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IN VITRO IMPACT OF CONCENTRATION AND ADDITION METHODS OF PLANT HORMONS ON PEACH (*Prunus persica* L. Batsch) MICROGRAFTING

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Abstract

Florin peach variety and Myrobalan 29C rootstock were included in the experiment. For the preparation of graft and rootstock, two methods were tested for this operation: I, submerging the explants resulting from the initiation step in solutions containing NAA and IBA in concentrations (0.00 control, 0.01, 0.50, 1.00 and 2.00) mg/l respectively and II, take the explants resulting from the initiation step and grow these explants into culture media tubes containing NAA and IBA in concentrations (0.00 control, 0.01, 0.50, 1.00 and 2.00) mg/l respectively. For the application of micrografting, two methods were tested: I, Micrografting before rooting and II, Micrografting after root formation. Results show the role of IBA in rooting the explants that gave the highest rate of root number (7.00 root/explant) and root length (9.07 cm root length/explant) at all concentrations studied with the same NAA concentrations. Also, the method of submerging explants (immersion in solutions containing rooting hormones and then planting them in hormone-free culture media) was superior to the number and length of roots produced by the method of adding rooting hormones to culture media shoots.

Key words: *explants, culture media, node, rootstock , Myrobalan 29C.*

INTRODUCTION

Grafting is the process of attaching a part of a desired plant to another plant that has some qualities that resist biotic and abiotic stresses. The first is called (scion) graft, while the second is rootstock. Used by the Chinese since 1560 B.C. (Melnyk and Meyerowitz, 2015; Kumari et al., 2015). Grafting is one of the methods of propagation in trees, we resort to grafting to propagate species and varieties with good specifications, high productivity, and diseases free (Murashige et al., 1972), which cannot be propagated by cuttings, Layering or other methods of vegetative propagation. The compatibility between rootstock and scion plays a major role in the success of the propagation process by grafting (Canan et al., 2006; Goldschmidt, 2014; Warschefsky et al., 2016). Grafting is a common method in the production of compound plants, and it depends on the growing season. The failure of the grafting process means waiting for the next growing season, and this means a loss of time, effort and money. To get rid of this problem, the technique of micrografting was used on *in vitro*, which is conducted carefully and under controlled conditions of temperature, humidity, lighting and free of pathogens (Murashige et al., 1972).

Micrografting technology is used to produce seedlings free of viral, bacterial and fungal infections and according to market needs (Navarro, 1981). The first successful micro-grafting process was carried out by Navarro et al., (1975) studies to obtain citrus seedlings free of pathogens, especially viruses.

Today, *in vitro* micrografting technology is widely used in the production of many types of fruit trees, especially citrus trees that are free of viral infection (Navarro, 1981).

This method is based on that the apical meristem does not contain viruses for the growth of meristem cells in the apical buds faster than the reproduction of viruses and that the grafting process is applied under controlled conditions and cultures. (DeLange, 1978). Many methods and techniques have been developed that help the success of the micrografting process on *in vitro* (for fixation of the grafting area), like filter paper bridge by Huang and Millikan (1980); elastic bands by Jonard et al. (1983); translucent silicon tubes by Gebhardt and Goldbach (1988); tubes, nylon strips, aluminum foil tubes, double-layer aluminum foil devices and absorbent paper by Obeidy and Smith (1991). The aim of the study is to know the methods of micrografting of peach seedlings and the effect of concentration and method of adding auxins on the rooting process of seedlings used in the micrografting process.

MATERIALS AND METHODS

The study was conducted at the micropropagation laboratory, Centre for Studies of Food and Agricultural Products Quality, USAMV of Bucharest, Romania. On Peach (*Prunus persica* L.), Florin peach variety and Myrobalan 29C rootstock were included in the experiment. Nodes explants were taken at 0.5-1 cm length. All explants were washed by tap water to 30 min. Primary sterilization was done by put the explants in 70% ethanol to 2-3 minutes, after which the alcohol was removed by washing with distilled water 3 times under the sterile hood. The explants were sterilized on the surface with NaOCI (10% v/v) for 15-20 minutes, and then rinsed with distilled water at least three times (AL Ghasheem et al., 2018).

MEDIA AND CULTURE CONDITIONS

Explants were grown on 1/2 MS (Murashige and Skoog, 1962) medium supplemented with 14 g/l Sucrose, 3.5 g/l Agar, pH adjusted to 5.7 was filled into test tubes and sterilized by autoclaving at 121°C for 20 min. The parameters of the growth chamber were maintained at 22°C, 2000-2500 lux with a relative humidity of 80-85% for each treatment.

Graft and rootstock preparation

Two methods were tested for this operation:

Method I: immersion of the explants resulting from the initiation stage in solutions containing hormones that stimulate rooting at NAA and IBA concentrations (0.00 control, 0.01, 0.50, 1.00 and 2.00) mg/l, respectively. Separated for 5 minutes, then cultured explants in tubes of culture medium containing GA3 1 mg/l and then placed the tubes of culture medium in the incubation room.

Method II: The explants resulting from the initiation stage were taken and cultured in tubes of culture medium containing GA3 1 g/l with NAA and IBA rooting hormones in concentrations (0.00 control, 0.01, 0.50, 1.00 and 2.00) mg/l respectively, then separately placed the tubes of culture medium in the incubation chamber.

Application of micro-grafting

Two methods were tested for this operation:

Method I: Micrografting was performed before root formation. The explants resulting from the initiation stage were taken, the shoots were removed from the node on the rootstock, after which the micro-grafting was performed by placing the graft (node containing 1 shoot) on the rootstock and then the explant was placed in test tubes with medium containing GA3 1 g/l and hormones that stimulate the rooting of NAA and IBA in concentrations (0.00 control, 0.01, 0.50, 1.00 and 2.00) mg/l respectively, then separated the culture medium tubes were placed in the growth chamber.

Method II: Micrografting was performed after root formation. The explants were transplanted into perlite and sand containers (1: 1) for the purpose of the acclimatization process, and then transferred to the greenhouse.

STATISTICAL ANALYSIS

A completely randomized design (CRD) was used in experiment with three repetitions, 10 replicates per treatment were used with 1 shoot/tube. After the shoots were incubated in the rooting medium for 8 weeks, data on the number of roots per shoot and the average root length (cm) were recorded. Significance of differences between the results was estimated by Analysis of Variance (ANOVA) on SPSS version 14 (SPSS 2005) program with the means compared with LSD test at < 0.05.

