirulina* powder in the composition of natural sourdough causes a decrease in the firmness of bread prepared with this natural fermentation agent, compared to Control bread. Thus, after 7 days from the date of manufacture, these bread samples had a firmness of 5.67 N, respectively, 5.84 N, compared to Control bread which had a firmness of 7.09 N. The firmness correlates sensorially with the softness of the core: a high firmness represents a denser, harder core. The elasticity of the core was not influenced by the fortification with *Spirulina* powder of the natural sourdough.

Physico-chemical analysis

The physico-chemical composition of "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder" is presented in Table 2.

Table 2. The physico-chemical indicators of the "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder"

Physico-chemical indicators	„Control natural sourdough" (C)	„Natural sourdough fortified with *Spirulina* powder"	
		V1- *Spirulina* 3%	V2- *Spirulina* 5%
Moisture (%)	49.34±1.23	48.76±1.22	48.88±1.22
Acidity (degrees)	11.0±0.17	13.0±0.20	14.6±0.22
Ash (%)	0.36±0.006	0.52±0.009	0.69±0.01
Protein (%)	5.95±0.09	6.74±0.10	7.28±0.11

Fortification with *Spirulina* of natural sourdough increases acidity, protein and ash content. Thus, "Natural sourdough fortified with *Spirulina* powder" has 1.44-1.92 times higher ash content, 1.13-1.22 times higher protein content and 1.18-1.33 times higher acidity, compared to "Control natural sourdough" (C). The "Natural sourdough fortified with *Spirulina* powder" has a protein content higher than that reported by Burnete et al. (2019), in the case of sourdough enriched in phenolic compounds and inulin (Protein = 5.83%). *Spirulina* is an important source of proteins, minerals, vitamins, antioxidants and polyunsaturated fatty acids Janda-Milczarek et al. (2023).

The physico-chemical indicators of the breads made with "Control natural sourdough" (C), respectively, with "Natural sourdough fortified with *Spirulina* powder" are presented in Table 3.

The physico-chemical analysis revealed that the white bread prepared with "Natural sourdough fortified with *Spirulina* powder" (V2-fortification level 5%) recorded the highest values of the physico-chemical indicators: volume (297 cm^3/100 g), porosity (77.85%), ash (0.60%), protein (8.85%). Also, this bread recorded the lowest value for the carbohydrate content (47.88%), respectively, available carbohydrates (47.28%). The volume of white bread prepared with "Natural sourdough fortified with *Spirulina* powder" is higher than that reported by Burnete et al. (2020) in the case of white bread prepared with natural sourdough (Volume = 272 cm^3/100 g), respectively that reported by Plessas et al. (2023) in the case of breads prepared with sourdough with the addition of freeze-dried *L. plantarum* (1-3%) (Volume = 245-252 cm^3/100 g).

Table 3. The physico-chemical indicators of breads made with "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder"

Physico-chemical indicators	Control bread	*Spirulina* 3% bread	*Spirulina* 5% bread
Nominal mass (%)	0.421±0.002	0.423±0.002	0.425±0.002
Volume (cm^3/100g)	275±1.38	289±1.44	297±1.49
Porosity (%)	71.30±0.29	73.30±0.29	77.85±0.31
Elasticity (%)	95±0.10	97±0.10	97±0.10
Moisture (%)	41.47±1.04	41.62±1.04	41.85±1.05
Acidity (degrees)	3.10±0.05	3.30±0.05	3.50±0.05
Ash (%)	0.47±0.007	0.52±0.008	0.60±0.009
Protein (%)	7.67±0.12	8.23±0.12	8.85±0.13
Fat (%)	0.76±0.01	0.80±0.01	0.82±0.01
Carbohydrates (%)	49.63±0.55	48.83±0.54	47.88±0.53
Available carbohydrates (%)	49.12±0.54	48.27±0.53	47.28±0.52
Total fiber (%)	0.51±0.008	0.56±0.009	0.60±0.01
Energy value (kcal/100g)	235	234	233
Energy value (kJ/100g)	998	995	989

The protein content of white bread prepared with "Natural sourdough fortified with *Spirulina* powder" is comparable to that reported by Lazo-Vélez et al. (2021) in the case of breads prepared with selenized chickpea sourdoughs (Protein = 8.89-8.98%). Also, this bread has a carbohydrate content, respectively, available carbohydrates, lower than that reported by Burnete et al. (2020) in the case of white bread prepared with natural sourdough (Carbohydrates = 49.79%, Available carbohydrates = 49.21%) and compared to that reported by Lazo-Vélez et al. (2021) in the case of breads prepared with selenized chickpea sourdoughs (Carbohydrates = 55.09-55.88%).

Bioactive compounds content

The content in bioactive compounds of "Natural sourdough control" (C) and "Natural sourdough fortified with *Spirulina* powder" is presented in Table 4. According to the results, "Natural sourdough fortified with *Spirulina* powder" (V2-fortification level 5%) recorded the highest values of total polyphenols (120.60 mg GAE/100 g), vitamin C (3.40 mg/100 g) and β-carotene (8.54 mg/100 g).

The total polyphenols content of the breads made with "Control natural sourdough" (C), respectively with “Natural sourdough fortified with *Spirulina* powder” is presented in Table 5.

Table 4. Bioactive compounds content of the “Control natural sourdough” (C) and “Natural sourdough fortified with *Spirulina* powder”

Bioactive compounds	„Control natural sourdough” (C)	„Natural sourdough fortified with *Spirulina* powder”	
		V1- *Spirulina* 3%	V2- *Spirulina* 5%
Total polyphenols (mg/100g)	55.23±1.38	109.21±2.73	120.60±3.01
Vitamin C (mg/100g)	Nd*	2.12±0.06	3.40±0.10
β-carotene (mg/100g)	Nd*	5.15±0.12	8.54±0.20

*Undetectable

Table 5. Total polyphenols content of the breads made with “Control natural sourdough” (C) and “Natural sourdough fortified with *Spirulina* powder”

Bioactive compound	Control bread	*Spirulina* 3% bread	*Spirulina* 5% bread
Total polyphenols (mg/100g)	21.75±0.54	35.44±0.88	46.67±1.16

White bread prepared with "Natural sourdough fortified with *Spirulina* powder" (V2 - fortification level 5%) recorded the highest values of total polyphenols content (46.67 mg GAE/100 g). The total polyphenol content of this bread is comparable to that reported by Plessas et al. (2023) in the case of bread with sourdough with the addition of freeze-dried *L. plantarum* (1%) (Total polyphenols content = 49 mg GAE/100 g), but lower compared to that reported by the same author in the case of bread prepared with sourdough with freeze-dried *L. plantarum* (1-3%) and freeze-dried pomegranate juice (6%) (Total polyphenols content = 54.4-90.1 mg GAE/100 g).
International epidemiological studies (Dini and Grumetto, 2022) suggested that the introduction in to the diet and the long-term consumption of foods containing polyphenols protect against cancer, osteoporosis, cardiovascular diseases, diabetes and neurodegenerative diseases. Bread has been a basic element in the human diet for thousands of years, being one of the most consumed foods in the world (Papadimitriou et al., 2019) and obtaining some types of bread, enriched in polyphenols, using "Natural sourdough fortified with *Spirulina* powder", is of real interest.

Antioxidant capacity

Due to its antioxidant content (polyphenols, vitamin C, β-carotene), "Natural sourdough fortified with *Spirulina* powder" has antioxidant capacity. The highest value of the antioxidant capacity was recorded in the case of "Natural sourdough fortified with *Spirulina* powder", V2 - fortification level 5% (Figure 6).

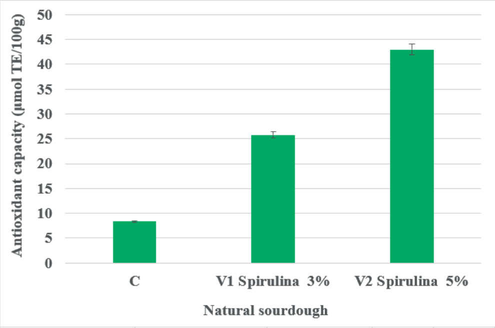

Figure 6. Antioxidant capacity of the “Control natural sourdough” (C) and “Natural sourdough fortified with *Spirulina* powder”

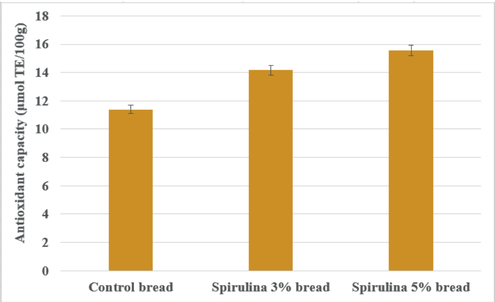

Figure 7. Antioxidant capacity of the breads prepared with "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder" (V1 - fortification level 3%; V2 - fortification level 5%)

Antioxidant capacity of the breads prepared with “Natural sourdough fortified with *Spirulina* powder” (Figure 7) is comparable with that reported by Burnete et al. (2019), for the white bread prepared with natural sourdough enriched in phenolic compounds and inulin (Antioxidant capacity = 13.68-14.8 µmol TE/100 g).

Microbiological analysis

The microbiological indicators of the “Control natural sourdough” (C) and “Natural sourdough fortified with *Spirulina* powder” are presented in Table 6.
The concentrations of yeasts, respectively lactic bacteria of "Natural sourdough fortified with *Spirulina* powder" are comparable to those

reported by Burnete et al. (2019), in the case of natural sourdough enriched in phenolic compounds and inulin: Yeasts = 2.8 x 10^6 CFU/g; Lactic acid bacteria = 1.7 x 10^8 CFU/g.

Table 6. The microbiological indicators of the “Control natural sourdough” (C) and “Natural sourdough fortified with *Spirulina* powder”

Microbiological indicators	„Control natural sourdough” (C)	„Natural sourdough fortified with *Spirulina* powder”	
		V1- *Spirulina* 3%	V2- *Spirulina* 5%
Yeast (CFU/g)	1.5x10^5	5x10^5	3x10^6
Lactic acid bacteria (CFU/g)	7.5x10^6	8x10^7	2.2x10^8
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10

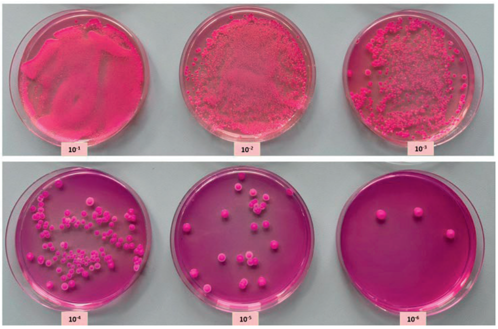

Figure 8. Yeasts on DRBC medium for the “Natural sourdough fortified with *Spirulina* powder” (V2 -fortification level 5%)

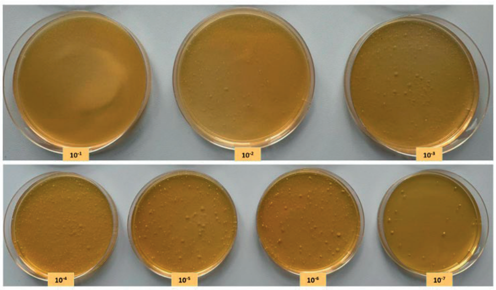

Figure 9. Lactic acid bacteria for the “Natural sourdough fortified with *Spirulina* powder” (V2 - fortification level 5%)

Based on microbiological analysis, in the case of "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder", yeasts of the genus *Saccharomyces cerevisiae* and *Zygosaccharomyces* spp. and lactic acid bacteria belonging to the genera *Lactobacillus* were identified.

Following the microbiological analysis of bread samples prepared with "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder" (V1 - fortification level 3%; V2 - fortification level 5%) packed in polypropylene bags, it was found that they fall within the provisions of the legislation in force even 9 days after the date of manufacture (Table 7).

Table 7. The microbiological indicators of breads with "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder"

Product	Yeast and molds (CFU/g)			*Enterobacteriaceae* (CFU/g)		
	2 days	5 days	9 days	2 days	5 days	9 days
Control bread	< 10	< 10	< 10	< 10	< 10	< 10
Spirulina 3% bread	< 10	< 10	< 10	< 10	< 10	< 10
Spirulina 5% bread	< 10	< 10	< 10	< 10	< 10	< 10

The microbiological stability of the bread samples is due to the acidity conferred by the use in their composition of "Control natural sourdough" (C) and "Natural sourdough fortified with *Spirulina* powder". Also, it should be mentioned that in the case of breads prepared with "Natural sourdough fortified with *Spirulina* powder", their microbiological stability is due to the antimicrobial activity of *Spirulina* powder. Recent international research has demonstrated the antioxidant and antimicrobial activity of extracts obtained from *Spirulina* (Abdel-Moneim et al., 2022).

CONCLUSIONS

“Natural sourdough fortified with *Spirulina* powder” (fortification level 3% and 5% *Spirulina*) stands out for the content in protein (6.74-7.28%), ash (0.52-0.69%), polyphenols (109.21-120.60 mg GAE/100 g), vitamin C (2.12-3.40 mg/100 g) and β-carotene (5.15-8.54 mg/100 g) and has antioxidant capacity (25.81-42.95 μmol TE/100 g). Also, “Natural sourdough fortified with *Spirulina* powder” is a natural dough fermentation agent, notable for its content in yeast (5 x 10^5-3 x 10^6) and lactic acid bacteria (8 x 10^7-2.2 x 10^8).

The fermentation capacity of "Natural sourdough fortified with *Spirulina* powder" was tested in the preparation of white bread, applying the biphasic process, obtaining products with superior sensory qualities and corresponding physico-chemical indicators. Thus, white bread prepared with "Natural sourdough fortified with *Spirulina* powder" (fortification level with 5%) has an elastic, dense core, pleasant taste and smell/aroma, has the largest volume (297 cm^3/100g), the highest protein content (8.85%), ash (0.60%), total polyphenols (46.67 mg GAE/100 g) and the highest antioxidant capacity (15.56 µmol TE/100 g).

"Natural sourdough fortified with *Spirulina* powder" can be considered a natural preservation agent, since the bread prepared with this fermentation agent, from a microbiological point of view, falls within the provisions of the legislation in force, and can be consumed up to 9 days after the date of manufacture.

Considering the results from this experimental study, "Natural sourdough fortified with *Spirulina* powder" can be successfully used for dough fermentation, in the case of bread and bakery products.

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RESEARCH ON THE FORTIFICATION OF A PASTRY PRODUCT WITH CAULIFLOWER LEAVES POWDER TO INCREASE NUTRITIONAL QUALITY AND ANTIOXIDANT POTENTIAL

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Abstract

Considering the exponential population growth and climate change, it is necessary to develop strategies to produce more foods with high nutritional value and antioxidant capacity, with less waste, using sustainable food production systems. This paper presents the results of the research carried out for the fortification of cakes with cauliflower leaves powder (fortification levels in the range of 4-23%). The fortified cakes have superior sensory qualities and are notable for their protein content (15.16-16.06%), total fiber (6.04-6.92%), total ash (1.89-2.30%), total polyphenols (92.85-157.15 mg GAE/100 g), glucosinolates (117.21-647.87 mmol/100 g), α-Tocopherol (0.638-0.682 mg/100 g), β-Carotene (0.24-1.43 mg/100 g), chlorophyll a (1.09-5.75 mg/100 g), chlorophyll b (1.76-10.06 mg/100 g). At the same time, the cakes fortified with cauliflower leaves powder have antioxidant potential (215.34-364.23 mg Trolox Equivalents/100 g). Due to their high nutritional value and antioxidant potential, these pastries are beneficial in a healthy diet, but also in the prevention and diet therapy of nutritional deficiencies and diseases caused by oxidative stress.

Key words: *cauliflower, leaves, cake, antioxidant potential.*

INTRODUCTION

International studies highlighted the fact that plant by-products are important sources of nutrients and bioactive compounds, with a high potential for use in the development of fortified food products (Amoah et al., 2023; San José et al., 2018; Tamasi et al., 2019). Cauliflower by-products (leaves, stems, stalks) are the most important from this point of view, as they are important sources of proteins, mineral elements, dietary fibers and bioactive compounds (vitamin C, polyphenols, glucosinolates, carotenoids), chlorophyll a and b (Montone et al., 2018; Munir et al., 2018; Drabińska et al., 2021).
Caliceti et al. (2019) mention that cauliflower leaves contain bioactive peptides exhibiting antioxidant properties, which may improve the viability of human vascular endothelial cells. At the same time, these authors highlight the fact that cauliflower by-products can be used as protein sources.
Due to the content in bioactive compounds and peptides, cauliflower by-products have antioxidant, anti-inflammatory and anticarcinogenic effects (Montone et al., 2018; Munir et al., 2018; Stojceska et al., 2008). At the same time, Sanlier & Guler (2018) highlight the fact that cauliflower by-products contain very high concentrations of glucosinolates up to 75,000 μg/g fresh weight.
Tukassar et al. (2023) made cauliflower by-products powder and characterized qualitatively this powder, which has a moisture content of 5.50% and stands out for its protein content (22.80%), crude fiber (15.32%), ash (5.81%), carbohydrates (47.62%), magnesium (36.3 mg/100 g), iron (20.03 mg/100 g), zinc (1.46 mg/100 g), total polyphenols (537.40 mg GAE/100 g), total flavonoid (252.20 mg CE/100 g). Catana et al. (2023) made a functional ingredient (powder) from cauliflower leaves that stands out for its content in protein (min. 28.5%), total fiber (min. 25%), ash (min. 10%), total sugars (min. 17.5%), mineral elements (potassium min. 3750 mg/ 100 g; magnesium: min. 110 mg/100 g; sodium: 475.58-525.73 mg/100 g; calcium:

min. 750 mg/100 g; iron: min. 40 mg/100 g; zinc: min. 5 mg/100 g), bioactive compounds (total polyphenols min. 5.5 mg GAE/g s.u.; vitamin C min. 70 mg/100 g; β-carotene min. 30 mg/100 g; glucosinolates min. 220 mmol/g.d.m.; lutein min. 10 mg/100 g).

Nartea et al. (2023) obtained flour from cauliflower leaves and stalks, using in their experiments two varieties: *Cheddar* (orange) and *Depurple* (purple). These flours rich in nutrients and bioactive compounds (glucobrassicin, carotenoids, phytosterols) were used for fortification (fortification levels 10% and 30%) of some pizza assortments. Pizza prepared with flour from violet stalks showed the highest glucobrassicin content (8.4 mg/ 200 g portion). Pizza prepared with orange stalks had the highest total content of phytosterols, a portion of 200 g providing 5.8% of the health claims imposed by EFSA (2009), for this class of bioactive compounds. Pizza prepared with cauliflower leaves flour had the highest content in β-carotene (4.09 mg/100 g d.w. of pizza) and lutein (3.04 mg/100 g d.w. of pizza).

This paper presents the results of the research carried out for the fortification of cakes with cauliflower leaves powder (fortification levels in the range of 4-23%).

MATERIALS AND METHODS

Materials

Cauliflower leaves powder was made within the Vegetable-Fruit Processing Pilot Experiment Station, from IBA Bucharest. To obtain the product "Minicake fortified with cauliflower leaf powder" the following ingredients were used: cauliflower leaves powder, white wheat flour, apple waste powder, hemp seeds, eggs, brown sugar, dehydrated cranberries, sunflower oil, cinnamon sugar, vanilla essence, baking powder and sea salt. The ingredients have been carefully selected, so that both their sensory and nutritional qualities, as well as the synergism of the bioactive compounds, are exploited. In parallel with the fortified product, the product "Control Minicake" (non-fortified minicake with cauliflower leaves powder) was also produced.

Minicake-making

The technological flow to obtain the products "Control Minicake" and "Minicake fortified with cauliflower leaf powder" includes the following operations: preparation of raw materials and auxiliary materials, dough preparation, dosing, baking, cooling, packaging, labeling and storage. To obtain the fortified product, the following fortification levels with cauliflower leaves powder were used: 4% (V1), 9% (V2), 18% (V3) and 23% (V4). Figure 1 shows cauliflower leaves powder.

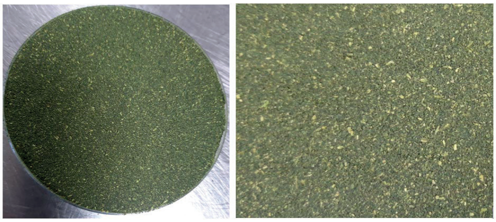

Figure 1. Cauliflower leaves powder

Figure 2. “Control Minicake” product

Figure 3. “Minicake fortified with cauliflower leaves powder” product

Methods

Statistical Analysis

“Control Minicake” and “Minicake fortified with cauliflower leaves powder” were analyzed in triplicate, the results beeing presented as arithmetic mean and standard deviation.

Sensory analysis

Sensory analysis (appearance, taste, color, smell, consistency) was carried out using the descriptive method and "Method of comparison with unit score scales" (Burnete et al., 2020).

The determination of instrumental color parameters (L*, a* and b*) was carried out using CM-5 colorimeter (Konica Minolta, Japan) with SpectraMagic NX software.

The determination of the texture parameters (firmness elasticity, cohesiveness and guminess) was carried out using the Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA) and Bluehill 3.13 software.

Physico-chemical analysis

The physical-chemical analysis was carried out using SR 91:2007 "Bread and fresh pastry products. Methods of analysis". Total fiber content was determined according to AOAC method 985.29. Total carbohydrate content was analytically determined using the following formula: Total carbohydrate (%) = 100 – moisture (%) – ash (%) – protein (%) – fat (%).

The determination of the energy value (expressed in kcal/100 g and kJ/100 g) was carried out according to the provisions of Commission Regulation no. 1169/2011 (European Commission, 2011).

The determination of the minerals was carried out by dry digestion of the samples and by high-performance analytical techniques: High-Resolution Continuum Source Atomic Absorption Spectrometry (in the case of magnesium, sodium, calcium and potassium) and Inductively Coupled Plasma Mass Spectrometry (in the case of manganese, zinc iron and copper).

Bioactive compounds content

Total polyphenol content was performed by extracting the sample in a methanol:water = 1:1 mixture, applying the Folin-Ciocalteau spectrophotometric method, using UV-VIS Jasco V 550 spectrophotometer (Horszwald and Andlauer, 2011). Determination of the absorbance of the extracts was performed at wavelength λ = 755 nm and a gallic acid calibration curve was used, in the concentration range 0-0.20 mg/mL. The content of total carotenoids, chlorophyll a and chlorophyll b was determined using a spectrophotometric method (Chinnadurai al., 2013). β-carotene content was determined using a chromatographic method (Catană et al., 2020). Vitamin E (α-tocopherol) content was determined using a chromatographic method (Popović et al., 2015). Determination of the total glucosinolates content was carried out using a spectrometric method (Mawlong et. al., 2017).

Antioxidant capacity

The antioxidant capacity was determined by applying the DPPH spectrophotometric method Horszwald and Andlauer (2011). The method is based on measuring the ability of antioxidants to scavenge stable radicals. The free radical DPPH (1,1diphenyl-2-picryl hydrazyl) is reduced to the corresponding hydrazine, when reacting with hydrogen donors, and this stability is visible by the discoloring test, which evaluates the decrease in absorbance at 517 nm produced by the addition of the antioxidant to the solution of DPPH in methanol. UV-VIS Jasco V 550 spectrophotometer and calibration curve of Trolox (0-0.4375 mmol/L) were used.

Microbiological analysis

The microbiological parameters were determined by using the following methods: SR ISO 21527-1:2009 (Yeasts and molds), SR EN ISO 21528-1:2017 (*Enterobacteriaceae*), SR ISO16649-2:2007 (*Escherichia coli*), SR EN ISO 6579-1:2017 (*Salmonella*), SR EN ISO 6888-1:2021/A1:2023 (*Staphylococcus coagulase* positive), ISO 21807:2004 (Water activity).

RESULTS AND DISCUSSIONS

Sensory analysis

Following the sensory analysis, it was found that the addition of cauliflower leaf powder (fortification levels of 4%, 9% and 18%) in the composition of the minicakes does not affect their taste and smell/aroma. Thus, minicakes fortified with cauliflower leaf powder, in the specified concentrations, have a pleasant taste and smell/aroma, characteristic of a well-baked pastry product, with the addition of cinnamon sugar, vanilla essence, dried cranberries and cauliflower leaf powder. At the same time, the product "Minicake fortified with cauliflower leaf powder" has a dark-brown uniform crust, and an elastic, dense core, with uniform pores, characteristic of a well-baked flour product and characteristic green color (in the core you can distinguish fragments of dehydrated cranberries burgundy color). Following the sensory analysis, it was found that the addition of cauliflower leaf powder in the composition of the minicakes, corresponding to 23% fortification level, affects their taste and smell/aroma. Following the sensory evaluation, using the "Method of comparison with unit scoring scales", the products "Control Minicake" and "Minicake fortified with cauliflower leaf powder" (V1, V2, V3) obtained the qualification "very good", recording the following scores (Figure 4):

- ✓ "Control Minicake": 19.68 points
- ✓ "Minicake fortified with cauliflower leaf powder" (V1): 19.60 points
- ✓ "Minicake fortified with cauliflower leaf powder" (V2): 19.52 points
- ✓ "Minicake fortified with cauliflower leaf powder" (V3): 19.60 points.

The experimental variant V4 (fortification level 23%) of the product "Minicake fortified with cauliflower leaf powder" obtained the qualification "satisfactory", registering 14.4 points.

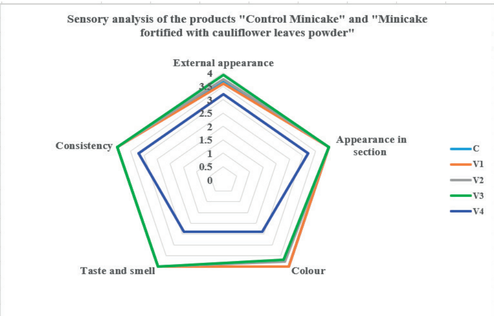

Figure 4. Sensory analysis of the products "Control Minicake" and "Minicake fortified with cauliflower leaves powder"

Following the instrumental analysis of the color, it was found that the addition of cauliflower leaf powder, in the composition of the minicakes, cause the green color of their core, which is reflected in the decrease of about 31.63% of the luminance value (L*) and the decrease with about 47.22% of the color parameter a* (a* - red-green color coordinate), compared to the Control minicake sample (Figure 5).

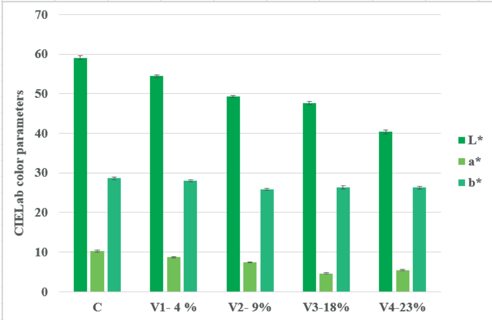

Figure 5. Colour parameters of the products "Control Minicake" and "Minicake fortified with cauliflower leaves powder"

The textural properties of the products "Control Minicake" and "Minicake fortified with cauliflower leaves powder" packed in a polypropylene bag, for 24 days from the date of manufacture, are presented in Table 1.

Table 1. The textural properties of the products "Minicake fortified with cauliflower leaves powder", compared to the control sample C

Minicake	Period (days)	Firmness (N)	Elasticity	Cohesiveness	Gumminess (N)
C	0	2.21±0.20	0.99±0.01	0,67±0.01	1.86±0.31
	3	3.43 ±0.33	0.99±0.01	0,62±0.01	2.09±0.21
	6	4.08±0.50	0.98±0.01	0.54±0.02	2.18±0.14
	9	4.38±0.43	0.98±0.01	0.50±0.02	2.22±0.16
	13	4.71±0.01	0.98±0.01	0.38±0.06	2.34±0.18
	24	6.04±0.61	0.98±0.01	0.33±0.04	2.58±0.15
V1	0	1.53±0.11	0.96±0.01	0.69±0.01	1.02±0.07
	3	2.45±0.08	0.98±0.01	0.60±0.05	1.45±0.07
	6	2.88±0.16	0.98±0.01	0.57±0.03	1.61±0.02
	9	3.59±0.69	0.98±0.01	0.53±0.03	1.85±0.26
	13	4.21±0.59	0.99±0.01	0.33±0.06	1.73±0.16
	24	5.87±0.26	0.99±0.01	0.35±0.07	2.00±0.48
V2	0	1.71±0.35	0.98±0.01	0.69±0.01	1.16 ±0.23
	3	2.74±0.04	0.98±0.01	0.59±0.01	1.59 ±0.02
	6	3.21±0.13	0.98±0.01	0.50±0.01	1.58 ±0.05
	9	3.48±0.38	0.98±0.01	0.52±0,08	1.78 ±0.08
	13	4.07±0.59	0.98±0.01	0.34±0.09	1.87±0.08
	24	5.68±0.41	0.99±0.01	0.37±0.06	2.07±0.66
V3	0	1.79± 0.12	0.98±0.01	0.64±0.06	1.13±0.03
	3	2.73±0.26	0.98±0.01	0.58±0.01	1.55±0.17
	6	3.31±0.19	0.98±0.01	0.55±0.01	1.79±0.09
	9	3.38±0.02	0.98±0.01	0.46±0.05	1.52±0.17
	13	3.98±0.59	0.99±0.01	0.45±0.05	1.68±0.23
	24	5.61±0.32	0.99±0.01	0.34±0.01	2.21±0.38
V4	0	1.85±0.39	0.98±0.01	0.63±0.04	1.15±0.29
	3	2.47±0.36	0.98±0.01	0.56±0.04	1.45±0.16
	6	2.64 ±0.09	0.98±0.01	0.53±0.01	1.54±0.08
	9	2.93±0.10	0.98±0.01	0.42±0.05	1.68±0.14
	13	3.71±0.14	0.98±0.01	0.35±0.03	2.04±0.17
	24	4.73±0.16	0.98±0.01	0.33±0.03	2.15±0.12

According to the results, addition of the cauliflower leaves powder, lead to a decrease in firmness of minicakes. Thus, after 24 days from the date of manufacture, the "Control Minicake" sample had a firmness about 1.27 times higher, compared to the "Minicake fortified with cauliflower leaves powder" sample (V4, fortification level 23%). Firmness correlates sensorially with core softness: a high firmness represents a denser, harder core.

Cohesiveness and elasticity were not influenced by the fortification of minicakes with cauliflower leaves powder. Also, according to the experimental results, the fortification of minicakes with cauliflower leaves powder lead to a reduction in their gumminess. The sample "Minicake fortified with cauliflower leaves powder" (V4, fortification level 23%), 24 days after the date of manufacture, had a 20% lower gumminess, compared to the sample "Control Minicake".

Physico-chemical analysis

The physico-chemical composition of the products "Control Minicake" and "Minicake fortified with cauliflower leaves powder" is presented in Table 2. The minicakes fortified with cauliflower leaves powder stand out for their protein content (15.16-16.06%), ash (1.89-2.30%) and total fiber (6.04-6.92%). The highest values of these chemical indicators were recorded in the case of the V4 variant of the "Minicake fortified with cauliflower leaves powder" product. At the same time, this product has the lowest content in carbohydrates, respectively, available carbohydrates.

Table 2. The physico-chemical indicators of the products“Minicake fortified with cauliflower leaves powder”, compared to the control sample C

Physico-chemical indicators	Control	V1	V2	V3	V4
Moisture (%)	19.44±0.49	19.65±0.49	20.00±0.50	20.12±0.50	20.56±0.51
Ash (%)	1.75±0.03	1.89±0.03	2.02±0.03	2.18±0.03	2.30±0.03
Protein (%)	14.92±0.22	15.16±0.23	15.45±0.23	15.81±0.24	16.06±0.24
Fat (%)	19.59±0.25	19.63±0.26	19.67±0.26	19.79±0.26	19.87±0.26
Carbohydrates (%)	44.30±0.53	43.67±0.52	42.86±0.51	42.10±0.50	41.21±0.49
Available carbohydrates (%)	38.52±0.46	37.63±0.45	36.65±0.44	35.45±0.43	34.29±0.41
Soluble sugars (%)	33.03±0.20	33.12±0.20	33.35±0.20	33.17±0.20	33.60±0.20
Total fiber (%)	5.78±0.10	6.04±0.10	6.31±0.11	6.65±0.12	6.92±0.12
Energy value (kcal/100g)	402	400	398	396	394
Energy value (kJ/100g)	1680	1672	1664	1657	1647

It is worth noting that the product "Minicake fortified with cauliflower leaf powder" (fortification levels according to the provisions of Regulation (EC) NO. 1924/2006 of the European Parliament and of the Council, is a source of protein because at least 12% (15.16-16.30%) of its energy value is represented by proteins and, at the same time, a source of fibers, as they have a fiber content of at least 6% (6.04-6.92%).

The ash content of the product "Minicake fortified with cauliflower leaves powder" is about 1.69 times higher than that reported by Na et al. (2023) in the case of rice muffins fortified with tigernut dietary fiber (Ash = 1.11-1.36%).

The product "Minicake fortified with cauliflower leaves powder" (fortification levels 4-23%) has a higher protein and total fiber content than that reported by Catană et al. (2021), in the case of the product "Minicake fortified with *Aronia melanocarpa* pomace powder" (Protein = 10.35%; Total fiber = 5.29%). At the same time, the product "Minicake fortified with *Aronia melanocarpa* pomace powder" (fortification levels 9-23%) has a lower carbohydrate and available carbohydrates content, compared to that reported by these authors (Carbohydrates = 42.43%; Available carbohydrates = 37.14%).

It is worth noting that the product "Minicake fortified with cauliflower leaves powder" (fortification level 4-23%) has a higher protein and fiber content compared to that reported by Tukassar et al. (2023) in the case of muffins fortified with cauliflower by-products powder (Protein= 8.93-9.70%; Total fiber = 1.17-3.74%), and the carbohydrate content is lower, compared to that reported by these authors (Carbohydrates = 53.46-55.23%). At the same time, the product "Minicake fortified with cauliflower leaves powder" has a fiber content comparable to that reported by Sławińska et al. (2024), in the case of shortcakes fortified with *Agaricus bisporus* powder (Total fibers = 5.67-6.87%). A diet rich in fiber is essential for the proper functioning of the human body. Thus, a diet rich in fiber determines a adequate lipid profile, causing the reduction of total cholesterol and LDL cholesterol (Gulati et al., 2017). At the same time, a high intake of fiber in the diet determines the reduction of blood pressure (Streppel et al., 2008), the reduction of the level of inflammatory markers and the risk of developing cardiovascular diseases (Ma et al., 2006). Saboo et al. (2022) mention that a high-fiber diet is vital for people with diabetes and associated diseases, and also is recommended for the prevention and management of type 2 diabetes.

The fortification of the minicakes with cauliflower leaves powder causes an increase in their content in mineral elements (K, Ca, Mg, Fe, Zn), compared to the Control sample (Figures 6 and 7). Among the analyzed mineral elements, in the case of fortified minicakes, potassium has the highest content. Thus, the potassium content of this product varied between 148.42-265.83 mg/100 g (the minimum value was recorded in the case of sample V1 - 4%, and the maximum in the case of sample V4 - 23%).

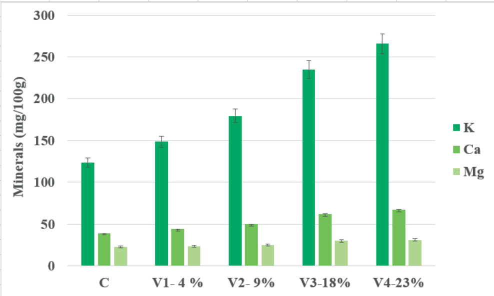

Figure 6. Mineral content (K, Ca, and Mg) of the products "Control Minicake" and "Minicake fortified with cauliflower leaves powder"

The potassium content of the product "Minicake fortified with cauliflower leaves powder" is higher compared to that reported by Tukassar et al. (2023) in the case of muffins fortified with cauliflower by-products (Potassium = 119.51-167.76 mg/100 g). These differences can be explained by the use in the composition of the product "Minicake fortified with cauliflower leaves powder" of some ingredients that come with an important intake of potassium: powder from apple waste, hemp seeds, dehydrated cranberries. The calcium content of the product "Minicake fortified with cauliflower leaves powder" varied in the range of 43.81-67.21 mg/100 g, and the magnesium in the range of 23.50-31.12 mg/100 g, being lower than those reported by Tukassar et al. (2023) in the case of muffins fortified with cauliflower by-products (fortification levels 10-30%): Calcium = 189.69- 244.56 mg/100 g; Magnesium=53.94-61.19 mg/100 g). The recorded differences can be explained by the fact that these authors used for fortification powder obtained from cauliflower by-products (leaves, stems, stalks) and not just cauliflower leaves powder, as in this experimental study, and the maximum fortification level of the muffins was 30%. At the same time, it is worth noting that "Minicake fortified with cauliflower leaves powder" has a higher calcium and magnesium content than that reported by Sławińska et al. (2024), in the case of shortbread cookies fortified with *Agaricus bisporus* and *Pleurotus ostreatus* powders (Calcium = 35.70-37.81 mg/100 g d.w.; Magnesium = 11.98 – 15.28 mg/100 g d.w.).

Fortification of the minicakes with cauliflower leaves powder lead to a increase in iron content of 1.27-2.39 times compared to the Control sample. The iron content of the product "Minicake fortified with cauliflower leaves powder" (V4 - 23%) is comparable to that reported by Catană et al. (2021), in the case of the product "Minicake fortified with *Aronia melanocarpa* pomace powder" (Iron = 3.25 mg/100 g).

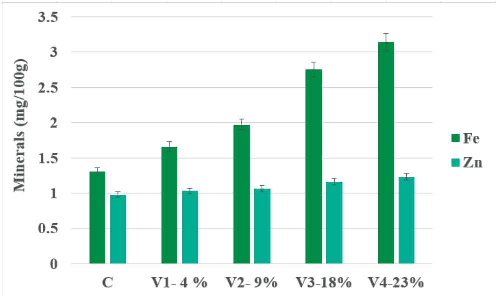

Figure 7. Mineral content (Fe and Zn) of the products "Control Minicake" and "Minicake fortified with cauliflower leaves powder"

It is worth noting that "Minicake fortified with cauliflower leaves powder" has a higher iron content of 2.24-3.24 times, compared to that reported by Sławińska et al. (2024), in the case of shortbread cookies fortified with *Agaricus bisporus* and *Pleurotus ostreatus* powders (Iron = 0.74-0.97 mg/100 g d.w.). At the same time, the iron content of the product "Minicake fortified with cauliflower leaves powder" (fortification levels 18 and 23%) is higher, compared to that reported by Catană et al. (2022) in the case of biscuits fortified with carrot pomace powder (Iron = 2.26 mg/100 g). Since the minicake is a product appreciated by several categories of consumers (children, teenagers, adults, elderly), its use as a vector for fortification with cauliflower leaves powder, for the purpose of prevention and diet therapy of nutritional deficiencies, is of real interest.