RESULTS AND DISCUSSIONS

Effect of IBA and NAA auxins on roots number formed

The results (Table 1) showed that contamination and no roots were formed for the grafts (Florin) in all the treatments used in the experiment, while the rootstock Myrobalan 29C was resistant to contamination and managed to form roots.

Table 1. Percentage of rooting and contamination on Florin variety and Myrobalan 29C rootstock.

Variety	% of contaminants	% of rooting
Florin	40.92%	0.00%
Myrobalan 29C rootstock	13.11%	81.56%

In addition, the hormone NAA showed a tendency to induce callus or bud death in high concentrations. It was confirmed by Swistowska and Kozak (2004) that the addition of NAA to the media leads to the formation of callus at the base of shoots in the tissue culture of *Columnea hirta*, while the hormone IBA has a tendency to take root. Analysis of variance (Tables 2 and 3) revealed that the treatment had highly significant effect on mean roots number and roots length. The results showed that the hormone IBA determined the formation of 7.00 roots/explant, higher than the average number of roots formed compared to the hormone NAA (3.66 root/explant).

Figure 1. Appearance of roots in Myrobalan 29C rootstock (0.50 mg/l IBA) by method of explants submerging in solutions containing hormones. Data were taken after 4 weeks of culture.

The results showed that the use of the method of immersing explants in solutions containing hormones is superior to the variant in which hormones were added in the culture medium, the highest root rate was recorded at the hormone IBA (7.00 roots/explant in method I compared to 5.66 roots/explant in method II) and the hormone NAA (3.66 roots/explants in method I compared to 3.00 roots/explant in Method II).

The study showed that the concentration (control 0.00 without hormone) did not show any root in the rootstock Myrobalan 29C compared to other concentrations; the concentration of 0.50 mg/l IBA gave the best results in root formation. The results showed a clear effect of the addition of auxins to the culture media compared to the hormone-free culture medium.

These are similar to what were confirmed by (Miri, 2018) studies to obtain from testing different concentrations of three auxins (IAA, IBA and NAA) on rooting apple roots stock M.9 and M.26 (*Malus pumila* Mill.) on *in vitro*, he was found that the treatment was (0.5+0.5 mg/l IAA+IBA and 0.1 mg/l NAA) respectively, gave the highest results. Also, similar studies by Ali et al. (2009) when different concentrations of auxin (NAA and IBA) were used on olive shoots, he was found that IBA auxin concentration (1.5 mg/l) gave the best results.

Auxins are necessary for the formation of roots, plants need it in small quantities the auxin indole-3-acetic acid (IAA) is producing at apex of the buds and is transferred to the base of the buds (Muday and DeLong, 2001; Casson and Lindsey, 2003). Auxins activate the synthesis of RNA, and mRNA provides energy through its activity, which in turn is associated with the processes of oxidation of nutrients and the formation of enzymes necessary for growth (Rabechault et al., 1976). Studies have confirmed that genes that contribute to the construction of auxin are expressed within the roots (root apex) and auxin, is turn contributes to the induction and growth of roots (Leung et al., 2005 and Peterson et al., 2009).

Figure 2. Emergence of root from callus in Myrobalan 29C rootstock, 2.00 mg/l NAA by placing the explants into a culture medium containing hormones. Data were taken after 6 weeks of culture

Effect of IBA and NAA auxins on the resulting root length

The results showed that the use of the method of immersing explants in solutions containing hormones is superior to the method in which hormones were added to the culture medium. The highest average root length was recorded with the hormone IBA (9.07 cm root length/explants in method I compared to 6.36 cm root length/explants in method II) and the hormone NAA (5.50 cm root length/explants in method I compared to 5.40 cm root length/explants in Method II).

Figure 3. Appearance of roots on Myrobalan 29C rootstock, 0.01 mg/l NAA by method of explants submerging in solutions containing hormones. Data were taken after 8 weeks of culture

The results showed that the hormone IBA leads to better results (9.07 cm root length/explants) compared to the hormone NAA (5.50 cm root length/explants). The concentration of 0.50 mg/l IBA gave the best results. Our results are similar to studies (Dabski and Parzymies, 2007) on *Hebe buchananii* (Hook) and *Hebe canterburiensis* (JBArmstr.) 'Prostra, when using different concentrations of auxins (IAA, IBA and NAA), IAA with IBA 2.5 and 5.0 mg/l treatments gave the highest average roots length.

Auxins effect has been confirmed in several studies by effect of IBA on root length such as (Ma et al., 1998; Leonardi et al., 2001; Swamy et al., 2002; Wawrosch et al., 2003; Dabski and Divya et al., 2008; Bhatt and Chouhan, 2012; Ullah et al., 2013).

Micrografting methods

Method I: grafting by placing the graft on the rootstock before root formation at the rootstock: The results showed the success of callus in the micrografting area, but it was observed that the roots were not formed by rootstocks, despite the passage of 8 weeks from the micrografting process.

Figure 4. Grafting by placing graft (Florin) on Myrobalan 29C rootstock before root formation on rootstock (Method I). Data were taken after 4 days of grafting

It was also found that most of the shoots stopped growing or withered after 4 weeks of micrografting, perhaps the reason for the stunted growth is due to the consumption of carbohydrates stored in the buds (Table 4).

Figure 5. Method I, Florin's micrografting on Myrobalan 29C rootstock. Data were taken after 4 weeks of grafting

Figure 7. Shoots of Myrobalan 29C rootstock after 4 weeks of culture

Method II: Grafting by placing the graft on the rootstock after root formation at the rootstock:
The results showed the success of the grafting process in this way, compared to the first method. The resulting shoots were placed in plastic containers containing previously sterilized perlite and sand for the purpose of the adaptation process.
Our results are similar to studies by Tangolar et al., (2003) the on micro grafting in two grape varieties (Early Cardinal and Yalova incisi) grafted on four rootstocks (Dogridge, Salt Creek, 1613 C and 41 B).
Micrografting has been used successfully in many studies such as: Apples (Huang and Millikan, 1980); pistachio (Abousalim and Mantell, 1992; Onay, 2002); cloves (Mneney and Mantell, 2001); grapes (Tangolar et al., 2003); avocado (Raharjo and Litz, 2003; 2005); apricots (Piagnani et al., 2006); cherry (Amiri, 2006; 2007); walnut (Wang et al., 2010); almonds (Yıldırım et al., 2013; Isıkalan et al., 2011).