The zinc content of the product "Minicake fortified with cauliflower leaves powder" varied between 1.03-1.23 mg/100g, being lower compared to that reported by Catană et al. (2021), in the case of the product "Minicake fortified with *Aronia melanocarpa* pomace powder" (Zinc = 1.62 mg/100 g).

Bioactive compounds content

The product "Minicake fortified with cauliflower leaves powder" stands out by its content in bioactive compounds: total polyphenols, glucosinolates, β-Carotene, chlorophyll a, chlorophyll b and α-Tocopherol (Table 3).

Table 3. Bioactive compounds content of the products "Minicake fortified with cauliflower leaves powder", compared to the control sample C

Bioactive compounds	Control	V1	V2	V3	V4
Total polyphenols (mg GAE/100g)	78.57±1.96	92.85±2.32	111.67±2.79	135.86±3.40	157.15±3.93
Glucosinolates (mmol/100g)	-	117.21±2.81	255.45±6.13	513.44±12.32	647.87±15.5
α-Tocopherol (mg/100g)	0.630±0.01	0.638±0.01	0.649±0.01	0.656±0.01	0.682±0.01
Total carotenoids (mg/100g)	0.10±0.001	0.74±0.007	1.70±0.017	3.32±0.033	4.27±0.042
β- Carotene (mg/100g)	0.04±0.00	0.24±0.005	0.58±0.013	1.12±0.025	1.43±0.031
Chlorophyll a (mg/100g)	-	1.09 ±0.011	2.31±0.023	4.50±0.045	5.75±0.058
Chlorophyll b (mg/100g)	-	1.76±0.018	4.03±0.04	7.86±0.079	10.06±0.01

The total polyphenol content of the product "Minicake fortified with cauliflower leaves powder" varied in the range of 92.6-157.15 mg GAE/100 g, the maximum value being recorded for the 23% fortification level. The content in total polyphenols of the product "Minicake fortified with cauliflower leaves powder" is higher compared to that reported by Troilo et al. (2022), in the case of muffins fortified with grape pomace powder (Total polyphenols = 64-69 mg/100 g) and that reported by Olawuyi and Lee (2019) in the case of functional rice muffins enriched with shiitake mushroom powder, fortification level 15% (Total polyphenols = 35.57 mg GAE/ 100 g). Polyphenols are phytochemicals that have numerous beneficial effects on the human body. Thus, polyphenols show antioxidant, immunomodulatory, antimicrobial and antiviral activity and also have anticancer properties. Polyphenols, such as catechins, flavonoids, phenolic acids and tannins, can be used as bio-preservative substances for food and beverages, causing an inhibition of stress oxidative, through various mechanisms (Rathod et al., 2023).

It is worth noting that the product "Minicake fortified with cauliflower leaves powder" is a source of glucosinolates, the content of these bioactive compounds, varying in the range of 117.21-647.87 mmol/100 g, the highest values being recorded in the case of experimental variant V3 (level of fortification 18%) and V4 (fortification level 23%). International studies (Connolly et al., 2021) highlight the fact that glucosinolates and their metabolites, isothiocyanates, are compounds with an important role in the prevention and treatment of chronic diseases. Thus, these compounds can improve the control of blood sugar, blood pressure and lipid profile. Glucosinolate metabolites, particularly sulforaphane, may also exert a beneficial effect on neurological and psychiatric conditions such as schizophrenia, depression, multiple sclerosis, autism, and Alzheimer's disease. Isothiocyanate, the main degradation product of glucosinolates, has been recognized for its anticancer potential (Rizwan et al., 2023).

At the same time, the product "Minicake fortified with cauliflower leaves powder" (fortification levels of 18% and 23%) has a high content of total carotenoids (3.32 mg/ 100 g, respectively, 4.27 mg/100 g) and β-Carotene (1.12 mg/100g and 1.43 mg/100 g, respectively). The total carotenoid content of this pastry product fortified with cauliflower leaves powder (18% and 23% fortification levels) is comparable to that reported by Olawuyi and Lee (2019) in the case of functional rice muffins enriched with carrot pomace, fortification level 5% (β-Carotene = 3.468 mg/100 g) and with that reported by Catană et al. (2022), in the case of "Biscuits fortified with carrot pomace powder" (β-Carotene = 1.30 mg/100 g).

International studies have revealed that carotenoids have various biological effects, such as antioxidant, anti-tumor, anti-diabetic, anti-aging and anti-inflammatory (Crup et al, 2023).

The α-Tocopherol content of the product "Minicake fortified with cauliflower leaves powder" varied in the range of 0.638-0.682 mg/100 g, being lower, compared to that reported by Lao et al. (2019), in the case of cakes fortified with sweet corn residue (α-Tocopherol = 0.937-1.022 mg/100 g).

It is worth noting that the product "Minicake fortified with cauliflower leaves powder" has a high content in chlorophyll a (1.09-5.75 mg/100 g) and chlorophyll b (1.76-10.06 mg/100 g). Studies by Martins et al. (2023) suggest that these pigments possess therapeutic properties: anticancer, antioxidant, antigenotoxic, antimutagenic and anti-obesity.

Antioxidant capacity

Due to its content in antioxidants, the product "Minicake fortified with cauliflower leaves powder" has antioxidant capacity. The antioxidant capacity of this product varied between 2.15-3.64 mg TE/g (the minimum value was recorded in the case of the experimental variant V1, and the maximum in the case of the experimental variant V4). The control sample of the minicakes recorded a value of the antioxidant capacity of 1.22-2.07 times lower compared to the fortified minicakes (Figure 8).

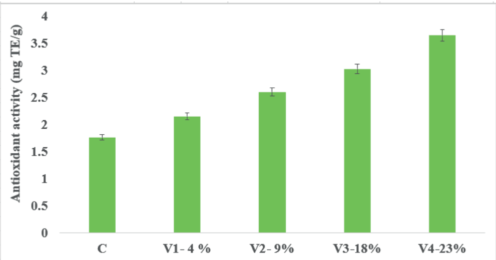

Figure 8. Antioxidant capacity of the products "Minicake fortified with cauliflower leaves powder", compared to the control sample C

It should be noted that in the case of the product "Minicake fortified with cauliflower leaves powder" between the total polyphenol content and antioxidant capacity it is a linear correlation ($y = 0.0234x-0.0526$; $R^2 = 0.9979$)

Microbiological analysis

Following the microbiological analysis of the products "Control Minicake" and "Minicake fortified with cauliflower leaf powder" it was found that they fall within the provisions of the legislation in force (Table 4). The values recorded for the water activity were in the range of 0.690- 0.764.

Corroborating the results of the microbiological analysis with those of the sensory and physico-chemical analysis, the content in bioactive compounds and the antioxidant capacity, the minimum period of validity of the product "Minicake fortified with cauliflower leaves powder", packed in a sealed polypropylene bag, was established at 15 days.

Table 4. Microbiological analysis of products "Minicake fortified with cauliflower leaves powder", compared to the control sample C

Microbiological indicators	Control	V1	V2	V3	V4
Yeast and molds (CFU/g)	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10
Escherichia coli (CFU/g)	< 10	< 10	< 10	< 10	< 10
Coagulase positive Staphylococcus (CFU/g)	< 10	< 10	< 10	< 10	< 10
Salmonella, in 25 g	Absent	Absent	Absent	Absent	Absent
Water activity	0.690	0.713	0.745	0.757	0.764

CONCLUSIONS

The results revealed that cauliflower leaves powder is a valuable functional ingredient, which can be used for the fortification of pastry products, in order to increase the nutritional value, the content in bioactive compounds and the antioxidant capacity.

Corroborating the results of the sensory, physico-chemical analysis, the content in bioactive compounds and the antioxidant capacity, in the case of the "Minicake fortified with cauliflower leaves powder" product, the experimental variant V3, level of fortification with cauliflower leaves powder, was selected as the optimal variant, 18%. The product corresponding to this level of fortification stands out for its sensory qualities, ash content (2.18%), protein (15.81%), total fiber (6.65%), and according to the provisions of Regulation (EC) NO. 1924/2006 of the European Parliament and of the Council, is a source of protein and fiber.

At the same time, the product "Minicake fortified with cauliflower leaves powder" (fortification level 18%) stands out for its content in mineral elements (Potassium = 234.93 mg/100 g; Calcium = 62.25 mg/100 g; Magnesium = 29.74 mg/100 g; Iron = 2.75 mg/100 g; Zinc= 1.16 mg/100 g), bioactive compounds (Total polyphenols = 135.86 mg GAE/100 g; Glucosinolates = 513.44 mmol/100 g; α-Tocopherol = 0.656 mg/100 g; Total carotenoids = 3.32 mg/100 g; β- Carotene = 1.12 mg/100 g; Chlorophyll a = 5.75 mg/100 g; Chlorophyll b = 10.06 mg/100 g) and antioxidant capacity (3.02 mg TE/ 100 g).

The product "Minicake fortified with cauliflower leaves powder" (fortification level 18%) falls microbiologically within the sanitary provisions in force and has a minimum validity of 15 days.

Due to the superior sensory quality, the complex composition and the fact that it has antioxidant capacity, the product "Minicake fortified with cauliflower leaves powder" (fortification level 18%) can be included in a healthy diet.

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RESEARCH ON THE IMPACT OF CLIMATE CHANGE ON THE ENVIRONMENT: A REVIEW

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Abstract

The climate changes in Romania are part of the global context, considering the regional conditions, with an increasing trend of dry summers. The research topic involves an analysis of the evolution of precipitation and periods of drought in recent years in conjunction with their impact on the environment, under the effect of climate change.
The increase in temperature, especially during the vegetation period and the large number of years in which evapotranspiration quantitatively exceeds precipitation, indicate the need for effective measures to regulate the water balance. From the distribution of precipitation in the vegetation periods, it was observed that in the warm period of the year the trend of decrease is greater than in the cold period, which implies a water deficit, now when the plants have the maximum water consumption (Smuleac Laura et al., 2020).

Key words: *temperature, soil, precipitation, CO_2, drought.*

INTRODUCTION

To date, predictions of European crop yields under climate change have been based almost entirely on the results of crop growth models. Although this strategy can provide good estimates of the effects of climatic factors, soil conditions and management on crop yield, these models usually do not capture all important aspects of crop management or relevant environmental factors.
For many environmental zones, there were signs of deteriorating agroclimatic conditions in terms of increased drought stress and a shortening of the active growing season, which in some regions is becoming increasingly tight between a cold winter and a hot summer. For most areas, there is a strong need for adaptation measures, either to increase soil water availability or to increase crop resistance to drought. The results suggest that there is a risk of an increase in the number of extremely unfavorable years in many climate zones, which could lead to greater inter-annual yield variability and pose a challenge to appropriate crop management (Trnka M. et al., 2011).
After surface water, groundwater is known to be the second largest freshwater storage in the world. Over the years, groundwater has come under pressure to meet human needs around the world. Meanwhile, the most visible footprint of human activities is the impact of climate change. They have the potential to alter the physical and chemical properties of groundwater, thereby affecting its ecological functions (Phuong, U.D., 2024).
Worldwide, more than half of the population has inadequate access to drinking water for at least one month a year. An estimate shows that by 2050, 57% of the global population will live in regions facing severe water shortages (Boretti A., L. Rosa, 2019). Given that groundwater accounts for ~20% of global water use, holding 70 times more freshwater than surface water (Earman S., M. Dettinger, 2011), declines in both the quantity and quality of groundwater lead to to severe pressure on global water resource management.
In arid and semi-arid regions where rainfall is limited, people use groundwater for domestic to industrial purposes. Along with the overexploitation of groundwater, its depletion has hindered economic development and created many difficult situations associated with health, education, and agriculture. As a result, the protection of groundwater becomes a matter of first concern, being even more critical under the influence of climate change.

Through changes in precipitation levels and atmospheric temperature, climate change affects both the quantity and quality of groundwater.

Climate change refers to the long-term alteration of global or regional climate components, which originates mainly from the increased accumulation of greenhouse gases, especially carbon dioxide (CO_2), in the atmosphere.

Climate change can also lead to an increase in rainfall and increase the possibility of flooding. Groundwater is naturally recharged from both diffuse recharge (i.e., rain-fed) and concentrated recharge through infiltration from surface water (Taylor R.G., 2013). Diffuse recharge occurs when precipitation falls on the ground and infiltrates into the water table. Both processes are dependent on precipitation, and therefore its change, either increasing or decreasing, can affect the quantity and quality of groundwater due to the transport of pollutants and the concentration of dissolved substances.

In arid and semi-arid regions, water from infiltration into the earth's crust is a large reservoir of soluble chloride and nitrate not only due to natural conservation over the years but also to long-term intensive agricultural practices (Gurdak J. et al., 2007). More severe torrential storms in these arid regions associated with climate change may encourage downward mobilization of these substances into the water table, making shallow aquifers susceptible to salinization and nitrate contamination (Cui Y. et al., 2020). If rainfall is more intense, there is a greater potential for surface runoff, which removes pesticides and other contaminants horizontally as well as vertically into deeper soil layers (Bloomfield J. et al., 2006).

Current climate simulations suggest that global warming will lead to a greater frequency of extreme hydrological events (floods and droughts). However, these results must be tempered by the fact that these current climate changes do not realistically represent many of the important processes for cloud and precipitation formation.

Warmed by sunlight and atmospheric radiation, water evaporates from the surface of oceans and land, moves with winds in the atmosphere, condenses to form clouds, and falls back to Earth's surface as rain and snow, some from it returning to the oceans via rivers (Rasmussen, R. M. et al., 2007).

Changes in the global and regional rainfall regime are expected to have a significant impact on the availability of water for plants and, implicitly, on the distribution, structure, composition, and diversity of populations (Foley, A., 2010; Vetter, T. et al., 2017; Bhaskaran, B. et al., 2012; Smuleac, Laura et al., 2020).

Some authors believe that decreases or increases in total precipitation because of climate change will affect hydrological cycles and plant growth (Alexander, L.V. et al., 2006). However, due to global climate change, rainfall intensity will increase, while total rainfall will remain unchanged (Asadieh, Behzad & Krakauer, Nir., 2015; Beck, F. et al., 2015; Bronaugh, D., 2014; Li, Xin et al., 2018).

Measures aimed at soil conservation must be considered a priority, as soil is widely exposed to global climate change. Considering that billions of people around the world rely on agriculture for their food supply, more investment in research is needed, both in soil conservation and protection, and in optimizing agricultural practices (Walter, L. F. et al., 2023). Different types of land use, especially agriculture and forestry, are responsible for almost a quarter of greenhouse gas emissions. Rising temperatures, prolonged drought and flooding are putting pressure on the soil.

According to some authors, climate change and land use change can negatively influence soil biodiversity. To increase the resilience of soil biodiversity in the context of climate change, soil needs to be well managed for resilient production systems. Agricultural zoning can also be a valuable tool within integrated systems to reduce the effects of climate change. However, it is essential to constantly monitor environmental variations so that producers are better prepared for climate change. Finally, appropriate water management is essential for soil functioning as climate change exacerbates water deficit (Walter L. F. et al., 2023).

Environmental changes are caused by the growth of the world population, the increasing rate of consumption by human society and technological changes. The most important component of global change is climate change due to the evening effect, which will have a major impact on the environment and economic and social activities. Global warming has led to an increase in the frequency of extreme events, with the rapid alternation between severe heat/drought and heavy rainfall/flooding becoming increasingly evident (Chen F., Chen Y., 2020).

Climate change affects all regions of the world. The ice sheet is melting, and the sea level is rising. In some regions, extreme weather and rainfall are becoming more common, while others are experiencing heat waves and drought (Apel H. et al., 2021; Jurcoane Maria Roana et al., 2023).

The evolution of precipitation in the Banat Plain in Romania was followed for a period of 146 years (1873-2019) and it turned out that the years are not constant, being years with more abundant precipitation, followed by dry years. It was found that in general the rainfall is decreasing, especially in the last 3 years. From the distribution of precipitation in the vegetation periods, it was observed that in the warm period of the year the tendency to decrease is greater than in the cold period, which implies a water deficit, exactly when the plants have the maximum water consumption (Smuleac, Laura et al., 2020).

The Banat hydrographic basin, located in the extreme south-west of Romania, occupies an area of 18393 km^2, which corresponds to a percentage of approx. 7.7% of the total surface of the country and includes the hydrographic network located between Mureş and Jiu, including the direct tributaries of the Danube between Baziaş and Cerna (http://www.old.anpm.ro/upload/121428_Mem_PPPDEI-).

From an administrative point of view, the Banat Hydrographic Basin is located in the West Development Region and the South-West Development Region, it fully occupies the administrative spaces of Timis and Caras-Severin counties and partially of Arad, Gorj and Mehedinti counties (https://inundatii.ro/bazinehidrografice/bazinul-hidrografic-banat/).

At the level of the Banat hydrographic basin, the effects due to climate change on freshwater resources have direct negative repercussions on the water circuit. These negative effects are observed by changing the distribution of precipitation, such as the fall area, their amount, as well as their distribution in time (vegetation period, cold period of the year). These changes in the water cycle in nature, because of climate change, have a stronger impact than global warming (Ziernicka-Wojtaszek, A., Kopcińska, J., 2020; Intergovernmental Panel on Climate Change, 2007; Smuleac, Laura et al., 2020).

The good quality of life, growth, nutrition, and development of all living beings depends directly or indirectly on the natural environment. Urbanization, agriculture, industrial activity, and greenhouse effects are the main causes of climate change worldwide. These climate changes are responsible for increasing carbon dioxide (CO_2) and temperature at the Earth's surface every year. All the components of the environment, i.e. air, water, and soil, are changing mainly due to human activities, especially due to changing lifestyles.

Climate change refers to any disturbance in the climate that can have a negative impact on living organisms, including humans, plants, and animals, which will have negative effects on the environment. With the increase in the earth's population and industrialization, the environment is disturbed daily. Due to carbon dioxide and other dangerous gases emitted by automobiles and industries, the air is continuously poisoned. Businesses release waste directly into the water without treating it properly, making it unsuitable for aquatic life. Plants act as filters that retain all pollutants to make the environment clean (Kabir M., 2023).

The processes that take up and those that introduce CO_2 into the air are balanced so that their percentage remains at 0.032% through a carbon cycle equilibrium (Kabir M., 2023; Ramsden, E., 1997) (Figure 1).

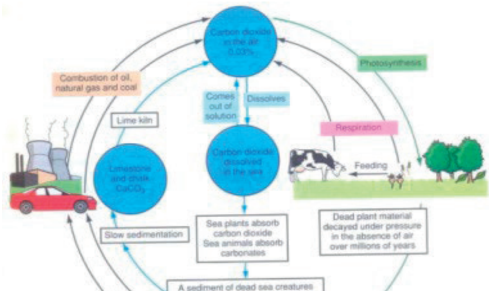

Figure 1. The carbon cycle (Ramsden, E., 1997; Kabir, M., 2023)

CONCLUSIONS

Increasing population without increasing plantations would completely affect the quality of life. Plants are natural purifiers of the environment. Due to the increasing concentration of carbon dioxide and global warming, the temperature of the earth is increasing day by day, which is causing disturbances in the environment.

This climate change is not beneficial, but rather causes damage to an ecosystem. So, human activities change the environment in a negative way.