Figure 8. Method II, grafting prepares, by placement of Florin graft on Myrobalan 29C rootstock after roots formation on Myrobalan 29C rootstocks.

Figure 6. Shoots that have formed have ceased to grow or have wilted - Method I, Florin on Myrobalan 29C. Data were taken after 6 weeks of grafting

Figure 9. Grafting process by placement Florin graft on Myrobalan 29C rootstock (Method II)

Table 2. Effect of IBA and NAA auxins on roots number formed/explant at rooting phase on the Myrobalan 29C rootstock. Data were taken after 8 weeks of culture

Hormons \ Concentrations		0.00	0.01	0.50	1.00	2.00	Mean
NAA	I	***	***	***	**3.66±0.28**	3.00±0.18	1.33±0.08
	II	***	**3.00±0.33**	***	***	***	0.60±0.07
IBA	I	***	4.00±0.46	**7.00±0.85**	4.66±0.21	***	3.06±0.18
	II	***	3.33±0.23	**5.66±0.68**	4.33±0.20	***	2.66±0.28
Mean		***	2.58±0.49	3.16±0.10	3.16±0.66	0.75±0.41	

***shoots did not produce roots.

Table 3. Effect of IBA and NAA auxins on roots length formed/explant (cm) at root stage on the Myrobalan 29C rootstock. Data were taken after 8 weeks of culture

Hormons \ Concentrations		0.00	0.01	0.50	1.00	2.00	Mean
NAA	I	***	***	***	4.22±0.39	**5.50**	1.92±0.10
	II	***	**5.40±0.57**	***	***	***	1.08±0.19
IBA	I	***	7.06±0.66	**9.07±0.78**	6.70±0.58	***	4.57±0.46
	II	***	5.30±0.52	**6.36±0.54**	4.43±0.33	***	3.22±0.32
Mean		***	4.44±0.33	3.85±0.23	3.83±0.37	1.35±0.11	

***Shoots did not produce roots.

Table 4. Percentage of callus formed at the graft area and rooting on Florin variety and Myrobalan 29C rootstock

Micrografting	% of callus formed at the graft area	% of Root formation	Grafting status
Method I	66.21%	0:00 %	Shoots stopped growing or withered after 4 weeks of micrografting.
Method II	73:55%	69:00%	Grafting success

Figure 10. Acclimatization stage, shoots resulting was placed in plastic pots covered with glass caps, Florin graft on Myrobalan 29C rootstock. Data were taken 1 week after grafting

CONCLUSIONS

The study demonstrated the success of the method of micro-grafting multiplication of peach varieties resulting from tissue culture technology and can be used in the production of a large number of seedlings without pathogens.

These results also show the role of IBA in rooting explants that gave the highest rate of number of roots formed (7.00 root/explant) and the longest root length (9.07 cm root/explant length) at all studied concentrations compared to the same NAA concentrations. Also, the method of immersing explants in solutions containing rooting hormones and then planting them in hormone-free culture media was superior to the number and length of roots produced by the method of adding hormones to culture media.

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AN OVERVIEW OF APRICOT BREEDING PROGRAMS FOCUSED ON PRODUCTION IMPROVEMENT, FIELD RESISTANCE AND HIGH-QUALITY FRUITS

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Abstract

Scientific knowledge through studies with fundamental and applied research has revolutionized plant breeding over the past decades. Given the importance of the fruits for their nutritional, health, and industrial value, the apricot breeding programs evolved continuously. The integrated use of actual gene banks, multiple crossings, selection, and the use of genetic engineering programs lead to improve apricot trees performance. Meanwhile, the production of resistant cultivars to harsh environmental conditions and climate changes, especially to hard winter temperatures and late frosts, disease-resistant, with early maturity, high-quality fruits, and large production is the main objective. This article aims to provide an overview of achievements in the breeding and improving the production of apricot fruits in the world, highlighting the modern challenges in apricot orchards. Recently, digitalization and mechanization can be involved as useful tools in breeding studies and orchard monitoring and management.

Key words*: core collection, crossing, early selection, disease-resistant, digitalization.*

1. Origin and distribution

The scientific name of the apricot is *Prunus armeniaca* L., being part of the Rosaceae family, and one of the most valuable fruit trees in the world (Zhang & Zhang, 2003). The word apricot chosen by the roman, is derived from the combination of the word "praecocia" meaning early matured, and "albarquq" meaning short ripening period (Guclu et al., 2009).

According to the available documents, the origin of apricots dates back to 5,000 years ago in China (Zhang & Zhang, 2003). Some sources consider this plant to be native to northern China. Wild species of this plant have been seen from Japan to Afghanistan. The Romans called this plant “the Armenian apple”, and based on this issue, some researchers believe that this fruit originates from Armenia (Punia, 2007).

The apricot has been planted at the beginning of the first century AD in this country. This plant was transferred to Anatolia in the fourth century BC due to Alexander the Great's travels to Iran. During the Roman-Iranian wars in the first century BC, the apricot plant was first transported to Italy and then to Greece. Finally, the apricot plant was transported to Spain and England in the thirteenth century and to France and the United States in the seventeenth century (Buttner, 2001). There are about 3,000 apricot cultivars in the world, 2,000 of which are found in China (Wang & Liu, 1998).

2. Nutritional value and production of apricots

Apricot is a fruit with high nutritional quality that is cultivated in the temperate climate of the world (Bhat et al., 2013). This fruit is highly valued among consumers due to its early ripening, beautiful colors, and high-nutritional-quality (Zhang & Zhang, 2003). Apricot fruit contains sufficient amounts of glucose, sucrose, fructose, antioxidant compounds such as lycopene, β-carotene, and vitamins A and E, and also minerals such as potassium, phosphorus, and magnesium (Muradoğlu et al., 2011).

Apricot is an important food source and its consumption was recommended in the presence of vitamin A deficiency, in case of depression, mental disorder, stress, anaemia, physical and mental fatigue (Iordanescu & Micu, 2012). In addition, the consumption of apricots strengthens the body's nervous system and increases the body's immune responses. Due to its alkaline properties, its consumption helps maintain the balance of acidity of blood and body tissues (Drogoudi et al., 2008).

Apricot fruit can be eaten fresh, industrial, dryed and deep freezing (Milošević & Milošević, 2019b). In recent years, in Henei Provence, as the second-largest area under cultivation of apricot in China, there has been a significant increase in the area cultivation and plant performance (Wu et al., 2018b).