Climate change is causing problems in many aspects, such as affecting the productivity rate of crops due to reduced rainfall, leading to losses in agriculture, impacting the country's economy. Rising temperatures cause many problems for humans, plants, and animals.

If we do not address rising CO_2 concentration and temperature as a critical issue, the destruction of habitat on the earth's surface will be even worse and the situation will spiral out of control.

In conclusion, climate change can be overcome by reducing greenhouse gas emissions, especially CO_2, through more plantations and by restoring water reservoirs.

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HORTIVOLTAICS - A ROAD TO GO OR NOT? A REVIEW

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Abstract

Climate change and disputes over land utilization are significant global obstacles. The impact of climate change on agricultural output is evident in the increasing frequency and severity of extreme weather phenomena, coupled with the ongoing escalation of temperature and carbon dioxide levels. At the same time, the increased demand for energy, especially the alternatives and greener forms, is always present.
There is hope in the form of agrivoltaic/hortivoltaic systems, a forward-thinking and truly innovative strategy. These systems integrate solar photovoltaic-based renewable energy generation with agricultural/ horticultural activities by positioning solar panels several meters above the ground.
This study aims to review these developed and implemented modern technologies, considering their long-term impact and efficiency. It will examine the dynamics of specific parameters such as abiotic stress and streamlined resource consumption, favorable microclimate through transparent photovoltaic panels, monitoring microclimate data, and early diagnosis of nutritional stress.

Key words*: photovoltaic panels, solar energy, crop production.*

INTRODUCTION

Climate change is one of the greatest challenges of the 21st century (Randers, 2021), with record global temperatures reaching approximately 1.4°C above pre-industrial averages in 2023. This rise in temperatures impacts agricultural production through increasingly frequent and intense extreme weather events, such as heatwaves, prolonged droughts, severe floods, and extended wildfire seasons, all contributing to rising sea levels (OECD, 2024). Intensive agriculture and climate change also risk soil quality and food production (Smith & Gregory, 2013; Cárceles, 2022). Food security is further endangered by the ongoing decline in arable land and the rapid growth of the global population (Durpaz, 2011). The primary driver of global warming is human activity, particularly greenhouse gas emissions (ICPP, 2023), highlighting the urgent need to expand renewable energy to meet global energy demand while replacing fossil fuels (Wydra, 2023). In this context, implementing renewable energy technologies and reducing global greenhouse gas emissions are essential measures for mitigating climate change and achieving net-zero emissions by 2050 (Kramarz, 2021; IEA, 2021). Among renewable resources, solar energy stands out for its significant potential (Lewis, 2006), with estimates indicating that by 2050, photovoltaic installations will supply over 40% of global renewable energy (IEA, 2021).

THE CONCEPT OF AGRIVOLTAICS

The attempt to produce electricity using photovoltaic systems has raised the issue of occupying arable land, thus increasing competition for limited land resources (Widmer, 2024). In this context, the combination of photovoltaic energy production with crop cultivation - often referred to as an agro-photovoltaic or agrivoltaic (AV) system - has emerged as an opportunity for the synergistic integration of renewable energy and food production (Dupraz, 2011). When crops are horticultural, we propose the term Hortivoltaic Systems (Miloș, 2022). This innovative approach maximizes land use efficiency by producing food and energy simultaneously (Dinesh & Pearce, 2016). The theoretical concept of photovoltaic systems was described as early as 1981 by Götzberger and Zastrow for a system that combines photovoltaic modules with potato production (Goetzberger et al., 1981). The first AV systems mounted on sloped spaces were invented in Japan in 2004. Small-scale AV development also began in Japan in 2011 (Nagashima, 2005; Sekiyama & Nagashima, 2019), followed by installations in Europe, with France and Italy in 2010 and 2011, respectively, as well as in Asia, Australia, and the United States of America (Barron-Gafford et al., 2019; Marrou et al., 2013; Elamri et al., 2018; Schindele et al., 2020; Toledo et al., 2022; Fraunhofer ISE, 2022; Al Mamun et al., 2022).

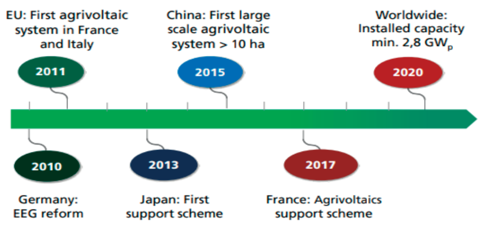

Figure 1. Development of Agrivoltaic Systems from 2010 to Present (Source: Fraunhofer Institute for Solar Energy Systems - ISE)

CLASSIFICATION OF AGRI AND HORTIVOLTAIC SYSTEMS

Agrivoltaic/hortivoltaic systems integrate agricultural/horticultural crop production with electricity generation on the same land, offering a promising sustainable energy and food production solution. However, a key challenge is the reduced light availability for crops growing under the panels, which can limit growth and yield potential. Research on shading impacts has shown that as shading intensity increases, crop yields tend to decrease (Prakash, 2023). Despite this, AV systems are particularly advantageous in hot and arid climates; strategically placed solar panels can reduce excessive thermal stress on crops during peak summer, enhancing yields and lowering water needs (Younas et al., 2019).

Central to AV systems are photovoltaic (PV) panels that convert sunlight directly into electricity using semiconductor materials, predominantly silicon-based solar cells (Dinesh, 2016; Al Mamun, 2022). These cells absorb sunlight and create electron-hole pairs, generating an electric current when connected to a circuit. Some advanced PV systems use artificial intelligence to optimize the panels' orientation and tilt, adapting to crop growth stages and weather patterns for better energy and crop performance (Klokov, 2023).

There are three primary types of agrivoltaic systems, each with distinct configurations suited to different agricultural needs (Sekiyama, 2019). The first type, *inter-row PV systems*, place solar panels between crop rows. This arrangement allows crops to grow in the spaces between the PV arrays, making it ideal for grasslands and certain crops like hay, silage, and those used for animal grazing. This setup maintains ample sunlight for crops and minimizes the shading impact on agricultural yield (Appelbaum, 2022).

Another type, *greenhouse-mounted PV systems*, incorporates PV modules onto greenhouse roofs. This system replaces part of the greenhouse's transparent covering with PV panels, which balance light transmission to support plant growth while generating electricity. This type is particularly advantageous for high-value horticultural crops, as it enables year-round production alongside energy generation (Scognamiglio, 2014).

The third type, *stilt-mounted PV systems*, involves installing solar panels on stilts above the crops. This design provides sufficient sunlight for photosynthesis, space for agricultural machinery, and easy dismantling of the structure if needed. This setup works well in larger-scale agricultural settings, allowing crops and energy production to coexist efficiently without significantly impacting crop yield (Toledo, 2021).

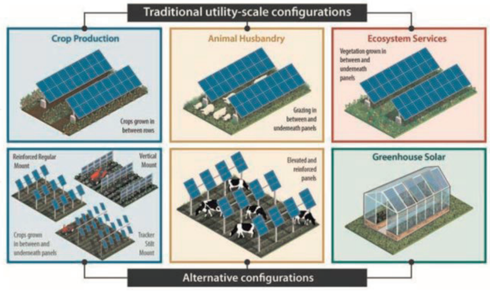

Figure 2. Types of AV systems (Wydra, 2023)

Furthermore, the types of photovoltaic panels employed can differ, each with specific characteristics and applications.

Opaque solar panels in agrivoltaic systems reduce sunlight reaching crops, helping to lower temperatures and mitigate heat stress (Uchanski, 2023). Gorjian et al. found that conventional opaque PV modules create shading that can harm crop growth (Gorjian, 2022). To mitigate this, strategies like using shade-tolerant crops, adjusting PV module layouts, employing tracking systems, and using advanced PV technologies have been tested to improve compatibility between agriculture and energy generation in AV systems (Chopdar, 2024).

Semi-transparent photovoltaic (STPV) modules represent a significant advancement in solar energy technology. They are designed to capture solar energy while permitting light transmission essential for crop growth. These modules are particularly beneficial for shade-tolerant crops such as *Rubus* spp. and *Pyrus communis* var. Conference, making them ideal for agrivoltaic applications (Reher, 2024). Innovations like Soliculture's LUMO greenhouse modules support photosynthesis by allowing specific wavelengths to pass through while converting the remaining light into electricity (Marucci, 2012; Chalgynbayeva, 2024).

Additionally, bifacial panels capture light from both sides and have proven effective in vertical configurations in countries such as Germany and Japan, increasing energy yield and optimizing land use (Younas, 2019; Li, 2021). Current STPV transparency levels are limited to 10-20%, yet research is progressing toward full transparency (Husain, 2018). Despite solar energy's promise, it currently meets only about 1% of global demand due to its low energy density. Transparent solar cells (TSCs) are being explored to transform glass surfaces into energy-generating materials, potentially increasing solar capacity and reducing reliance on non-renewable sources (Pulli, 2020).

Transparent solar panels in agrivoltaic systems balance light transmission with electricity generation, allowing sunlight to reach crops while still producing energy. These panels, often made from thin-film materials, are designed with spaces between cells to increase transparency and allow photosynthetically active radiation (PAR) to reach plants (Vasiliev, 2023).

Solar panels used in agrivoltaic systems can be fixed or adjustable, each offering specific benefits. Fixed panels, mounted with a north-south (N/S) inclination, are simple and less costly to install, providing stability and reducing maintenance costs. However, they can create an uneven distribution of light and soil moisture, which may affect crop yield (Younas, 2019). In contrast, adjustable panels allow tracking of the sun's path from sunrise to sunset, providing superior yields.

Agrivoltaic/hortivoltaic (AV) systems combine agricultural/horticultural crop production and electricity generation on the same land, with particular benefits in hot and arid climates where optimized panel coverage can protect crops from excessive heat, thereby improving yields and reducing water consumption (Younas, 2019). However, a primary challenge in AV systems is the reduced light availability for crops beneath the panels, which can restrict growth and yield. Studies indicate crop yield decreases as shading intensity increases, so choosing shade-tolerant species is crucial for successful AV integration (Prakash et al., 2023; Uchanski, 2023).

The effectiveness of AV systems relies heavily on the interaction between agricultural and photovoltaic components, particularly in managing the shading effects of panels on crops. Understanding this shading impact requires an in-depth analysis of the spatial distribution of solar radiation between the panels and crops. Pulido-Mancebo et al. (2022) introduced a methodology to estimate solar radiation distribution based on panel geometry and diffuse and direct irradiation levels. Research shows that shade-tolerant crops, which require lower light intensities, can enhance AV system productivity by preventing crop damage from

high solar intensity, often leading to scorching on leaves or fruits.
Integrating shade-tolerant crops in AV systems not only supports agricultural yield stability but can also enhance the economic value of farms by over 30% compared to conventional farming. This dual land-use strategy could significantly increase solar energy output, with potential national production gains of 40 to 70 GW (Dinesh et al., 2016).

PROGRESS IN SOLAR PV TECHNOLOGY

2010 global solar cell production ranged from 18 GW to 27 GW, reflecting a substantial increase since 2000, with yearly growth rates of 40-90%. Between 2008 and 2011, PV system costs dropped by 40%, while global electricity demand is expected to grow by 2.4% annually through 2030 (Tyagi, 2013). The worldwide installed PV capacity rose 21% from 483.1 GW in 2018 to 580.2 GW in 2019 (IRENA, 2020). Asia holds the lead in PV installations, particularly in China, Japan, and India, followed by Europe, where Germany, Italy, and the UK contribute significantly (Allouhi, 2022). By 2022, the installed capacity of agrivoltaic (AV) systems will exceed 14 GW. Deploying AV on only 1% of Europe's arable land could generate over 900 GW of solar power, vastly surpassing current installations (Klokov, 2023).
The IEA forecasts that PV power will generate around 6000 TWh by 2050, meeting about 16% of global energy needs. Due to solar energy's diffuse nature, achieving this target will require extensive land use (Denish, 2016).

AGRI AND HORTIVOLTAIC SYSTEM APPLICATIONS

The efficient functioning of agrivoltaic systems relies on effective light management and sharing between solar panels and the crops below. Tracking systems are essential in this context, as they offer the flexibility to balance energy generation with plant growth. This thesis explores various tracking optimization methods focusing on light distribution and availability. By ensuring equal emphasis on irradiation reaching both the crops and the solar panels, shading levels can be kept below 40% throughout the year, resulting in only an 8% decrease in electrical output compared to traditional backtracking setups (Bruno, 2023).
Recently, the conversion of photovoltaic installations with horizontal NeS trackers into agrivoltaic systems by cultivating orchard hedges between collector rows has been analyzed to study shading effects on the panels. A zone between the collectors was identified where the crops do not shade the panels, and a new tracking strategy was proposed to avoid shading outside this area. In a facility in Cordoba, with olive trees up to 3 meters tall, the land equivalent ratio could increase by 28.9-47.2%, making photovoltaic systems adaptable and sustainable in orchard agriculture (De La Torre, 2022).
Installing dynamic photovoltaic panels over apple orchards can address climate protection challenges and support the energy transition, but the impact of shading on apple performance needs investigation. In a three-year study (2019-2021) in southern France, 'Golden Delicious' apple trees experienced fluctuating shading (4-88% daily, averaging 50-55%). Results showed reduced air temperature and increased humidity, improved frost protection, and less alternate bearing, with more fruit-bearing trees (+31%) and higher fruit counts (+44%) in 2021. However, shading lowered photosynthetic capacity, carbohydrate storage, and dry matter content, resulting in suboptimal yields under 40 t/ha across all years (Juillion, 2022).
A study conducted in a kiwi plantation evaluated the impact of different levels of photovoltaic (PV) shading on growth, yield, and water productivity. With shading densities of 19%, 30.4%, and 38%, it was found that low shading (19%) minimally affected fruit growth and yield while improving water productivity by reducing evapotranspiration. In contrast, higher shading levels (30.4% and 38%) significantly negatively affected fruit volume and yield. The study concluded that 19% shading can support energy production and efficient agricultural yields, making it a suitable solution for isolated areas (Jiang, 2022).
The Lake Constance agrivoltaic study (2017-2018) evaluated AV system impacts on microclimate and yields for winter wheat, potato, grass-clover, and celeriac. Bifacial solar panels reduced photosynthetically active radiation by 30% and lowered air and soil

temperatures, leading to moderate yield reductions. However, during the hot, dry summer of 2018, yields for winter wheat and potatoes rose by 2.7% and 11%, respectively, highlighting AV systems' potential to stabilize yields under extreme weather conditions (Welesek, 2021).

Pascaris et al. conducted in-depth, semistructured interviews with 11 agricultural professionals to explore perspectives on agrivoltaic systems. They identified key barriers, including concerns about long-term land productivity, market uncertainties, and compatibility with current agricultural practices. Despite these challenges, participants recognized potential benefits such as increased revenue and dual land use. The study emphasizes the need for flexible system designs and strong partnerships between the solar and agriculture sectors to address concerns and promote adoption. The conclusion highlights that overcoming these barriers is crucial for successfully implementing agrivoltaic technology (Pascaris et al., 2020).

Established in 2010, the agrivoltaic experimental model in Montpellier, France, integrates photovoltaic (PV) panels with crops on 820 m². Featuring monocrystalline PV panels mounted 4 meters high at a 25° tilt, the setup includes full-density (FD) and half-density (HD) configurations. During the wheat growing season, the FD system received 43% of incident light, while the HD system received 71%. The STICS model predicted a 29% reduction in dry matter, a 19% yield reduction under FD panels, and 11% and 8% reductions under HD panels. The findings suggest significant land use efficiency and productivity improvements with agrivoltaic systems (Dupraz, 2011).

CONCLUSIONS

Hortivoltaic systems represent a promising solution to address the challenges of climate change and land-use competition, efficiently combining horticultural production with renewable energy generation. These systems are particularly beneficial in arid regions, where panel shading reduces thermal stress on crops, thus optimizing water consumption and plant yield. Additionally, hortivoltaics are ideal for crops sensitive to intense solar radiation, protecting them from burns and other adverse effects of excessive sun exposure. Technological advancements, such as semi-transparent and bifacial panels alongside artificial intelligence, ensure efficient light management. These systems provide significant economic and environmental benefits, and ongoing research can further expand their applicability in horticulture and sustainable energy.

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ASSESSING THE INFLUENCE OF 2,4-D AND BAP VARIATIONS ON *SOLANUM TUBEROSUM* 'BLUE CONGO' NODAL SEGMENTS

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Abstract

This study explores the effects of different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6 benzylaminopurine (BAP) on the tissue culture of Solanum tuberosum cultivar 'Blue Congo'. Nodal segments derived from in vitro shoots were cultured on 9 variants of MS medium with varying concentrations of 2,4-D (0.5, 0.7, and 1.0 mg/L) and BAP (0.1, 0.2, and 0.5 mg/L). The explants were evaluated under different light conditions to determine their viability, shoot regeneration, and micro-tuber formation. Results indicated that light conditions significantly influenced explant viability and shoot growth, with optimal shoot regeneration observed in specific hormone combinations. Additionally, micro-tuber formation was highest in certain variants. Further studies will be published to evaluate the ability of the obtained callus to produce somatic embryos and, ultimately, to develop into plantlets.

Key words*: germplasm conservation, callus, in vitro, microtubers, tissue culture, potato*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is cultivated worldwide and it is considered one of the most important staple food (Aksoy et al., 2021). This species was domesticated around Lake Titicaca, in southern Peru and northern Bolivia, between 7,000 and 10,000 years ago, originating from a species within the *Solanum brevicaule* complex. (Council et al., 1989; Spooner et al., 2005). Potatoes rank as the third most important food crop globally for human consumption, following rice and wheat (Shiwani et al., 2021).
A fundamental requirement for producing certified seed potatoes is that the planting stocks come from pathogen-tested materials grown *in vitro* (Westra et al., 2020), making plant tissue culture a standard method of certified potato propagation.
Mini-tubers are the primary material used for propagation to produce the first generation of seed potatoes in the field, as they are easier to plant and have a lower mortality rate than plantlets obtained in tissue culture (Westra et al., 2020). They do however have a dormancy issue, as they have a longer dormancy period compared to those grown in the field, leading to slower emergence (Westra et al., 2020). On the other hand, this prolonged dormancy is rather useful during *in vitro* conservation, as they can be easily stored in the fridge, even up to 210 days without loss of viability (Estrada et al., 1986). Micro-tuber dormancy can be reduced by pre-warming the mini-tubers or treating them with gibberellic acid (Westra et al., 2020).
The production of *in vitro* micro-tubers was first achieved more than 60 years ago, and factors inducing the *in vitro* tuberization are well-known and studied (Donnelly et al., 2003), the most important factor being the concentration of sucrose and growth regulators used in the medium. Abu Zeid et al., 2022 obtained a high percentage of *in vitro* potato tuberization using halved strength MS medium supplemented with 80-90 g/L sucrose, with 3.0 mg/L KIN and 1.0 mg/L PBZ (paclobutrazol) for 'Rosetta' and 'Victoria' cultivars.
Another growth regulator from the auxins group, 2,4-dichlorophenoxyacetic (2,4-D) aids the process of *in vitro* micro-tuber formation. When added to MS medium supplemented with 22.19 μM BAP and with 80 g/l sucrose, it has been observed to increase the formation of micro tubers from nodal segments of *Solanum*

tuberosum and *Solanum acaule* (Anjum, 1997), highlighting the synergic effect of both BAP and 2,4-D on the *in vitro* tuberization of potatoes. 2,4-D is an auxin that is mainly used for its ability to induce callus formation during the early stages of somatic embryogenesis. The role of 2,4D in somatic embryogenesis is well documented, as it is the most used auxin-type phytohormone for organogenic callus induction (Raghavan, 2004; Vondráková et al., 2011; García et al., 2019).