According to FAO statistics (2022), in the last twenty years, the average apricot production in the world was 3,719,974 million tons., the highest percentage (57.5%) being of Asia mainly due to the large farms established (Turkey, Iran, Uzbekistan, Pakistan) and Africa (Algeria, Morocco, Egypt). It is followed with 25.4% by the European production, that increased at a lower rate, while in North America and Oceania production has decreased. Turkey ranks first in the world in production with an average of 624,256 tons among the ten apricot-producing countries in the past twenty years (FAOSTAT, 2022).

3. The purpose and research conducted to enhance apricots crop quality

Today, apricot research has been done in various fields, such as: evaluation and improvement of fruit quality characteristics (Ilhan et al., 2020; Wang et al., 2018), the effect on the apricot based on morphological and physiological characteristics of the scion (Yordanov et al., 2018), identification, management and resistance to various types of diseases (Lambert et al., 2020; Suran et al., 2020), investigation of physiological characteristics of the plant and environmental conditions affecting it (Kwon et al., 2020), identification of genomes of different apricot cultivars and study on native and wild cultivars (Ruiz et al., 2020; Li, 2018), introduction of new cultivars (Milatović et al., 2018), fruit postharvest studies (Ledbetter, 2012).

4. Breeding methods

Plant breeding is done by two groups including farmers and specialized breeders (Breseghello & Coelho, 2013). The goal of apricot breeding programs was to develop cultivars for fresh and processed use, starting with the ability to adapt to different ecological conditions and the ability to cultivate in different areas. The first controlled apricot pollination took place in the Soviet Union. Kostina's collection of apricot cultivars at the Nikiski Botanic Garden prompted her to work as a breeding researcher.

More than 150 years ago, apricot breeding programs began in the United States, Canada, and Romania, followed by Hungary, Yugoslavia, France, Italy, Greece, and Yugoslavia (Layne et al., 1996). Hybridization techniques such as pollination, seed collection, and check seedling were first used in the apricot breeding process (Layne et al., 1996).

In traditional plant breeding, the selection is based on the appearance of the plant. However, plant breeding is controlled by specialists through controlled fertilization and sequential checks of new compounds using molecular markers (Breseghello & Coelho, 2013; Batnini et al., 2016).

In the early years of breeding activities, apricot breeding focused on the collection and exploitation of germplasm resources from all geographical areas of China. From the 1980s, hybridization programs began at several pomology institutes. However, new cultivars with acceptable features emerged through controlled interbreeding. Since the 1990s, biotechnology, marker-assisted selection (MAS) and molecular breeding have been widely used in fruit tree breeding programs (Wang et al., 2014b; Liu et al., 2015).

4.1. Hybridization

In apricot breeding, standard breeding techniques are used such as: cross-breeding by emasculation, manual pollination, self-pollination, open pollination (Krška & Vachun, 2016). Hybridization is one of the important methods in apricot breeding. By selecting the optimal parent, the obtained hybrids that have their own unique feature can be used in subsequent breeding programs and also in cultivation for commercialization (Nesheva et al., 2020). In a study conducted in Bulgaria at The Fruit Growing Institute, the use of the hybridization method was able to produce hybrids with better quality fruits. Parents 'Lito' and 'Silistrenskaranna', 'Modesto' and 'Harcot' and 'Harlayne' and 'Harcot' were used in this study, and at the end of the experiment, it was found that the hybrids from the parents of 'Modesto', 'Harcot' and 'Harlayne'×'Harcot' had larger fruits and its TSS content was more than 15° Brix. In addition, the highest inheritance of PPV-resistant alleles was observed in hybrids derived from 'Harlayne'×'Harcot' parents. Interspecific hybrids between *Prunus salicina* Lindl and *Prunus armeniaca* L. are called 'Pulmcot'. This hybrid produces high-quality fruit for sale, and some of them are resistant to the PPV virus and do not show any incompatibility (Bassi & Audergon, 2006). In the Czech Republic, hybridization programs have been used to select suitable parents to produce hybrids that are resistant to disease, cold, and frost and have a long dormancy period and also high-quality fruit (Nečas et al., 2020).

After phenological, pomological studies, and performance analysis in 3718 hybrids from 42 crosses with the aim of producing late cultivars, it was found that 12 genotypes had promising results (Cross et al., 2018). In Turkey, the hybridization method in 'Hacıhaliloğlu' cultivar and cultivars resistant to monilinia disease has been used to produce monilinia resistant cultivars; according to phenological observations and characteristics of fruits and plants, promising results have been obtained (Bilgin et al., 2020a). In a study conducted in the Samara region, after interspecific and interstitial hybridization, 9 cultivars of apricot were produced, and finally, 4 cultivars were registered in the Russian Federation. The flower buds of these cultivars and their wood had a high resistance to low temperature during dormancy. The 'Samarskij', 'Vnuchok', and 'Elita no.1' cultivars had high performance. Ripening time, weight, and taste of fruit varied according to cultivar, air temperature, and rainfall. The best fruit flavor was observed in 'Andryushka', 'Bojcovyj', 'Seyanec Voenkomovskij', 'Trofej' cultivars, and the largest fruits were observed in new 'Avdeevskij', 'Andryushka', 'Bojcovyj', 'Seyanec Voenkomovskij' cultivars (Mini et al., 2020). 16 plants were obtained from hybridization of native plum and black apricot (*Prunus domestica* L. x *Armeniaca dasycarpa* Ehrh), and finally, 12 plants were introduced as hybrids. In the resulting hybrids, a wide range of dominance of morphological characteristics of native plums was observed. Black apricot-related traits in hybrids were poorly displayed in seven hybrids. However, some of the most important characteristics such as resistance to biological and abiotic stresses, growth inhibition, performance, maturity, and high fruit quality were combined in these hybrids. The aim of obtaining the hybrids from this study was to select and modify new cultivars of local plum, to produce tetraploid and hybrid cultivars of black apricot, and to modify and select ordinary apricots with some valuable traits of plum (Soldatov & Salaš, 2007).

Interspecific cross-breeding is one of the most common methods in breeding fruit trees (Arbeloa et al., 2006). The intraspecific hybridization method is also used to produce scions in apricots and the interspecific crossing method between *Prunus* species is used to improve the rootstock and the features of the scion (Bassi & Audergon, 2006). At interspecific cross-breeding, the number of fruits formed is generally lower than the pollinated flowers (Szymajda et al., 2015).