For callus induction, several types of explants have been used in potato tissue culture: leaves (Ghomari et al., 2013; Manisha & Nailwal, 2015; Kumlay & Ercisli, 2015; Singh et al., 2017; Kaur et al., 2018), internodes (Sharma & Millam, 2004; Ghomari et al., 2013; Manisha & Nailwal, 2015; Kaur et al., 2018) nodal segments (Anjum, 1997; Khatun et al., 2003; Kumlay & Ercisli, 2015), tuber segments (Khalafalla et al., 2010).

The crucial step during the process of somatic embryo formation appears to be the trans-differentiation of the pro-embryogenic masses into SE, which begins with the removal of auxin from the culture medium and is marked by the formation of globular embryos (Sharma et al., 2008; JayaSree et al., 2001). The medium used after the callus induction can be either hormone-free or various cytokinins might be added to aid the process (JayaSree et al., 2001; Sharma et al., 2008). Sharma et al. (2008) were able to obtain somatic embryos in potato cultivar 'Desire' by culturing internodes for 2 weeks on a medium with 5 μM 2,4-D and then sub-cultivating the induced callus on a hormone-free medium. Although 2,4-D alone should be able to produce embryogenic callus mass, growth regulators from the cytokinins group might be supplemented to the medium. JayaSree et al. (2001), observed that when applied alone, 2,4-D produces a white callus that is friable, without meristematic centers, whereas the addition of BA generated a nodular callus that was further able to sustain the development and germination of embryos in other species. Callus induced on medium with 2,4-D was successfully used for generating shoots when cultivated on medium with TDZ at a concentration of 5 mg/L, with better results compared to the mediums supplemented with BA (Khalafalla et al., 2010). Abu Zeid et al. (2022) obtained high percentages of callus formation using MS medium supplemented with 1.5 and 2 mg/L 2,4-D in combination with 0.5 mg/L KIN from leaf explants of 'Rosetta' and 'Victoria' cultivars. JayaSree et al. (2001) used for the induction and maturation of embryos medium supplemented with 22.8 μM zeatin 10 μM BA and obtained complete plantlets by placing the embryos in the cotyledonary stage on a hormone-free medium. Low concentrations of zeatin (for example, 9.1 μM), can develop the embryos into the globular stage, while, at higher concentrations (over 13 μM), zeatin was able to generate.

Regarding the type of explant used for callus induction, Manisha & Nailwal (2015) observed leaf explants to be more efficient compared to internodal segments. Explants exercised from stems are more potent for generating organogenic callus (Ghomari et al., 2013). The composition of the culture medium in relation to the type of explant is also an important factor to be taken into account.

At the same time, if present in high concentrations or for a prolonged time, 2,4-D can be responsible for causing several abnormalities in somatic embryos, mainly by disrupting the endogenous auxin balance and the auxin polar transportation interfering with the embryo apical-basal polarity (García et al., 2019).

Some of these abnormalities can be both genetic and epigenetic, for example, DNA methylation and mutations and, if present in high concentrations, they can disturb the typical genetic and physiological functions within cells and might obstruct the normal development of the embryo (Loschiavo et al., 1989; Cruz et al., 1990; Tokuji & Masuda, 1996; Gaj, 2004; Leljak-Levanić et al., 2004; Vondráková et al., 2011) or causing somaclonal variations. For example, albinism in *Agave anguslifolia* has been associated with the environments present in tissue culture, where the absence of chlorophyll pigments was linked to increased levels of DNA methylation (Duarte-Aké et al., 2016).

This study aims to examine the response of *Solanum tuberosum* 'Blue Congo' (purple variety) explants to different combinations of 2,4-D and BAP concentrations using nodal segments of *in vitro*-derived shoots, cultivated under light conditions or in the absence of light.

MATERIALS AND METHODS

To assess the influence of combinations of 2,4-Dichlorophenoxyacetic acid (2,4-D) and 6-Benzylaminopurine (BAP) and light conditions on the response of potato nodal segments of *Solanum tuberosum,* 'Blue Congo', 9 variants of medium were prepared, which are detailed in Table 1. The culture medium for this experiment was prepared according to the recipe described by Murashige & Skoog (1962) with the concentration of thiamine HCl modified to 1 mg/L, solidified with 3% agar, and with different concentrations of 2,4-D (0.5, 0.7, 1.0 mg/L) and BAP (0.1, 0.2, 0.3, 0.5 mg/L). Hormones were added in the concentrations presented in Table 1, from stock solutions prepared at concentrations of 100 mg/L and stored in the refrigerator.

Table 1. The composition of medium variants used for the nodal segments

No.	**Medium variant**	Concentration of growth regulators (mg/L)	
		2,4-D	**BAP**
1.	DB 1	0.5	0.1
2.	DB 2	0.5	0.2
3.	DB 3	0.5	0.5
4.	DB 4	0.7	0.1
5.	DB 5	0.7	0.2
6.	DB 6	0.7	0.5
7.	DB 7	1.0	0.1
8.	DB 8	1.0	0.2
9.	DB 9	1.0	0.5

After the addition of hormones, the pH of the solutions was adjusted to 5.75 and autoclaved at 121°C and 1.1 bar atmospheric pressure for 20 minutes.
After sterilizing the medium, while still in liquid form, it was distributed in sterile petri plates inside the laminar vertical airflow hood.

Inoculation of explants on the medium

The biological material used in this experiment was represented by *in vitro*-grown shoots of *Solanum tuberosum* 'Blue Congo' (Figure 1). Shoots were cultivated on MS medium for 30 days from *in vitro* cultures, maintained in the Plant Micropropagation Laboratory of the Research Center for Studies of Food Quality and Agricultural Products from the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

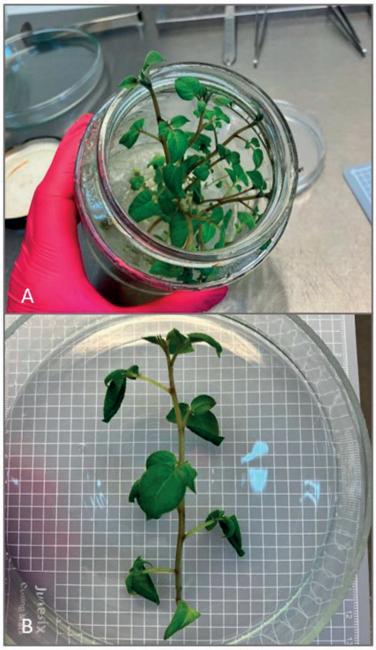

Figure 1. *In vitro* obtained shoots of *Solanum tuberosum* 'Blue Congo', used as a source for the nodal segments

The shoots were cut into nodal segments of 3- 4 mm in length, and, after the leaves and leaf petiole (Figure 2A), they were placed horizontally in the culture medium prepared in sterile Petri plates., in number of 10 explants per plate (Figure 2B).

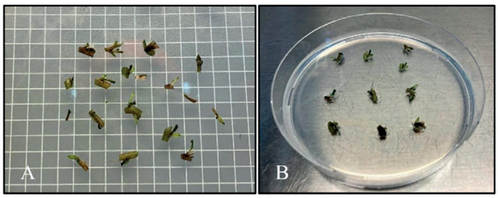

Figure 2. Nodal segments obtained from the *in vitro*-grown shoots (A); Nodal segments placed in the growing medium (B)

Explants were cultured either in dark conditions, at 22°C, or in the growing room at a temperature of 22-25°C, with a light intensity of 5023 lx, and a photoperiod of 16 hours of light followed by 8 hours of darkness. The light source was represented by a combination of white, blue, and red light-emitting diodes (LEDs).

Microscopical observations and measurements
Pictures regarding the microscopical observations and measurements were made using The Leica Application Suite (V4) using Leica S8AP0 stereomicroscope and the Leica DFC925 connected to it.

Statistical Analysis
The statistical analysis was conducted using The Real Statistics Resource Pack (https://real-statistics.com/) for Excel 2019. Due to the unequal sample size across the different variants, the Kruskal-Wallis test was employed for the analysis of variance instead of ANOVA.

RESULTS AND DISCUSSIONS

Viability of explants cultured on medium with combinations of 2,4-D and BAP
We refer to the viability of explants as explants that were both able to generate shoots and/ or callus mass and had cells that were still viable after 40 days of culture. No data is available for the explants cultivated on variant DB7 (1.0 mg/L 2,4-D and 0.1 mg/L BAP), as all plates were contaminated and thus, they were removed from the experiment.

Figure 3. Influence of light conditions on explant viability (A); Influence of culture medium on explant viability (B);. Explant viability (%) after 40 days of culture for all experimental variants (C)

Explants cultured under light conditions had an overall higher viability, of 97.56%, compared to the one cultured in the absence of light, which had a viability of only 83.29% Regarding the influence of the composition of the culture medium, high percentages of 100% were recorded on DB5, DB6, and DB8, under both light conditions. Variants DB1, DB3, and DB 9 recorded 100% viability, but only when cultured under light, highlighting the importance of this factor, as only 1 variant, DB2, recorded higher viability when cultured in the absence of light.

Average number of shoots and shoot length of explants cultured on medium with combinations of 2,4-D and BAP

The highest values regarding the average number of shoots regenerated from each explant were recorded on variants DB2 and DB9 (1.53 shoots/explant), followed by DB1 with 1.41 shoots and DB6 with 1.33 shoots/ explant, all cultivated under light conditions. All variants recorded higher values for this parameter under light conditions, except for variants DB3, DB4, and DB5, where the light conditions did not influence the capacity of shoot growth, the values being either equal (1.0 shoots/ explant in DB3 and 1.05 shoots/ explant in DB4) or very similar (DB5, with 1.20 shoots/ explant in darkness and 1.18 shoots/explant under light). Between the medium variants, Kruskall-Wallis recorded no significant differences between the explants grown under light or dark conditions.

Concerning the average shoot length of the explants, all measured values were higher for the shoots grown without light (Figure 4).

Figure 4. The average length of the shoots regenerated from the nodal segments, after 40 days of culture

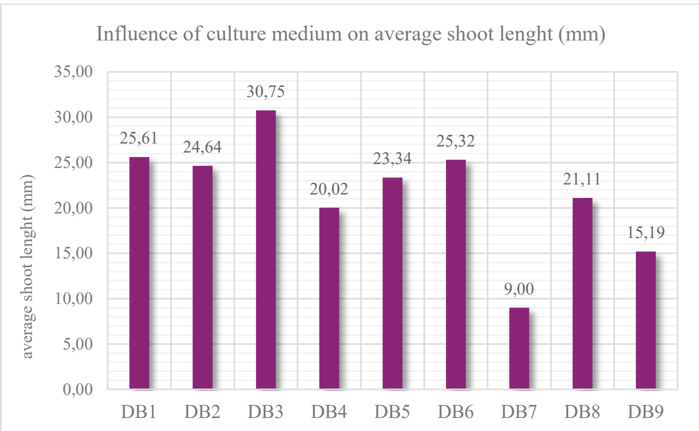

Figure 5. The overall influence of growth regulators in the culture medium on the average length of shoots regenerated from the nodal segments, after 40 days of culture

The explants cultured under no light recorded the highest values, with 54.33 mm on DB3 (with 0.5 mg/L 2,4-D and 0.5 mg/L BAP) and lowest on DB 7, with only 9.00 mm. The growth of the shoots decreased as the concentration of 2,4 increased in the medium, as the highest values were obtained on the variants with only 0.5 mg/L BAP (DB1, DB2, and DB3), and the lowest values were recorded on the variants with 1.0 mg/L 2,4-D (DB7, DB8, and DB9). The explants cultivated under light followed the same decreasing trend, the lowest values being obtained on the variants with higher concentrations of 2,4-D. However, these shoots cultivated under light tended to grow more in width leaning to create tuberous tissue than to grow in length (Figure .6).

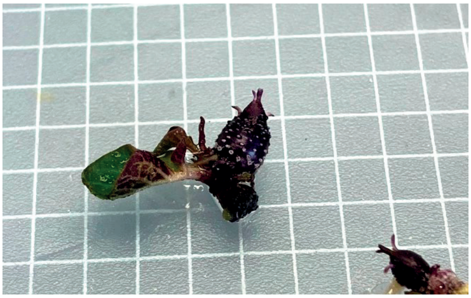

Figure 6. Explant on variant DB3, grown under light

Influence of combinations of 2,4-D and BAP on micro tuber formation

The highest capacity to produce micro tubers was observed on variant DB8 (with 1.0 mg/L 2,4-D and 0.2 mg/L BAP), in which 71.43% of the explants could generate micro tubers, as shown in Figure 7.

Figure 7. Influence of medium composition on the capacity of explants to produce micro tubers (%)

Following this value, explants on variant DB3 (equal amounts 2,4-D and BAP: 0.5 mg/L) recorded 60.00% and explants on DB2 and DB4 recorded similar values, of 50.00% and 52.17%, respectively. Two variants with higher concentrations of 2,4-D recorded 0 % capacity to produce micro tubers (DB7 and DB9) and the lowest values were obtained on DB6 (10%) and DB5 (14.29%).

Shoots cultivated in the dark produced between one and a maximum of 3 micro tubers/ explant (on DB2). The culture medium that produced the highest number of micro tubers was DB2, with an average value of 1.67 micro tubers/ explant, as shown in Figure 8.

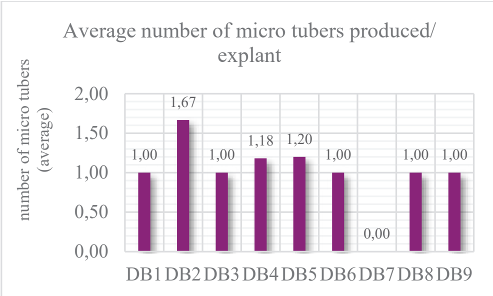

Figure 8. Influence of medium composition on the average number of micro tubers produced from each explant

The statistically significant differences between the medium variants were verified by applying the Kruskall-Wallis test. Dunn's post-hoc test confirmed statistically significant differences between variant DB2 and all other variants, except for DB5, case in which there are no significant differences. Other significant differences were revealed between DB4 and DB8.

On average, the length of the micro tubers measured 3.02 mm and the width 2.45 mm, and the Kruskal-Wallis test pointed out significant differences between the variants, both in terms of length and width.

The values for the length of the micro tubers ranged between 0.5 mm (the lowest, on DB8) and 6.1 mm (the highest, on DB2). The highest average lengths were recorded on variants DB2, with 3.94 mm in length, and DB6 with 3.80 mm in length (Figure 8), but with no significant differences between those two variants, according to Dunn's test. Lowest values were observed on DB4 (1.88 mm) and DB5 and DB8 (2.38 mm and 2.52 mm, respectively), as presented in Figure 9 A.

Width values were measured in the range between 0.30 mm, on DB4 and 3.80 mm on DB6. Values are mostly proportionate with the length one, except for DB2. The highest ones were obtained on variant DB6 (3.78 mm), followed by DB3, with 3.17 mm and, with no significant differences between them, and lowest recorded were on variant DB4, with 1.48 mm, and DB2 with 2.10 mm (Figure 9 B).

Visually, the shape of the tubers appears to be mostly isodiametric, and width values are similar or slightly lower than the length, except for variant DB2, which had average dimensions of 3.94 mm in length x 2.10 mm in width, with the tubers appearing rather elongated.

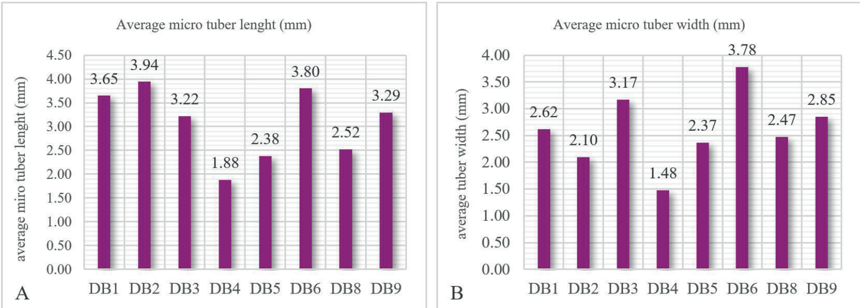

Figure 9. Influence of culture medium on the average micro tuber length (mm) (A); Influence of culture medium in the average micro tuber length (mm) (B)

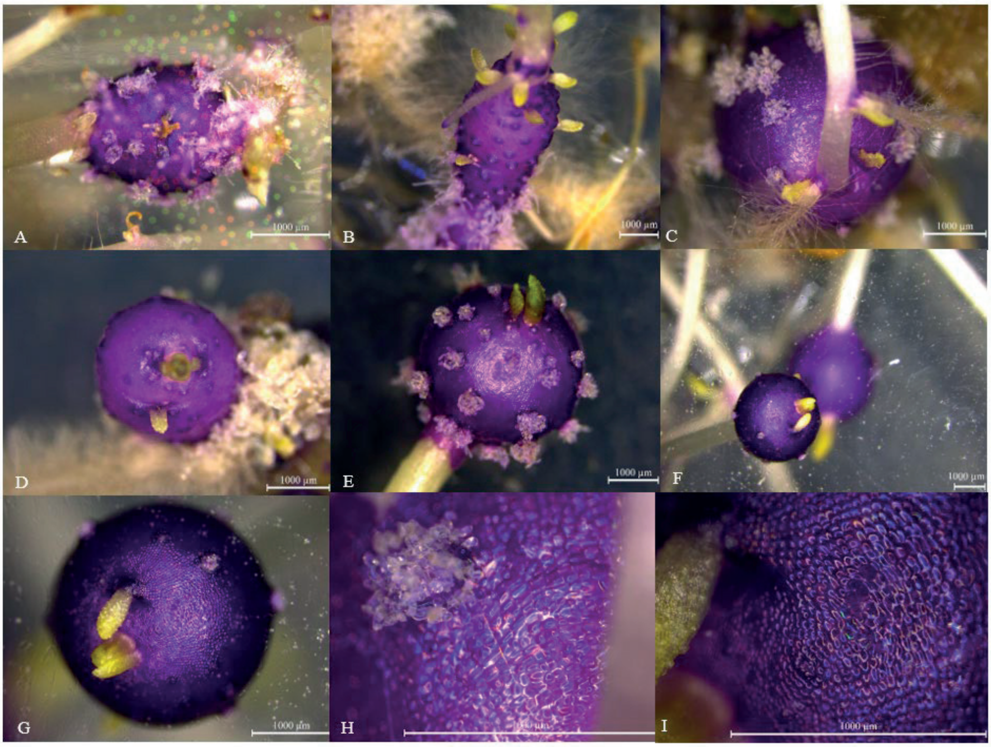

Figure 10. Micro tubers obtained on culture medium with 2,4-D and BAP: A: DB1, magn. 25X; B: DB2, magn. 12.5X; C: DB3, magn. 20X; D: DB5, magn. 20X; E: DB4, magn. 16X; F:DB9, magn. 10X; G: DB9, magn. 25X; H and I: tuber surface of DB3 and DB9, respectively, mang. 80X

Explants cultivated under light conditions did not produce micro tubers, but rather their main shoots grew in width and created tuberous tissue around them (Figure 11).

Figure 11. Tuberous shoot regenerated from nodal segment on variant DB2

Regarding the capacity to produce callus, all explants generated callus, either cultivated under light or in the absence of light, but statistical data and observations will make the subject of another paper.

Compact and dark callus developed on both ends of the nodal segment, from the internodal tissue, as can be observed at the end of the explants in Figure 6 and Figure 11. On shoots and nodal segments, friable calli emerged, with high multiplying capacity and undifferentiated, elongated cells. Depending on the light conditions and age, they appeared to be either white, with no pigments, light purple to purple on explants cultivated on both light conditions or pale green with chlorophyll pigments on the explants exposed to light (Figure 12).

As an overview, two types of callus groups were observed: compact and dark colored and friable, high multiplying white/light purple.

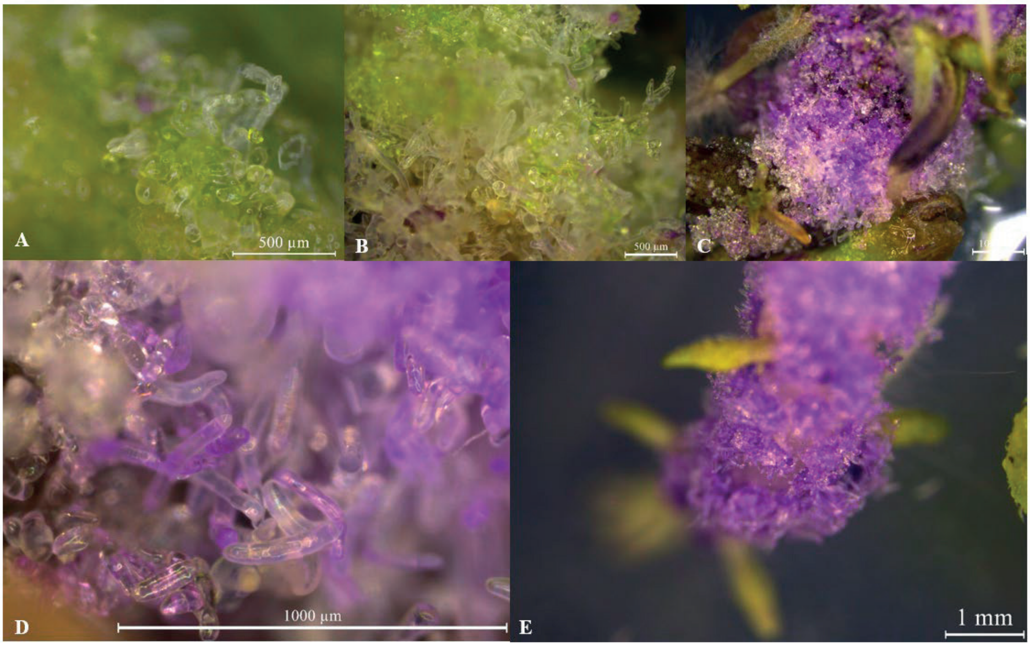

Figure 12. Friable callus obtained on culture medium with 2,4-D and BAP. A: DB2 (light); B: DB4 (light); C: DB2 (light); D: DB4 (darkness) and E: DB6 (darkness)

CONCLUSIONS

The combinations of 2,4-D and BAP and light conditions (cultivated under light or in the absence of it depend greatly on the type of tissue that needs to be obtained. The higher number of shoots were obtained on DB2, with 0.5 mg/L 2,4-D and 0.2 mg/L BAP, but the overall highest values in terms of shoot length were recorded on variant DB3, with equal concentrations of 2,4-D and BAP (0.5 mg/L).

Concerning the capacity to produce micro tubers, variant DB8, with 1.0 mg/L 2,4-D and 0.2 mg/L BAP showed a high rate of 71.43% explants that generated tubers, but the variants that were more suitable for tuber growth were observed to be DB2 (0.5 mg/L 2,4-D and 0.2 mg/L BAP), DB6 (0.7 mg/L 2,4-D and 0.5 mg/L BAP) and DB3 (0.5 mg/L 2,4-D and 0.5 mg/L BAP). With regards to the callus that was induced, further studies will be published to assess the capacity of these tissues to generate somatic embryos and future plantlets.

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EVALUATION OF PHOTOSYNTHETIC RATE AND CHLOROPHYLL CONTENT IN FIVE FOREST SPECIES

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Abstract

The photosynthesis and chlorophyll content are fundamental to understanding ecological processes in forest ecosystems, providing valuable clues to the health and functioning of these complex systems. The study of photosynthetic rates and chlorophyll content in five different tree species (Carpinus betulus, Fraxinus excelsior, Robinia pseudoacacia, Acer campestre and Acer pseudoplatanus) conducted in two different locations, namely in the Iron Gates Natural Park in Moldova Nouă (PNPF) and in Timișoara, brings to the fore a detailed analysis of the adaptations and responses of vegetation to environmental variability. Across the whole study, Acer pseudoplatanus revealed a significantly higher value of chlorophyll content, followed by Robinia pseudoacacia and Carpinus betulus with similar values and Acer campestre and Fraxinus excelsior with the lowest values, respectively. Acer campestre had a significantly higher photosynthesis rate than the other species, while Acer pseudoplatanus had the lowest values in both locations.

Key words: *Carpinus betulus, chlorophyll, Fraxinus excelsior, photosynthesis, Robinia pseudoacacia.*

INTRODUCTION

Chlorophyll (Chl) is an important photosynthetic pigment for plants, strongly determining photosynthetic capacity and consequently plant growth. This concept has not been widely tested in natural forests (Li et al., 2018.).

Photosynthesis is the most important source of energy for plant growth since Chl is an important pigment for photosynthesis (Baker et al., 2008; Li et al., 2018.). The photosynthetic reaction is mainly divided into three stages (1) primary reaction, (2) electron transport and photophosphorylation, and (3) carbon assimilation. Chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) are essential for the light reactions of photosynthesis. Chl *a* and Chl *b* absorb sunlight at different wavelengths (Chl *a* mainly absorbs red-orange light and Chl *b* mainly absorbs blue-violet light) (Lichtenthaler & Buschmann, 2001.). Thus, it is assumed that the total amount of chlorophyll content in leaves (Chl *a*+*b*) and the ratio of chlorophyll *a* to *b* (Chl a/b) directly influence the photosynthetic capacity of plants (Croft et al., 2018).

Excessively high or low temperatures inhibit the enzyme reaction, even destroying chlorophyll. The optimal temperature for general plant chlorophyll synthesis is 30°C (Nagata et al., 2005.).

Therefore, temperature has an influence on chlorophyll synthesis as well. Precipitation could affect the photochemical activity of chloroplasts (Zhou, 2003), as water is used for nutrient transport in plants because mineral salts must be dissolved in water to be absorbed by plants. Therefore, chlorophyll synthesis and water are closely linked. Lack of water in leaves influences chlorophyll synthesis and favours its breakdown, accelerating leaf turning yellow. There is also indirect evidence that chlorophyll is controlled by climate and soil. Thus, chlorophyll could be an indicative trait for characterizing how plants respond to climate change (Li et al., 2018.).

During periods of drought, forest plants experience a decrease in water availability, leading to a reduction in photosynthesis and other physiological processes (Brodribb et al., 2009). As a result, plant growth is suppressed and plants may wither (Bigler et al., 2006; Choat et al., 2018). Studies have shown that drought can also cause changes in plant anatomy, morphology and physiology, such as reduced leaf surface area, increased root-to-shoot ratio and decreased stomatal conductance (Schäfer, 2011; Urban et al., 2023).

One impact of low light on forest species is the alteration of chlorophyll content, which affects photosynthetic rate (Sui et al., 2012). Low light decreases chlorophyll content in plants (SPAD value) (Park et al., 2018). Some results have reported that chlorophyll a and total chlorophyll contents for two maize leaf varieties increased after shading treatment (Wang et al., 2017.). Chlorophyll content, especially chlorophyll *b* content, in tomato seedlings increased under low light (Wang et al., 2010). Consequently, low light is a key factor in plant photochemical efficiency and has different influences on leaf chlorophyll content (He et al., 2020). In Central European forestry, the establishment of mixed broadleaved forests is becoming increasingly important, but there is little information on the gas exchange characteristics of some tree species. In an old-growth forest in central Germany (Hainich, Thuringia), photosynthetic parameters of saplings and adult trees (lower and upper tree level) were analysed in four species (*Acer pseudoplatanus* L.*, Carpinus betulus* L.*, Fraxinus excelsior* L. and *Tilia platyphyllos* Scop.) (Hölscher, 2004).

The hornbeam (*Carpinus betulus*) is a deciduous species of the Betulaceae family. It is also an invasive species due to its high budding and draining ability.

The roots live in symbiosis with various fungi or bacteria. The leaves are alternate, simple with deciduous stipules. The flowers are unisexual, grouped in mixed inflorescences. The fruit of the hornbeam is of the achene type (Croft et al., 2017). It is an indigenous tree, native to Europe and Southwest Asia.

In Romania the hornbeam is found from the plain area (100-400 m altitude) to the mountain floor at 1,000-1,200 m altitude (Croft et al., 2017).

Common ash (*Fraxinus excelsior* L.), also known as European ash, is a species of the genus *Fraxinus,* native to most of Europe, from Portugal to Russia, with the exception of northern Scandinavia and southern Iberia. It is also considered native to southwest Asia, from northern Turkey to the Caucasus mountain range, as far north in the Trondheim Fjord region of Norway. The species is widely cultivated and naturalised in New Zealand and locations in the United States and Canada (Nova Scotia, New Brunswick, Quebec, Massachusetts, Connecticut, New York, New Jersey, Maryland, Ontario, Ohio, Kentucky and British Columbia) (Pautasso et al., 2013).

The acacia (*Robinia pseudoacacia* L.), a member of the Fabaceae family, is a honey tree with tall stems up to 25-30 metres and sparse thorny branches, acclimatised in North America, Europe, South Africa and Asia. The acacia originates from the North American continent. The genus Robinia is named after the French royal gardeners Jean Robin and his son Vespasien Robin, who introduced the acacia to Europe in 1601.

Sârbu and Oprea stated that even in North America, where it is native, outside the primary habitat, it poses a serious threat to native vegetation in dry and sandy meadows (Sârbu et al., 2011). Acacia grows spontaneously on almost any type of land. With high frost resistance, it loves light, tolerates drought, prefers permeable, light, fertile soils, but does not tolerate calcareous soils. Acacia grows well on light soils, even on loose sands, and is cultivated for fixation. It has the ability to fix nitrogen from the atmosphere, and the seeds retain their ability to germinate for many years. It has compound leaves and white, clustered, strongly fragrant flowers, and the fruit are flattened, reddish-brown pods. It flowers in late spring, in May-June. The acacia lives for around 100 years and belongs to the category of hardwood trees, being hard, tough and particularly resistant to moisture.

Jugastrum (*Acer campestre* L.) is a tree of the genus *Acer*, family Acerceae. It is also called field maple or common maple. The leaves are glabrous, 5- or 3-lobed palmate-lobed, up to 8 cm long. The flowers appear in spring arranged in compound corymbs, are polygamous and greenish in colour.

Jugastrum is a hard white tree with reddish bark, which grows frequently in the lowlands and hills up to altitudes of 1000 m. At maturity it is 15-25 m high. The crown of the tree forms low and provides good shade.

The mountain ash (*Acer pseudoplatanus*) is a tree of the family Acerceae with thick, palmate leaves, disseminated fruit and white, very resistant, elastic and fine wood, used for making furniture and musical instruments. It is considered the tree with the highest sound vibration. Also known as the "singing paltin", the mature tree is in great demand among the lute players of Bihor. The famous bugle violins are made from it.

It usually reaches heights of 20-30 metres. The leaves are large, palmate, five-lobed and dark green. They are similar to the leaves of the common maple. The flowers are arranged in large inflorescences and are greenish-yellow in colour, but are not as decorative as other maple species.

It is adorned by its large, rich leaves in spring. Its leaves provide dense shade in summer. The leaves turn shades of yellow and red before they fall.

It is commonly planted in alleys or along streets. It is a popular choice for parks and gardens due to its impressive size.

It prefers sun or semi-shade exposure. Also it needs regular watering, especially in the first year after planting. Pruning may be necessary to improve shape or control size.

The objective of this study was to assess the photosynthetic rates and chlorophyll content in five tree species (*Carpinus betulus, Fraxinus excelsior, Robinia pseudoacacia, Acer campestre and Acer pseudoplatanus*) in two locations, namely in the Iron Gates Natural Park in Moldova Nouă and in Timișoara, in order to obtain information regarding the physiological responses of these species to the particular environments.

MATERIALS AND METHODS

The biological material studied was represented by five tree species of similar age, hornbeam (*Carpinus betulus* L.), ash (*Fraxinus excelsior* L.), acacia (*Robinia pseudoacacia* L.), juniper (*Acer campestre* L.) and mountain ash (*Acer pseudoplatanus* L.). Representative leaves were measured from 5 trees of each species, during April-August, from two locations, namely Timişoara and from the Iron Gates Natural Park (PNPF) in Moldova Noua.

During the study period the temperatures in Timişoara ranged from 14°C in April to 26.5°C in August, and from 15.5 to 28.4°C in PNPF, respectively (Table 1). The cumulative rainfalls in Timişoara were 240 mm during April-June and 100 mm in July-August. In PNPF the amount of rainfalls was lower, ranging from 90 mm in July-August to 215 mm in April-June.

Table 1. Average temperature and rainfall from Timişoara and PNPF during April-August 2023

Location	Timişoara		PNPF	
Month	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)
April	14.0	70	15.5	60
May	18.5	80	19.7	75
June	23.0	90	24.5	80
July	26.0	55	27.2	50
August	26.5	45	28.4	40

Every month, we selected for measurements 10 representative leaves from different parts of the crown from each of the five trees per species. The measurement was repeated three times in different parts of each leaf to obtain an average for each leaf. The amount of total chlorophyll (SPAD) in leaves was estimated using the Konica Minolta SPAD-502 handheld chlorophyll meter. This meter determines the relative chlorophyll content by measuring the absorbance of a leaf in two wavelength ranges. The apparatus measures the light absorbance of the leaf in the range of red and near-IR light radiation. Using this principle the chlorophyll meter calculates a numerical value, SPAD (single photon avalanche diode) which is directly proportional to the amount of chlorophyll present in the leaf. The process highlights the nitrogen uptake, but also the amount needed to reach the maximum yield potential.

Photosynthetic capacity (%) was determined with the EARS Plant Photosynthesis Meter (PPM) which measures the photosynthetic light use of plants. The measurement is based on chlorophyll fluorescence, a very weak optical signal emitted by the plant, but which can be detected by the meter. Because of its light weight, the instrument is very suitable for laboratory and field use. In addition, long measurement series can be carried out automatically.

The data regarding photosynthetic rate and chlorophyll content were evaluated using three-factor ANOVA, followed by LSD test for post hoc comparison of means and determine the significance of differences at $p \leq 0.05$ (Ciulca 2006).

RESULTS AND DISCUSSIONS

It was observed that all three factors (species, location and study period - month) and their interactions had a significant influence on photosynthesis rates. Species had a higher contribution to the variability of photosynthetic rate compared to location and month, while among the interactions, species x month and location x species x month had a high influence. The results for this trait were influenced to an extent of about 8.15% by other sources of variation.

Also, in the case of chlorophyll content, a significant influence of all sources of variation was observed, against the background of a higher contribution of species to the variability of this character and close effects of location and period. Considering the different interactions, a very high variation in chlorophyll content of the different species is observed in the studied locations. Chlorophyll content values were influenced to an extent of about 3.39 SPAD by other uncontrollable variables.

From Table 2 it can be concluded that all three factors (species, location and study period - month) and their interactions had a significant influence on photosynthesis rate. Species had a higher contribution to the variability of photosynthetic rate compared to location and month, while among the interactions, species x month and location x species x month showed a high influence. Also in the case of chlorophyll content, a significant influence of all sources of variation is observed, amid a higher contribution of species to the variability of this character and close effects of locality and period. Considering the different interactions, a very high variation in chlorophyll content of the different species in the studied locations is observed.

Table 2. Analysis of variance for photosynthetic rate and chlorophyll content in five forest species and two locations during April-August

Source of variation	DF	Photosynthetic rate (%)			Chlorophyll content (SPAD)		
		SS	MS	F value	SS	MS	F value
Location (L)	1	3104.64	3104.6	270.96**	80.66	80.66	68.00**
Species (S)	4	4625.94	1156.49	100.93**	2048.98	512.24	431.84**
Month (M)	4	2638.06	659.52	57.56**	147.44	36.86	31.07**
L x S	4	1343.18	335.79	29.31**	3417.36	854.34	720.25**
L x M	4	2032.74	508.18	44.35**	30.36	7.591	6.40**
S x M	16	5473.22	342.08	29.85**	307.35	19.21	16.19**
L x S x M	16	6608.94	413.06	36.05**	724.89	45.31	38.19**
Residual	200	2291.6	11.46		237.24	1.19	
Total	249	28118.3			6994.29		

**significant at $p<0.01$

Table 3. Variation of photosynthetic rate and chlorophyll content in five forest species and two locations

Parameter	Photosynthetic rate (%)			Chlorophyll content (SPAD)		
Species	Location			Location		
	PNPF	Timișoara	Mean	PNPF	Timișoara	Mean
Carpinus betulus	55.36 a	56.04 b	55.70 B	29.25 b	24.10 c	26.68 B
Fraxinus excelsior	54.24 a	57.56 b	55.90 B	14.55 e	24.41 bc	19.48 D
Robinia pseudoacacia	48.32 c	56.44 b	52.38 C	28.46 c	24.80 b	26.63 B
Acer campestre	52.28 b	66.20 a	59.24 A	21.81 d	26.19 a	24.00 C
Acer pseudoplatanus	41.92 d	51.12 c	46.52 D	32.64 a	21.52 d	27.08 A

Photosynthetic rate: Species LSD5%=1.34; Location x Species LSD5%=1.89
Chlorophyll content: Species LSD5%=0.43; Location x Species LSD5%=0.61
Different letters (a-e) in the columns indicate significant differences ($p<0.05$) between species.
Capital letters were used for species means (A-D) comparisons

The photosynthesis rate at Iron Gates Natural Park had variation amplitude of 13.44% with values ranging from 41.92% for *Acer pseudoplatanus* to 55.36% for *Carpinus betulus* which was significantly equal to the value recorded for *Fraxinus excelsior*. *Acer campestre* produced a higher photosynthesis rate than both *Robinia pseudoacacia* and *Acer pseudoplatanus* (Table 3). In the Timișoara conditions the amplitude between species was higher, against a significantly higher photosynthesis rate in *Acer campestre*. The species *Carpinus betulus*, *Fraxinus excelsior* and *Robinia pseudoacacia* recorded small and insignificant variations of this parameter, but significantly higher by 4.92-6.44% compared to the value for *Acer pseudoplatanus*.

In *Carpinus betulus* a stable photosynthesis rate was observed, amidst a reduced variation between the two locations. Local environmental conditions had the highest influence on this parameter in *Acer* species (*Acer campestre* and *Acer pseudoplatanus*), with higher values in Timișoara. Overall, *Acer campestre* showed a significantly higher photosynthesis rate than the other species, while *Acer pseudoplatanus* showed the lowest values in both locations.

In terms of chlorophyll content at the Iron Gates Nature Park, the five species showed an average of 18.09, with ranges from 14.55 SPAD for *Fraxinus excelsior* to 32.64 SPAD for *Acer pseudoplatanus*. Chlorophyll content in *Acer pseudoplatanus* was significantly higher than in the other species, amid significant variation between species. In *Carpinus betulus* the value of this parameter was significantly higher than in *Robinia pseudoacacia*, *Acer campestre* and *Fraxinus excelsior*, which differed from each other. At Timișoara the variability between species for this trait was considerably lower, ranging from 24.1 SPAD in *Carpinus betulus* to 26.19 SPAD in *Acer campestre*, which showed a significant superiority over the other species. *Carpinus betulus*, *Fraxinus excelsior* and *Robinia pseudoacacia* showed values close to and significantly higher than *Acer pseudoplatanus*.