In interspecific hybridization in *P. armenia* L., *P. salicina* Lindl, *P. cerasifera* Ehrh, and *P. salicina* × *P. cerasifera* hybrids it was found that the percentage of fruit formed was directly related to the pollination of crossed species. Most fruits were observed in the cross between *P. salicina* × *P. armeniaca* and *P. salicina* × *P. cerasifera*. According to the results, adaptation and efficacy in crossbreeding were more affected by the mother's parent genotype of *P. salicina* compared to the paternal parent genotypes of *P. armeniaca* and *P. cerasifera* (Szymajda et al., 2015). In a study that aimed to investigate pomological factors and fruit formation, the effect of intra-species hybridization in some apricot cultivars (*Prunus armeniaca* L.) and interspecific hybridization in apricot and paternal parent plum cultivars (*Prunus salicina* L.) was studied and it was found that crossbreeding combinations of 'Black Amber' plum cultivar provided better results. In crossbreed hybrids, 'Palstein' group hybrids showed better performance index (Yaman & Uzun, 2020).

According to breeding principles, apricot breeding by hybridization is very slow due to the long seedling period and high heterozygosity.
Some research was done to introduce suitable cultivars. 'Darina', 'Dobrzyńska', 'Harostar', 'KijewskijKrasen', 'Krojczynka', 'Apricot from Ukraine', 'Pietropawłowskij', 'Poleskij Krupnopłodnyj', 'Veharda', 'Hungarian Early' cultivars are capable of growing in Polish climates and are suitable for cultivation in these climatic conditions (Sitarek, 2020). In order to produce late-maturing cultivars, the following cultivars can be used: 'Da-Huang-Hou' (from China); 'Geogdzhanabad' and 'Voski' (Iranian-Caucasian group); 'ManduleRogni', 'Morava', 'Hersonsky 23', '16-15-9a', 'Original', 'Saratovsky Rubin' (European group); 'Luchak Sumbarsky', 'KokPshar', 'Khurmai', 'Zard' (Central Asian group) (Gorina et al., 2020). 'Ananasny Avgustovsky', 'Bagration', 'Zapozdaly', 'Kazachok', 'Stepnyak Orangevy', 'Professor Smykov', 'Altair', 'Pasynok', 'Jupiter', 'Krasny Krym', 'Iskorka Tavridy', 'Vynoslivy', 'Ozornik' cultivars can also be used in variable climates, but it should be noted that these cultivars are late or medium flowering (Gorina et al., 2020).

4.2. Molecular markers

Molecular markers have made possible to study genetic diversity and association in plants and improves the use of different genotypes in breeding programs and ultimately facilitates the design of new crosses (Sánchez-Pérez, 2005). The use of isozymes was first introduced by Byrne & Littleto (1989) (cited by Zhebentyayeva et al., 2012). In a short time, the use of molecular markers replaced the use of isozymes (Zhebentyayeva et al., 2012; Ruiz et al., 2011).
Apricot genomic sequencing is also one of the strategies that lead to shorter stages of breeding and production of new cultivars (Teber et al., 2020). Today, molecular markers can be used in apricot breeeding. RLFP (Restriction Fragment Length Polymorphisms), AFLP (Amplified Fragment Length Polymorphisms) and SSRs (Simple Sequence Repeats), AFLP-RGAs (AFLP markers targeting the Resistance Gene Analogs), AFLP-cDNA are molecular markers used for this purpose (Zhebentyayeva et al., 2012).
The use of SNP (single nucleotide polymorphisms) marker is one of the ways that can be used in apricot breeding programs with the aim of selecting PPV resistant seedlings and the result is a faster selection of cultivars (Lambert et al., 2020). Nowadays, selection using molecular markers in the breeding programs has made significant progress and is one of the efficient ways in experiments to improve the quality of apricot fruit in new cultivars (Ruiz et al., 2020).
The use of SSR molecular markers in different apricot cultivars from different regions showed that the use of this method made it possible to molecularly identify all apricot genotypes. According to this method, apricot genotypes were divided into seven main groups according to origin and pedigree (Sánchez-Pérez, 2005). Examination of 14 apricot hybrids and 6 of their parents based on morphological and molecular indices revealed that Ay × P3 cross was a promising genotype related to early maturity and fruit indices. Finally, 224 bands could be scored with 8 combinations of SRAP primers (25 bands), 8 DAMP primers (81 bands) and 18 ISSR bands (118 bands); which showed a great variety between hybrids and cultivars. In addition, a total of 4 S-RNase alleles (SC, S2, S3, S6) were identified that Sc and S3 alleles were more widely present than other alleles (Pinar et al., 2017). Another efficient molecular method in apricot breeding programs is Marker-Assisted Selection (MAS) (Ruiz et al., 2011; Zhebentyayeva et al., 2012). This method can be used in traditional hybridization techniques, especially in interspecific crosses (Zhebentyayeva et al., 2012). The MAS method is very efficient and practical for fruit trees with long seedlings such as apricots. The MAS method has been used to identify PPV-sensitive cultivars in some studies (Auvinet et al., 2020).
Gene mapping in some apricot hybrids is another breeding method in this plant. In this method, two characteristics of PPV resistance and self-incompatibility have been investigated (Zhebentyayeva et al., 2012). Preserving plant genetic resources and using them in research should be a priority for agricultural research. In research on apricots, DNA extraction from

older cultivars and the use of SSR polymorphisms make it possible to preserve and identify these cultivars and prevent genetic erosion in the plant. This method is also effective in identifying cultivars that have only seeds (Martín et al., 2011).

Genetic analysis of a group of 202 apricot plants using 13 microsatellite markers or SSRs, which included introduced breeding cultivars and native cultivars in several countries and it was found that the diversity of parameters in modified plants was higher compared to native plants. However, a decrease in genetic diversity was seen in recent breeding programs. Using phylogenetic analysis based on Nei genetic distance, two main groups were created. The first group consisted mainly of traditional American and European cultivars, and the second group included commercial species created by breeding programs. Population distribution in each group was close to each other, which confirmed the results of molecular phylogenetic analysis. Finally, it should be noted that genetic diversity in apricots is currently declining and one of the most important solutions is to preserve traditional cultivars and local germplasms as genetic resources in breeding programs (Herrera et al., 2021).

In the apricot plant, microsatellites have been used to study the evolutionary history of the plant, to describe and study traditional cultivars, and to study local cultivars in Spain, Turkey, Tunisia, and Iran (Bourguiba et al., 2020; Liu et al., 2019; Hormaza., 2002; Murathan et al., 2017; Bourguiba et al., 2013; Batnini et al., 2016; Raji et al., 2014).