For all five species there is a significant variation in chlorophyll content between the two locations. Thus, for *Robinia pseudoacacia*, *Carpinus betulus* and *Acer pseudoplatanus* in the conditions of the Iron Gates Natural Park significantly higher values were recorded by 14.76-51.37 %. For *Acer campestre* and *Fraxinus excelsior*, the conditions of Timișoara favoured an increase in chlorophyll content by 20.08-67.77%. In the whole study, *Acer pseudoplatanus* showed a significantly higher value of chlorophyll content, followed by *Robinia pseudoacacia* and *Carpinus betulus* with similar values and *Acer campestre* and *Fraxinus excelsior* with the lowest values.

Considering the variation in photosynthesis rate of the five species during the active vegetation period, it can be seen that in April values ranged from 53.1% in *Acer pseudoplatanus* to 68.8% in *Fraxinus excelsior* followed by *Acer campestre* (Table 4). In May the range between species was 19.7% associated with a grouping into three categories, namely *Fraxinus excelsior* and *Acer campestre* with the highest values followed by *Carpinus betulus* and *Robinia pseudoacacia* with close values and *Acer pseudoplatanus* with the lowest photosynthesis rate. During June *Acer campestre* recorded the highest photosynthesis rate, followed by *Fraxinus excelsior* and *Robinia pseudoacacia* with close values and *Carpinus betulus* and *Acer campestre* with significantly lower values. In July *Carpinus betulus* and *Acer campestre* showed significantly higher values compared to *Fraxinus excelsior* and *Acer pseudoplatanus* respectively, with *Robinia pseudoacacia* showing the lowest values. During August the range between species was between 44% in *Fraxinus excelsior* and 58.8% in *Carpinus betulus*, followed by *Robinia pseudoacacia* and *Acer campestre* with values close to 51-51.7%.

Table 4. Variation of photosynthetic rate (%) in five forest species during April-August

Species	Month				
	April	May	June	July	August
Carpinus betulus	56.20 c	49.00 b	48.90 c	65.60 a	58.80 a
Fraxinus excelsior	68.80 a	58.00 a	54.30 b	54.40 b	44.00 d
Robinia pseudoacacia	55.70 cd	51.10 b	56.90 b	47.20 c	51.00 b
Acer campestre	60.80 b	57.60 a	60.50 a	65.60 a	51.70 b
Acer pseudoplatanus	53.10 d	38.30 c	43.80 d	49.50 c	47.90 c

LSD 5%=2.99; Different letters in the columns indicate significant differences ($p<0.05$) between species.

In terms of chlorophyll content, *Acer pseudoplatanus* and *Robinia pseudoacacia* had the highest values in April, followed by *Carpinus betulus* and *Fraxinus excelsior* with the lowest (Table 5). *Robinia pseudoacacia* also had a significantly higher value in May than the other species, with significantly equal values for *Acer campestre*, *Acer* *pseudoplatanus* and *Carpinus betulus*. During June, *Acer pseudoplatanus*, *Robinia pseudoacacia* and *Carpinus betulus* showed a high chlorophyll content with values of 25.92-26.2 SPAD, while *Fraxinus excelsior* and *Acer campestre* showed values of 20.18-22.31 SPAD. Chlorophyll content variation in July ranged from 19.18 SPAD in *Fraxinus excelsior* to 27.02-27.73 SPAD in *Acer pseudoplatanus* and *Carpinus betulus*. Also in August *Carpinus betulus* showed the highest chlorophyll content, followed by *Robinia pseudoacacia* and *Acer pseudoplatanus* with close values and respect-tively *Fraxinus excelsior* with the lowest content.

Table 5. Variation of chlorophyll content (SPAD) in five forest species during April-August

Species	Month				
	April	May	June	July	August
Carpinus betulus	26.78 b	25.52 b	26.20 a	27.73 a	27.15 a
Fraxinus excelsior	19.88 d	21.04 c	20.18 c	19.18 d	17.12 d
Robinia pseudoacacia	29.32 a	27.67 a	25.92 a	24.42 b	25.81 b
Acer campestre	23.97 c	26.22 b	22.31 b	23.20 c	24.30 c
Acer pseudoplatanus	29.93 a	26.45 b	26.12 a	27.02 a	25.86 b

LSD 5%=0.96; Different letters in the columns indicate significant differences ($p<0.05$) between species.

The dynamics of photosynthesis rate at Iron Gates Nature Park in Figure 1 shows that *Carpinus betulus* showed a significant reduction from April to June followed by a 20.8% increase in July and a 12.6% reduction in August. *Fraxinus excelsior* showed a significant reduction from month to month with a variation from 73.6% in April to 33.8% in August. For *Robinia pseudoacacia* there is a fluctuation in photosynthesis rate, characterised by an 18.6% reduction in April-May, followed by an 8.4% increase in June and then an 18.8% reduction. In *Acer campestre*, a 13.6% decrease in photosynthesis rate in April-May was followed by a gradual increase of 7.2-9.6% until July and a 12% decrease in August. The same trend is observed in *Acer campestre* and *Acer pseudoplatanus*, with lower values than in *Acer campestre*.

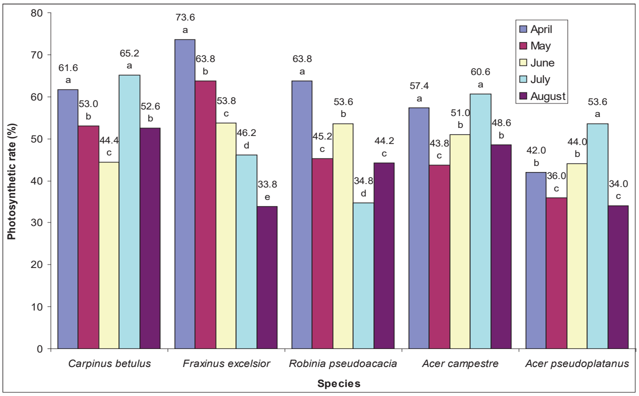

Figure 1. Dynamic of photosynthetic rate (%) in each of the five forest species during April-August in Iron Gates Natural Park
LSD 5%=4.22; Different letters indicate significant differences ($p<0.05$) within species

In the conditions observed in Timişoara, *Carpinus betulus* showed a reduction in photosynthesis rate of 5.8% in April-May, followed by an increase of 8.4-12.6% by July and a similar level in August (Figure 2). In *Fraxinus excelsior*, against a value of 64% in April, there was a reduction of 9.2-11.8% in May-June , followed by an increase of 7.8% in July and a reduction of 8.4% in August. For *Robinia pseudoacacia*, the 9.4% increase in photoresist rate in April-May was followed by stability in May-August. A similar trend was observed for *Acer campestre*, except that photosynthesis stability in May-July was followed by a 15.8% decrease in August. In *Acer pseudoplatanus*, the variation in photosynthesis rate shows a different pattern from the other species, characterised by a considerable reduction of 23.6% in April-May, followed by a reduced variation of 4.8% in May-July and an increase of 16.4% in August.

Regarding the dynamics of the chlorophyll content at the Iron Gates Nature Park in Figure 3 it can be seen that *Carpinus betulus* showed an increase of 4.1 SPAD from April to May, followed by a reduction of 3.4 SPAD in June and then increases of 2-3 until August. In *Fraxinus excelsior*, against a background of reduced values compared to the other species, the chlorophyll content was stable, with the exception of June where there was an increase of 3.5-5.2 SPAD compared to the other months. For *Robinia pseudoacacia* a fluctuation in photosynthesis rate is observed, characterized by a progressive and significant reduction from month to month in April-July, followed by a 5.5% increase in August. In *Acer campestre*, a decrease in photosynthesis rate of 6.5% in June-July was followed by an increase of 2% in August. Against the background of the highest values in *Acer pseudoplatanus* there was a 6% reduction in April-May, followed by a gradual increase until July and a 4.9% reduction in August.

Based on the conditions in Timișoara, *Carpinus betulus* shows a 6.6 SPAD reduction in chlorophyll content in April-May, followed by a 4.7 SPAD increase by June-July and a 3.1 SPAD reduction in August (Figure 4).

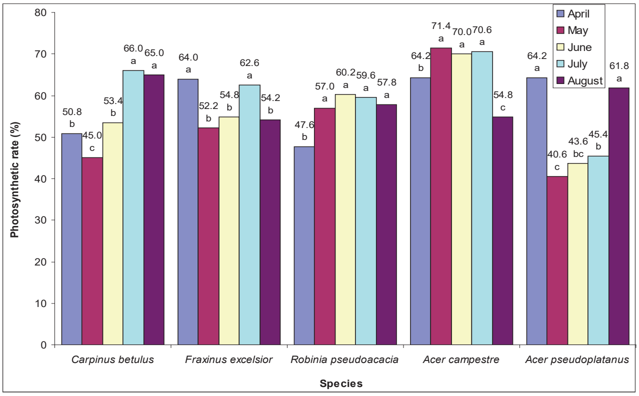

Figure 2. Dynamic of photosynthetic rate (%) in each of the five forest species during April-August in Timișoara
LSD 5%=4.22; Different letters indicate significant differences (p<0.05) within species

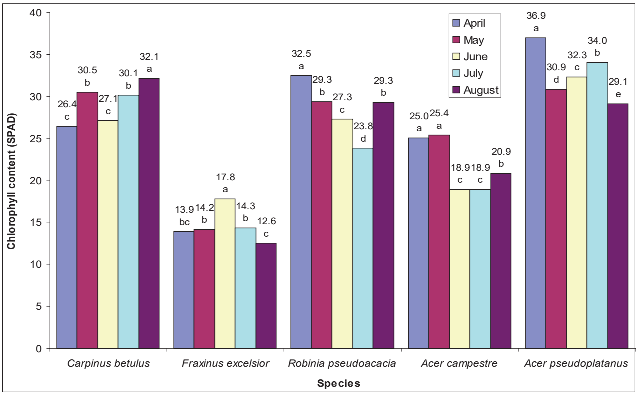

Figure 3. Dynamic of chlorophyll content (SPAD value) in each of the five forest species during April-August in Iron Gates Natural Park
LSD 5%=1.36; Different letters indicate significant differences (p<0.05) within species

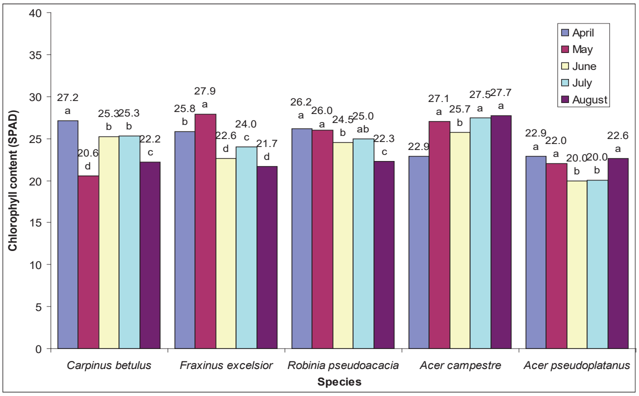

Figure 4. Dynamic of chlorophyll content (SPAD value) in each of the five forest species during April-August in Timisoara LSD5%=1.36; Different letters indicate significant differences ($p<0.05$) within species.

In *Fraxinus excelsior*, against a value of 25.8 SPAD in April, there was an increase of 2.1 SPAD in May, followed by a reduction of 5.3 SPAD in June and a reduced variation until August. For *Robinia pseudoacacia*, relative stability was observed from April to July, associated with a change of 1.7 SPAD, followed by a reduction of 2.7 SPAD in August. For *Acer campestre*, an increase in chlorophyll content of 4.2 SPAD is observed in April-May, followed by a reduced variation in May-August.

For *Acer pseudoplatanus*, the chlorophyll content variation was characterized by a level of 22-22.9 SPAD in April-May, followed by a reduction by 2 SPAD in June-July and an increase in August, reaching the April-May level.

Environmental factors such as temperature, precipitation, and light influence both the synthesis and functioning of chlorophyll in the leaves of the trees under study.

This theme can be further developed in relation to plant adaptations to changing environments and the consequences of these changes on forest ecosystems. This need is also highlighted by the fact that climate change can influence the production and functioning of chlorophyll.

The ecological characteristics of the tree species in this study highlight specific adaptations to different habitats and their interactions with environmental factors.

CONCLUSIONS

The results obtained from the data analysis indicate a significant influence of several factors on photosynthetic rates and chlorophyll content. In particular, the species appeared to have a major contribution to the variability of these characters compared to the location and study period (month). Interactions between these factors also revealed significant influences, highlighting the complexity and interdependence of ecological processes in the forests analysed.

Variation in photosynthetic rates and chlorophyll content between the five tree species and the two locations studied is highlighted. These variations reflect species-specific adaptations to environmental conditions and provide a detailed picture of ecological diversity within forest ecosystems.

The overall photosynthesis rate of *Acer campestre* was significantly higher than that of the other species, while *Acer pseudoplatanus* was observed to have the lowest values at both locations.

Across the entire study, *Acer pseudoplatanus* revealed a significantly higher value of chlorophyll content, followed by *Robinia pseudoacacia* and *Carpinus betulus* with similar values and *Acer campestre* and *Fraxinus excelsior* with the lowest values.

Analysis of the dynamics of photosynthetic rates and chlorophyll content during the period of active vegetation reveals seasonal changes and variations between species and locations. This information is essential for understanding how environmental factors influence plant physiological processes and for identifying potential vulnerabilities or adaptations to climate change and other disturbances.

Particularly, in the light of these results, our research aims to provide new insights into forest ecology, contributing to the sustainable management and conservation of these important terrestrial ecosystems.

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THE BENEFITS OF USING USEFUL MICROORGANISMS IN DROUGHT MITIGATION AND INCREASING SOIL FERTILITY - AN OVERVIEW

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Abstract

In recent decades, drought has represented a major setback for the world's agricultural economy, causing substantial reductions in global agricultural production that threaten the amount of food and food security. Thus, are necessary new alternatives to improve the sustainability of agricultural yield, to cope with various stress factors, including drought and high temperatures. Beneficial microorganisms (fungi, rhizobacteria) play an important role in mitigating the stress caused by drought by modulating the enzymatic and non-enzymatic antioxidant systems of plants and by producing phytohormones, osmolytes which represent the primary mechanisms through which they mitigate the effects of water stress, improving plant growth parameters and soil characteristics. In this study, we presented a review of studies found in the literature regarding the currently known implications of beneficial microorganisms for drought tolerance, including their mechanisms of action and also implication of them in increase of soil fertility.

Key words: *water stress, yield, crops, nutrients, plants.*

INTRODUCTION

The increasing demand for food to sustain a growing global population necessitates the maximization of agricultural production, even under extreme conditions, which have become more frequent due to global climate change, while simultaneously conserving non-renewable resources along with the environment (Raza et al., 2019).

In nature, plants confront numerous stress factors (drought, extreme temperatures, heavy metals, salinity, limited availability of soil nutrients) individually or in combination, factors which can have a negative impact on plant growth and development, productivity, and soil ecosystem (Bera et al., 2022; Teshome et al., 2020).

In recent decades, drought (water deficit) has represented a major setback for the global agricultural economy, causing losses worth hundreds of millions of dollars each year and substantial reductions in global agricultural production (Zhang et al., 2022). Additionally, drought results in the loss of diversity in rhizospheric microbial flora, soil fertility, and nutrient availability, with negative consequences on the functional traits (morphological, physiological, and biochemical) of the plant (Ali et al., 2022).

Therefore, it is essential to find and develop alternative methods to mitigation drought, improve soil fertility, and retain water in the soil, methods which can contribute to enhancing plant performance under extreme environmental conditions.

Lately, researchers focused on various approaches and methods of enhancement to increase drought resistance in different crops (Qaim, 2020). However, this requires time and is a long-term approach. For instance, employing biotechnological methods to develop drought-resistant and tolerant varieties in cereals and vegetables has made significant contributions to improving drought resistance traits in both model and crop plants. Nevertheless, their accessibility to farmers has been limited due to high costs, complexity, ethical considerations, and concerns regarding toxicity.

An innovative technique increasingly studied and implemented in sustainable agricultural systems, is the use of beneficial microorganisms (rhizobacteria, symbiotic bacteria, mycorrhizal and non-mycorrhizal fungi) that promote and enhance plants' tolerance mechanisms to adverse environmental conditions (Tripathi et al., 2021; Poudel et al., 2021; Hanaka et al., 2021; Poveda, 2020; Guler et al., 2016; Mastouri et al., 2012; 2010; Bae et al., 2009), stimulating plant growth, development, and resistance to abiotic factors. This approach is novel and efficient for enhancing plant tolerance to climate change and can play a significant role in the development of sustainable agricultural systems (Rajesh et al., 2016).

Among these groups of microorganisms, *Trichoderma* spp. is a rhizospheric fungus of significant agricultural and environmental importance, capable of conferring numerous beneficial effects through secondary metabolites (xylanases, peptaibols, epipoly-thiodioxopiperazines, terpenes, pyrones, siderophores, etc.) in promote plant growth and nutrition (Harman et al., 2004), induction systemic resistance to abiotic and biotic factors (Kashyap et al., 2017).

Species of *Trichoderma* spp. are fungi found in soil ecosystems and rhizosphere (Harman et al., 2004). They are avirulent and form symbiotic relationships with plants, colonizing their roots and leading to significant alterations in metabolism by modifying hormone content, soluble sugars, phenolic compounds, and amino acids, photosynthetic intensity, transpiration rate, and water content (Brotman et al., 2013).

Based on these arguments, the present study evaluated current knowledge regarding the use of various *Trichoderma* species to mitigate the negative effects of drought on the nutrition, physiological, and morphological parameters of different crop and horticultural plants, as well as soil fertility improvement.

THE USE OF BENEFICIAL MICROBIAL AGENTS TO ALLEVIATE THE EFFECTS OF DROUGHT

Recently, researchers have focused their activity on identifying, using, and applying beneficial microbial agents to improve the sustainability of agricultural production, increase crop nutrition efficiency and productivity, and new evidence suggests that these agents help plants cope with a variety of stress factors, including drought, high temperatures, salinity (Rubin et al., 2017; Colla et al., 2015).

Beneficial microorganisms play a crucial role in mitigating drought stress by modulating plants enzymatic and non-enzymatic antioxidant systems and by producing compounds such as phytohormones (indole 3-acetic acid, cytokinins, gibberellins, ethylene, ABA, salicylic acid, jasmonic acid), osmolytes, which represent the primary mechanisms through which they alleviate the effects of water stress and improve plant growth characteristics under drought conditions (Ullah et al., 2019). During drought stress, plants generate excessive amounts of reactive oxygen species (ROS), which cause oxidative damage to cells, such as membrane injury, increased lipid peroxidation, protein degradation, and ultimately cell death. Plants, on the other hand, defend themselves by activating antioxidant systems (both enzymatic and non-enzymatic) to protect against damage and maintain normal cellular function under drought stress.

In general, water deficiency reduces nutrient absorption through roots and transport from roots to stems. However, the use of certain microorganisms can aid in nutrient uptake from the soil even under water restrictions (Khoshmanzar et al., 2020). Mastouri et al. (2010) speculated that since the interaction between plant and *Trichoderma* mostly occurs in the rhizosphere, such a mechanism is likely linked to an increase in water absorption efficiency due to enhanced root capabilities, thus increased water uptake. Nevertheless, it has been demonstrated that *Trichoderma* spp. enhances plant drought tolerance even from the germination phase.

Inoculation of sugar cane plants with *Trichoderma asperellum* showed higher absorption of nitrogen and sulfur in both unstressed and drought-stressed plants (Scudeletti et al., 2021). Increased absorption of nitrogen and sulfur can occur directly through the production of hydrolases (proteases and chitinases), which are responsible for degrading rhizospheric proteins and chitin (Khoshmanzar et al., 2020), as well as indirectly assisting in the provision of simple compounds for degradation by heterotrophic nitrogen-fixing microorganisms. Multiple pieces of evidence have shown that *Trichoderma* spp. species are directly involved in nitrogen metabolism, mediated by the activation of nitrate reductase in plants (Sherameti et al., 2005).

Trichoderma spp. can enhance plant drought tolerance by activating the antioxidant system against dehydration-induced damage (Guler et al., 2016; Brotman et al., 2013; Mastouri et al., 2012), solubilizing minerals (including solubilization through acidification, redox, chelation, and hydrolysis), and delaying drought-induced changes by promoting stomatal opening, increasing leaf chlorophyll content, and enhancing photosynthesis.

Another valuable and interesting characteristic of plant root colonization by *Trichoderma* spp. strains is the improvement of root development (Contreras-Cornejo et al., 2009), proliferation of secondary roots, accompanied by alterations in root architecture and increased pH, providing better water and nutrient absorption and increase in seedling fresh weight and leaves surface (Pelagio-Flores et al., 2017).

It has been reported that crop productivity in the field increased by up to 30% after the addition of strains of *Trichoderma hamatum* or *Trichoderma koningii*. Numerous reports have indicated that *Trichoderma* stimulates plant growth in radishes, cucumbers, peppers, lettuce, and tomatoes (Contreras-Cornejo et al., 2016; Brotman et al., 2013; Bae et al., 2009).

Recently, the positive effects of using *Trichoderma* strains to mitigate drought stress effects on tomatoes has been described in published studies (Rawal et al., 2022; Cornejo-Ríos et al., 2021; Mona et al., 2017).

Furthermore, plant colonization by *Trichoderma* has stimulated the antioxidant mechanism (Mastouri et al., 2012), thereby reducing plant sensitivity to stress and/or delaying stress-induced changes in gene expression. Increased tolerance to abiotic stress in several plant species after inoculation with *Trichoderma,* has also been associated with increased osmolyte production (Zhang et al., 2019; Mona et al., 2017).

Scudeletti et al. (2021) results have shown that inoculating sugar cane plants with *Trichoderma asperellum* improves not only agronomic and nutritional parameters but also physiological metabolism and plant production under drought conditions. Additionally, numerous other studies have supported that *Trichoderma* spp. can increase tomato production and quality (Khoshmanzar et al., 2020), wheat (Shukla et al., 2015), rice (Shukla et al., 2012) under drought conditions.

Trichoderma spp. species are widely recognized as producers of phytohormones (e.g., auxin) and hormone-like compounds called harzianolide and svolenine, substances that, together with auxin, aid in cell wall extension (De Sousa et al., 2020). These compounds not only enhance stem weight but also, promote root and lateral root development (De Sousa et al., 2020), which are involved in water and nutrient absorption.

On the other hand, it has been found that IAA-producing bacteria (*Bacillus*) mediate drought tolerance through various mechanisms, such as increasing water permeability, water absorption, ROS scavenging, improving root architecture, as well as inducing a large number of stress-related genes (Etesami and Maheshwari, 2018).

Corn plants inoculated with beneficial microorganisms under drought conditions showed greater plant biomass, soluble substances, sugars, and higher leaf water content and osmotic potential compared to uninoculated corn plants (Sandhya et al., 2010). A subsequent study also demonstrated similar findings in corn plants after inoculation with drought-tolerant bacterial strains such as *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus thuringiensis*, and *Paenibacillus favisporus* during drought stress (Sandhya et al., 2011). For example, cucumber plants inoculated with microbial strains of *B. subtilis* SM21, *B. cereus* AR156, and *Serratia* sp. XY21 increased leaf proline content three to four times, protecting them from dehydration during drought (Wang et al., 2012).

Thus, the application of microbial consortia significantly improves plant drought tolerance by modulating their antioxidant system. The use of microbial consortia is a long-term solution, a cost-effective strategy, and environmentally beneficial as it boosts plant immunity, assimilates nutrients, and helps crops tolerate abiotic stress factors.

Specialized literature describes several successful research studies on the use of bio-

preparations based on *Trichoderma* spp. formulations for managing abiotic stress. Both solid and liquid formulations are utilized to produce active and viable inoculants using conidia of *Trichoderma* strains, which are more tolerant to unfavorable environmental conditions during the application of the formulated product in the field (Aamir et al., 2020).
Applying these formulations in the early stages of crop growth provides maximum benefits regarding root development and nutrient absorption. However, plant response to treatment with *Trichoderma* spp. has varied depending on the crop, variety, application method (i.e., seeds, roots, and soil), inoculum concentration, formulation type, soil and environmental conditions, etc. (López-Bucio et al., 2015).

THE PROTECTIVE MECHANISMS USED BY MICROORGANISMS UNDER DROUGHT CONDITIONS

Beneficial microorganisms with specific characteristics in agricultural ecosystems can increase drought resistance through various mechanisms that improve water circulation in the soil and its absorption by the plant (Chepsergon et al., 2014).
Vilela et al. (2017), demonstrate that in drought-stressed plants, reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide, and hydroxyl radicals can increase to toxic concentrations, which can significantly affect cell membranes and DNA, deactivate antioxidant enzymes, and lead to lipid peroxidation, resulting in morphological, physiological, and metabolic changes in plants.
Some researchers, highlighted the existence of multiple pathways in plants that transform toxic levels of ROS into a less toxic form. Thus, *Trichoderma* strains stimulate the activation of these pathways by enhancing the expression of genes encoding the component enzymes, increasing the capacity for ROS removal (Mastouri et al., 2010). For example, if metabolic pathways in chloroplasts are improved, it is expected that photosynthetic efficiency will increase by reducing damage caused by superoxide anion and other reactive species involved in photosynthesis.
Trichoderma spp. colonizes the roots and remains limited to the cortex and outer layers of the host plant root epidermis, modulating gene expression both in roots and stems. Roots inoculated with *Trichoderma* have shown increased levels of antioxidant enzymes, primarily superoxide dismutase (SOD), as well as elevated levels of peroxidase (POD), glutathione reductase, glutathione-S-transferase (GST), and other detoxifying enzymes in leaves. SOD represents the first line of cellular defense against abiotic stress generated by ROS, being the primary scavenger of superoxide radicals, which converts the toxic superoxide (O_2^-) into hydrogen peroxide and oxygen through a process called dismutation reaction.
Arora et al. (2002) explained that the enzymes catalase and peroxidase are capable of converting toxic H_2O_2 into water and oxygen under water stress conditions. However, increased SOD activity alone cannot protect plants from the toxic effects of free oxygen radicals, hence CAT and POD are necessary to eliminate the toxicity of H_2O_2.
Gusain et al. (2014), observed increased drought tolerance in rice plants due to the application of *Trichoderma harzianum* T35 and highlighted that *T. harzianum* stimulated the activity of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), thus preventing oxidative damage in rice by rapidly removing reactive oxygen species (ROS). Mastouri et al., (2012), observed increased tolerance of tomatoes to water stress induced by the application of *T. harzianum* T22, which was attributed to its ability to eliminate harmful reactive oxygen species, accompanied by an increase in antioxidant enzyme activity.
It has been reported that crop productivity in the field increased by 30% after the addition of *T. hamatum* or *T. koningii* due to their production of cytokinin-like molecules (e.g., zeatin), indole- 3- acetic acid a precursor of auxin, and gibberellin GA3, or related molecules. The production and controlled application of these compounds could be useful for enhancing the biological fertility of crops (Tucci et al., 2011).
In a previous experiment conducted by Björkman Bjorkman (1998), showed that maize seeds subjected to oxidative stress had significantly reduced vigor; however, subsequent treatment with *Trichoderma -T22*

restored vigor. *Trichoderma* spp. helps plants withstand stress caused by drought and high temperatures by activating the antioxidant system and enhancing defense signaling pathways in roots and stems, thereby strengthening plant growth (López-Bucio et al., 2015; Contreras-Cornejo et al., 2009). Additionally, plants colonized by *Trichoderma* secrete various compounds (such as auxins, gibberellins, enzymes, antioxidants, soluble substances, and compounds like phytoalexins and phenols) that provide tolerance to abiotic stress and enhance root system branching capacity (López-Bucio et al., 2015; Brotman et al., 2013).

Trichoderma strains applied to plants under water stress, improve the content of antioxidant pigments and proline (Mona et al., 2017; Mastouri et al., 2010), with positive effects also observed on seed germination and seedling vigor, inducing physiological protection against oxidative damage.

Numerous studies have demonstrated that *Trichoderma* spp. can enhance plant drought tolerance by stimulating root development, promoting secondary root proliferation, increasing root density, and altering root system architecture, while also activating antioxidant protection against dehydration-induced damage (Guler et al., 2016; Brotman et al., 2013; Mastouri et al., 2012; Contreras-Cornejo et al., 2009).

THE USE OF BENEFICIAL MICROBIAL AGENTS AS BIOFERTILIZERS

Although significant results have been achieved through the use of drought-resistant and heat-tolerant varieties (Fullana-Pericas et al., 2019), the use of biofertilizers has garnered increased attention due to their capacity to sustainably increase crop production and quality when applied both to the soil and to vegetation (Itelima et al., 2018).

A proper management of beneficial microorganisms in agriculture to allow for increased assimilation of minerals from the soil without production losses will lead to a more sustainable production system. Optimal growth and development of plants require nutrients in the soil to be available and in balanced quantities (Chen, 2006). Soil fertility can be restored by applying the concept of integrated soil fertility management based on managing soil nutrients through the conservation of natural resources and increased input efficiency.

Biofertilizers (microbial inoculants) are important components of integrated soil management, playing a key role in regulating soil nutrient activity through enzymatic activity followed by nutrient dynamics in the rhizosphere. This helps maintain soil structure and fertility, making it an environmentally friendly measure with low costs and a renewable source of nutrients for plants, successfully replacing chemical fertilizers in sustainable agriculture systems (Mahanty et al., 2017).

Biofertilizers are products containing living or latent cells of various beneficial microorganisms (bacteria or fungi) that, when applied to seeds, plants, soil, or foliage, colonize the rhizosphere, soil, or interior of the plant. They stimulate plant growth and development by transforming nitrogen and phosphorus nutritional elements into assimilable forms through biological processes such as phosphate solubilization (Verma and Pandey, 2022; Simarmata et al., 2015; Colla et al., 2015; Chen, 2006) and the production of bioactive compounds capable of inducing various plant responses leading to the development of induced systemic tolerance conditions, making the plant more resistant to adverse environmental conditions (Figure 1).

The use of biofertilizers for plants containing beneficial microorganisms (*Trichoderma* spp., *Bacillus* spp., and rhizobacteria) increases the accessibility and efficiency of nutrient absorption, maintains soil moisture and soil health, enhances crop production, and can be considered a sustainable and environmentally friendly approach for production stability through minimal input usage (Colla et al., 2015).

The specialized literature provides numerous pieces of evidence that the application of biofertilizers offers numerous benefits to the soil-plant system, including the improvement and stabilization of soil physical functions and properties, enhancing the soil's capacity to sequester carbon, and long-term productivity enhancement (Figure 1). Numerous researchers have experimented with the beneficial effects of applying *Trichoderma* in soil fertilization for various vegetable and cereal varieties. *Trichoderma* and its secondary metabolites enhance the absorption and efficiency of macronutrients, such as nitrogen (Fiorentino et al., 2018) or phosphorus (Garcia-Lopez et al., 2018), and oligo/micronutrients, such as iron, zinc, copper, and manganese (De Santiago et al., 2011).

Trichoderma spp. plays a vital role in the soil nutrient cycle through their mobilization and absorption. Numerous studies have indicated that *T. harzianum* can solubilize a range of nutrients for plants (Khan et al., 2016; Saravanakumar et al., 2013; Altomare et al., 1999). The colonization of cucumber roots by *T. asperellum* increases the availability of P and Fe through a significant increase in plant biomass (Yedidia et al., 2001). Seed treatment with *Trichoderma* spp. can reduce nitrogen requirements by 30–50% in various crops (Shoresh and Harman 2008).

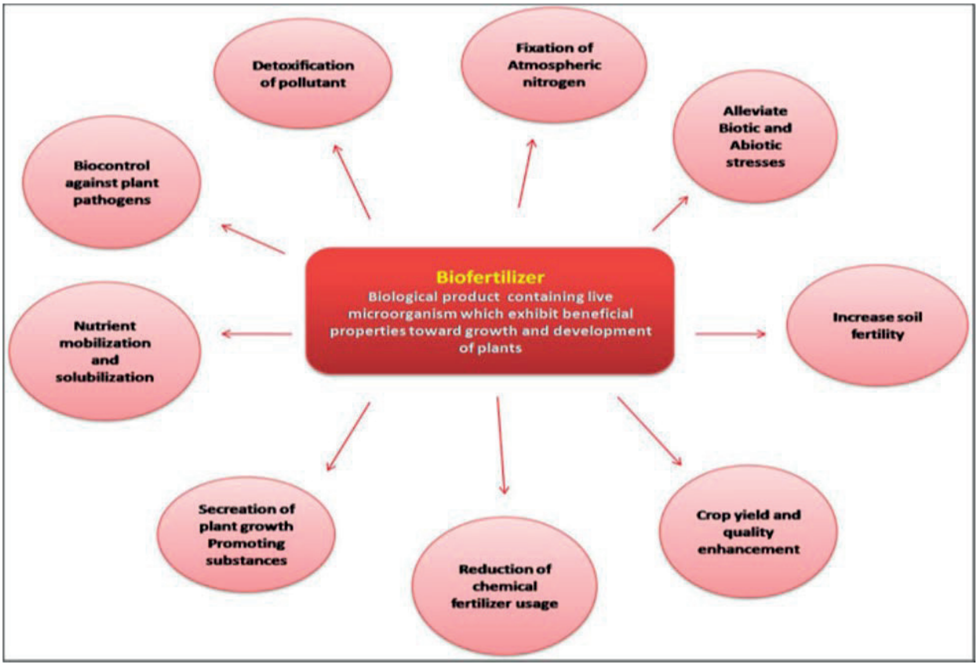

Figure 1. A summary on mechanism of biofertilizer and its importance in agriculture (according to Verma and Pandey, 2022)

The application of the *T. harzianum* (Th 37) formulation (20 kg ha^{-1}) in sugar cane increased the availability of primary nutrients N, P, and K by 27, 65, and 44%, respectively (Singh et al., 2010). The role of *Trichoderma* spp. in solubilizing tricalcium phosphate and other phosphates has been well investigated, with results indicating increased phosphorus availability for plants (Saravanakumar et al., 2013). The application of *T. harzianum* in combination with other beneficial microbial agents has led to higher contents of N, P_2O_5, K_2O, Fe, and Mg in chickpea leaves (Mohammadi et al., 2010). Yadav et al. (2009), demonstrated that *T. viride* has great potential to restore soil fertility and stimulate the growth of sugar cane plants.

Biofertilizers enriched with *Trichoderma* spp. have intensified plant growth in tomatoes, assimilatory pigments in leaves, and mineral content (P, K, Ca, Mg, Cu, Fe, Mn, and Zn) in tomato roots, increasing production by 12.9% compared to recommended NPK doses. Taken together, these reports suggest that *Trichoderma* spp. enriched bioproducts can reduce the application of chemical fertilizers and, therefore, can be considered an important practice in sustainable agriculture.

Trichoderma spp. has attracted special attention because it mineralizes organic nutrients by producing large quantities of extracellular enzymes that allow them to use plant residues as nutritional material (Contreras-Cornejo et al., 2009). This may be due to their abundance in soil under diverse climatic conditions, their ability to degrade a variety of organic substrates in soil, metabolic versatility, and competitive saprophytic capacity. Thus, *Trichoderma* spp. represents one of the viable options of modern crop cultivation technologies, where considerable emphasis is placed on environmental impact. The low cost and ease of obtaining *Trichoderma*-based biofertilizers are of particular importance in producing stress-resistant and high-quality agricultural crops in the current context of global climate fluctuations and population explosion.

CONCLUSIONS

Fungal strains from the genus *Trichoderma* induce drought tolerance, and their abundance increases under water-limiting conditions.

Reduction of oxidative stress by increasing the activity of antioxidant enzymes and attenuating reactive oxygen species in plants under water stress has been reported as one of the mechanisms of action of the *Trichoderma* fungus. Another mechanism includes the production of phytohormone analog metabolites that enhance plant growth and development in the presence of water stress. Increased water absorption efficiency due to modifications in root density, length, and number of secondary roots has also been proposed as another mechanism used by this fungus.

Microbial biofertilizers stimulate plant growth and nutrition by increasing the availability or absorption of nutrients, through the action of phytohormones, or by decomposing organic residues.Microbial biofertilizers can replace or reduce the use of chemical fertilizers and, therefore, can be considered an important practice in sustainable agriculture, preventing environmental pollution.

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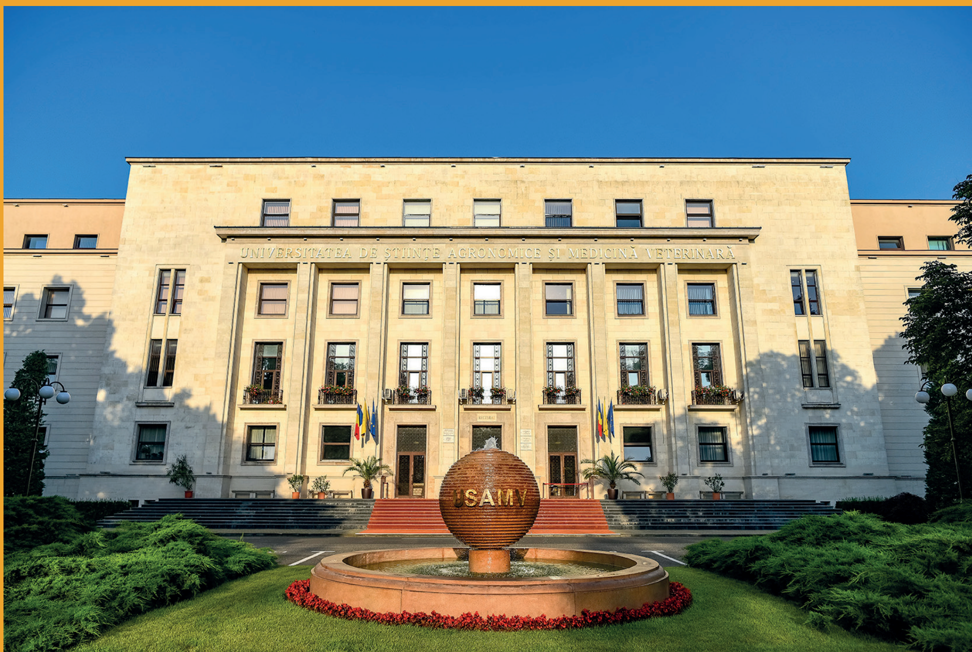

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