The study of genetic diversity in apricots is performed by SSR (Sheikh et al., 2021a).

In a study, sexual incompatibility and S allele diversity were studied using PCR (polymerase chain reaction) and sequencing techniques in 24 cultivars of Turkish apricot, Paviot and Sakit-1 as parents and 127 F1 breeds. The results showed that most Turkish cultivars were self-incompatible. The Sc allele, indicating self-compatibility in the plant was observed in only three cultivars. In 124 F1 breeds, 2 S alleles of Paviot were inherited by half of the breeds; 76% of the breeds inherited the S52 allele from Sakit-1 and less than 24% from the S20. Amplifying was successful with all 18 SSR primers (Murathan et al., 2017).

The SSR molecular marker was effective in differentiating 48 apricot genotypes and finally UPGMA cluster analysis grouped the genotypes based on pedigree information and their origin (Hormaza, 2002). Molecular markers make it possible to describe the germplasm in apricots. In a study, 890 apricot genotypes with different origins were used. Finally, 5 different genetic clusters were obtained based on the Bayesian model. Accessions from China and Central Asia are in the same cluster and show the highest diversity resulting from a region and origin. In general, the results showed that apricots originated and expanded from China and Central Asia. Identification of specific alleles outside the centre of origin confirms the existence of secondary variability.

The existing database provides the conservation of apricot genetic resources and is also used in its breeding programs (Bourguiba et al., 2020).

In a study of 27 Iranian apricot cultivars, based on morphological indices and molecular markers, it was found that there is a high diversity in relation to fruit weight, total soluble solids (TSS) and titratable acidity (TA). High correlations were observed between fruit size, pit and kernel, TSS and TA. In the molecular study, 53 alleles were identified and UPGMA cluster analysis divided the studied apricots into seven groups (Raji et al., 2014).

Molecular markers have been used in the study of domestication of wild apricot cultivars and it has been found that apricot is a suitable model for the study of speciation and domestication in perennial fruit trees with long life (Liu et al., 2019).

In a study using SNP markers, a total of 267 allelic compounds were identified in 37 studied apricots. The use of SNP markers is effective in studying genetic diversity in apricots and in determining the relationship between their accessions, which is due to pedigree relationships (Salazar et al., 2015). In the study of genetic diversity and determining the relationship between different apricot cultivars using SRAP (Sequence related amplified polymorphism) it was found that the similarity between Turkish apricot cultivars and foreign cultivars was between 0.77 to 0.97%.

Significant genetic diversity was identified in Turkish cultivars and between Turkish and foreign apricot cultivars. The four cultivars with high cold requirements originated in eastern Turkey and were placed in separate clusters. No clear grouping was observed between European, South African, North American, and other Turkish cultivars. Finally, it can be concluded that these cultivars have similar genetic backgrounds due to their different geographical origins (Uzun et al., 2010).

The AFLP molecular marker has been successful in identifying the apricot gene mapping in some of the studied traits, such as disease resistance (Hurtado et al., 2002). Evaluation of genetic diversity in older Spanish cultivars was performed using microsatellite molecular markers and the results showed that the samples were derived from four closely related genotypes and one of them includes 89% of the samples (Martínez-Mora et al., 2009). Molecular markers in the apricot plant play an important role in the study of molecular properties and genetic linkage mapping (Ruiz et al., 2011). Apricot breeding programs focus primarily on several specific purposes for the production of commercial cultivars: Self-compatibility, PPV resistance, fruit quality and development of fruit ripening period (Herrera et al., 2021).

4.3. Transgenic plants and *in vitro* culture

Other breeding methods in apricot include transgenic (Zhebentyayeva et al., 2012). Biotechnological techniques are used in the analysis of gene function or the introduction of new agronomic traits in apricots that are not introduced by hybridization or selection (Pérez-Casellaes et al., 2021). Transgenic plants are generally produced through *Agrobacterium*-mediated transmission (Pérez-Casellaes et al., 2021) and direct gene transfer (Ruiz et al., 2011). Genetic engineering allows plants to grow and develop faster, also vegetative propagation of these plants allows them to grow in wider areas. The first plant produced by genetic engineering was introduced to the market in 1994 (Lobato-Gómez et al., 2021). The first transfer in apricot was done by Petri in leaf explants (Petri et al., 2008). Cultivation *in vitro* plant allows the researcher to produce disease-free and virus-free plants. Soma clonal diversity is another method of producing plants with a new phenotype (Ruiz et al., 2011).

Micropropagation is also commonly used in the production of rootstocks (Burgos & Ledbetter, 1993) and virus-free plants in apricots (Mitrofanova et al., 2020). *In vitro* embryo culture can be successfully used as a tool in apricot growing program for large-scale plant production (Burgos & Ledbetter, 1993). By comparing traditional breeding methods with modern breeding methods, it was found that in modern breeding methods, fruit size was increased using controlled fertilization, while self-compatibility and firmness were acceptable. Cluster and structural analysis of SSR data, identified and distinguished different genotypes based on their geographical origin and pedigree. Finally, some alleles related to fruit weight, firmness, and fruit ripening were also identified using SSR molecular markers (Batnini et al., 2016).

5. Breeding programs in apricot plant

One of the important breeding goals in apricot breeding programs includes: improving apricot cultivation, resistance to biological stresses, increasing fruit harvest time, improving fruit quality for fresh eating and processing, and productivity, resistance to bacterial diseases caused by *Pseudomonas* spp., or *Xanthomonas arboricola* pv. *pruni* Smith, biological stresses (Sharka virus, root Brown caused by *Monilinia* spp.), Chlorotic Leaf Roll Phytoplasma, and Apricot Decline Syndrome, and adaptation to different environmental conditions (Zhebentyayeva et al., 2012; Bassi & Audergon, 2006).

5.1. Evaluation and improvement of fruit quality characteristics

Numerous studies have been performed to improve fruit quality in apricots and their focus is on improving fruit quality in the market, improving skin color, fruit flavor, TSS, TA, fruit size, fruit firmness, fruit scent and fruit ripening time and after fruit harvest time. In most studies, the hybrid fruits produced fruit weight, flesh firmness, skin color and marketability are considered (Oprita et al., 2020). Fruit quality is one of the most important goals in breeding program in apricot.

Fruit size and firmness are two influential factors in the performance, marketability, and value of the product (Roussos et al., 2011).

In fresh eating cultivars, breeding goals have been changed from increasing performance to improving fruit quality such as taste, attractiveness, firmness, and aromatic compounds. Using China's unique resources, kernel-yielding apricots are an option used in recent breeding programs. The goal of these breeding programs is to increase performance, sweet kernel, large kernel, and hardness. Also increasing the fruit harvest season and attractive appearance have been other goals of breeding in recent programs in China (Sun et al., 2018). In China, from 2006 to 2013, 46 new cultivars with new features were introduced (Sun et al., 2018). The features of these cultivars were precocity, disease resistance, dwarfism, resistance to various environmental conditions, attractive appearance, long life after fruit harvest, improved fruit quality features, and increased fruit size (Sun et al., 2018).

The goal of apricot breeding programs in Spain is to produce and introduce early maturing and Sharka resistant cultivars (Egea et al., 2012). Many studies have provided satisfactory results in improving the quality of fruits, it was found that spraying the tree with 1 mmol/l salicylic acid is effective in delaying fruit ripening and increasing its shelf life by reducing fruit maturity index (Elsabagh et al., 2020). Also, pre-treatment of apricots using oxalic acid seven days before harvest improved fruit quality traits compared to the control and the concentration of 1 mM oxalic acid had the greatest effect on fruit quality (SevinçÜzümcü et al., 2020).

The use of benzyl adenine (BA) as a foliar spray in the pit hardening stage, improves the quality of the fruit. Fruits treated with 100 ppm benzyl adenine were larger in size and firmer (Canli et al., 2014). Foliar spray of the apricot tree at a concentration of 0.1 mMmelatonin, improves fruit quality and maintains the quality characteristics of the fruit during its storage life and maintenance period (Medina-Santamarina et al., 2021). In a study conducted by Ilhan et al. (2020) in Turkey, the physiochemical properties of wild apricots were investigated and it was found that apricots have considerable antioxidant properties and phenolic compounds, and vitamin C (Ilhan et al., 2020).

Today, the use of molecular markers in improving fruit quality is an effective tool. In addition, molecular methods have been effective in post harvesting apricots and determining genes associated with firmness and softness of fruit tissue (Wei et al., 2018). In a study conducted in the CEBAS-CSIC breeding program to identify genes related to fruit quality in apricots, a combined genetic, genomic and transcriptomic approach was performed. This process had two stages; the first step involved making genetic linkage maps and identifying quantitative trait loci and the second step was RNA sequencing to identify the genes involved. Finally, in this study, the genes responsible for fruit white / orange color, anthocyanin content and carbohydrate metabolism were identified (Ruiz et al., 2020). Hybridization is also an efficient method of producing high quality fruit hybrids.

In 'Hacıhaliloğlu' × 'Boccucia hybrids', high diversity of fruit zone, fruit color, fruit firmness and fruit harvest time were observed. Two factors, fruit color and harvest time, were distinct parameters in genotype selection. Finally, cluster analysis placed 20 genotypes in the same group and other genotypes in different groups (Bilgin et al., 2020b). Hybrid production was produced from the parents of 'Lito' and 'Silistrenska ranna', 'Modesto' and 'Harcot', and 'Harlayne' and 'Harcot' with the aim of investigating PPV resistance and pomological traits. 'Harlayne' × 'Harcot' was the hybrid with the highest frequency of inheritance of PPV-resistant alleles. Full flowering time, fruit ripening time, fruit weight, fruit shape, fruit color and TSS were examined in the resulting hybrids. The results showed that the hybrid fruits of the crosses 'Modesto' × 'Harcot' and 'Harlayne' × 'Harcot' had the largest size (more than 45 g). In addition, the TSS level in these fruits was higher than 15 °Brix (Nesheva et al., 2020).

In another study found that fruit ripening in 'Ariel®', 'Leda®' and 'Alissa®' apricots occurred 95, 89 and 102 days after full bloom, respectively. Also, all of these cultivars are self-fertile. The fruits produced in all three apricots had 30-40% bright red color and orange background, firm but juicy and also had good taste and smell (Bassi & Foschi, 2020). The selection of early apricot cultivar as the maternal rootstock is effective in transmitting this trait to the breeds (Wu et al., 2018a). 'Jinyu' and 'Jinhe' cultivars are new early cultivars derived from the early maternal rootstock in the Plum and Apricot Laboratory of SFRIHAAFS. 'Jinyu' cultivar has high freshness, sweet taste and high content of vitamin C. This cultivar is derived from the intersection of 'Yuzhouhong' cultivar as the maternal rootstock and 'Sungold' cultivar as the paternal rootstock. 'Jinhe' cultivar has a large size fruit, good appearance and high flesh quality and this cultivar is derived from the intersection of 'Zihe' cultivar as maternal parent and 'Xinshiji' cultivar as paternal parent (Wu et al., 2021).

In the study of hybrids obtained from Irano-Caucasian and European groups and some important cultivars of the Eastern Mediterranean, it was found that 'Çağataybey' hybrid cultivar had the highest TSS, phenolic content and the highest levels of antioxidant capacity. In addition, more than 50% of the fruit skin was red (Caliskan et al., 2012). In a research program in Spain, three cultivars 'Mirlo Blanco', 'Mirlo Naranja', and 'Mirlo Rojo' due to their high fruit quality, ripeness and resistance to Sharka were introduced. These cultivars were obtained from a cross between cultivars with moderate cold requirements and early cultivars with low cooling requirements and good quality (Egea et al., 2012).

5.2. Genetic erosion and reduction of genetic diversity in apricots

One of the ways to increase the quality of apricots in the market is to improve the cultivars and select seedlings with high quality. Another way is to introduce cultivars from all over the world in order to select useful and suitable genotypes for cultivation (Krska & Vachun, 2016). In recent years, the introduction of new cultivars has become necessary in most breeding programs. New cultivars are needed to produce apricots that improve performance and profitability (Krska & Vachun, 2016). Research by Herrera et al. (2021) has shown that genetic erosion and reduction of genetic diversity in apricots are currently occurring, and the best method for solving this problem is to pay more attention to existing traditional cultivars and local germplasm as future genetic resources in apricot breeding (Herrera et al., 2021). Decreased diversity in modernization products is defined as genetic erosion. The two main reasons for genetic erosion are the replacement of traditional cultivars with modern cultivars and functions related to breeding programs. Genetic erosion occurs at three levels of product, cultivar and allele (Wouw et al., 2009). Rich genetic diversity is an important resource for breeding programs in plants. However, it should be noted that the reduction of genetic resources and artificial selection in plants, reduces the genetic diversity and potential of plant germplasm (Laido et al., 2013). Consequently, evaluation of the genetic diversity of plant germplasm is necessary to select superior genotypes and conserve endangered resources (Sheikh et al., 2021b; Martín et al., 2011). Preservation of traditional fruit cultivars is essential due to genetic erosion. The use of molecular methods plays a significant role in identifying, retrieving and preserving traditional cultivars (Martín et al., 2010). SSR molecular markers were used to analyze the genetic diversity of the structural population of 120 apricot genotypes in India. Molecular analysis of variance showed that the rate of change within individuals, between individuals and between populations was 73, 25 and 2%, respectively (Wani et al., 2021). ISSR markers were able to separate native genotypes from foreign genotypes and divided the modern apricot genotypes studied in India into two completely separate groups. Determining genetic divergence between native and non-native genotypes plays a significant role in apricot breeding programs (Sheikh et al., 2021). Kashmir Valley and Ladakh region in India is one of the rich germplasms in apricots. Some important apricot genotypes in this region include: Afghani, Raktsey-Karpo, Halman, Australian Sweet, and Charmagz (Wani et al., 2020).

Domestication is one of the reasons for the decrease in genetic diversity due to genetic drift and extinction of wild ancestors (Bourguiba et al., 2012; Li et al., 2020). In a study by Bourguiba et al (2012), it was found that genetic diversity in the Iranocaucasian gene pool as one of the secondary centres of diversity is declining (Bourguiba et al., 2012). In the study of apricot genetic diversity using single-nucleotide polymorphisms (SNPs), it was found that Dzhungar-Ili ecological group had the highest genetic diversity related to the number of private alleles, heterozygosity and nucleotide diversity. The researchers said that the Central Asian ecological group originated from the Dzhungar Ili ecological group and became native. In addition, it has been suggested that the population structure and gene flow of the North China and European ecological genetic group have *P. sibirica* background. *P. armeniaca* originated in Northwest China and later expanded to Central Asia and then to Europe (Li et al., 2020).

Germplasm genetic diversity is necessary to search phenotypic and genetic resources and plays an important role in the sustainability and improvement of crop breeding. It seems that the genetic diversity of ordinary apricot germplasm grown in China is greater than the Western countries. Research studies showed that 27% of the studied genome was identified as introgressed. These introgressed regions have created a high diversity in the germplasm of common apricots grown in China through the introduction of different genes associated with distinct phenotypes from other cultivated groups. Introgressed regions can be said to be an important source of genetic resources and can be used in breeding programs (Zhang et al., 2021).

A study of Siberian apricots in China from 22 populations using molecular markers showed that the number of alleles per loci varied from 5 to 33 (mean 19,323 alleles). Structural analysis showed that all populations are divided into 4 genetic clusters. Based on hierarchical analysis of molecular variance, 94% variation was observed within populations. There was no significant difference between wild and semi-wild groups, which is probably due to the current cultivation conditions of Siberian apricots. In addition, according to Mantel test, genetic distance between populations was not significantly correlated with geographical distance (Wang et al., 2014a). It should be noted that breeding programs with specific pomological aim and the production of new cultivars have reduced genetic diversity in apricots (Corrado et al., 2021).

5.3. Precocious maturity

Precocity is another breeding goal in this plant. According to the consumer market, precocity apricots have more commercial value among consumers (Wu et al., 2021). In fact, many programs focus on improving very precocity apricots. 'Zaojinyan' homologated by Zhengzhou Fruit Research Institute or 'Chunhua' from the Shandong Institute of Pomology in China are results of these programs (Yuan et al., 2019; Huang et al., 2019). 'Chunhua' cultivar is obtained from the intersection of 'Jintaiyang' × 'Honghebao' and the fruit ripens in mid-May. The duration of fruit ripening in this cultivar is about 55 days (Yuan et al., 2019). Two new and early cultivars of 'Jinyu' and 'Jinhe' have been produced at Plum and Apricot Laboratory of SFRIHAAFS. 'Jinyu' cultivar is derived from the cross between 'Yuzhouhong' cultivar as maternal parent and 'Sungold' cultivar as paternal parent. 'Jinhe' cultivar is derived from the cross between 'Zihe' cultivar as maternal parent and 'Xinshiji' cultivar as paternal parent (Wu et al., 2021).

5.4. Disease resistant

Production of disease-resistant cultivars is also one of the important goals of breeding in this plant (Ruiz et al., 2011). Resistance to Sharka (plum pox virus) was studied. Sharka viral disease caused by Plum pox virus (PPV); is one of the significant cases in apricot production in Central European regions that should be considered (Krska & Vachun, 2016). Sharka is one of the most important viral diseases in apricots. The disease was first introduced in Bulgaria in 1917 and then it was seen in some other parts of the world. Undoubtedly, one of the effective ways to control this disease is to create resistant varieties. Recent research has shown that 'Stark Early Orange' and 'Stella' cultivars do not show signs of viral infection after two years of graft (Syrgiannidis et al., 1991). Recently, resistant cultivars such as 'Rojo Pasión' and 'Murciana' have also introduced in Spain. Based on field farm, and artificial experiments, 'Harcot' and 'Bebecou' cultivars were introduced as cultivars resistant to this viral disease (Karayiannis et al., 2008). Three cultivars resistant to PPV were introduced in Spanish breeding programs, including: 'RojoPasión', 'Selene', 'Murciana' (Zhebentyayeva et al., 2012). In Italy, resistant cultivars include: 'Antonio Errani', 'Cafona', 'Fracasso', 'Noumo', 'Paviot', 'Pelese di Giovanillo', 'Portici', 'Stark Early Orange', 'Stella', and 'Veecot' were also introduced using the artificial inoculation-chip budding method on woody indicator GF-305 (Faggioli et al., 2009). 'Goldrich', 'Henderson', 'Harlayne', 'Stark Early Orange' (SEO), 'Stella', and 'LE-3276' are cultivars that are naturally resistant to PPV and can be used in other research (Gorina et al., 2020).

One reliable way to detect viral infection in apricot trees is to use the High-throughput sequencing (HTS) method for sequence analysis (Canbulat et al., 2020). Screening is one of the methods used to find disease-resistant cultivars in apricots (Karayiannis et al., 2008).

Finding resistant cultivars was done based on biological tests described by Audergon & Morvan (1990). In this method, peach seedling 'GF-305', which is very sensitive to this disease and shows the symptoms of the disease quickly, is selected as an indicator of the disease. The studied apricots are grafted on peach seedlings and after inoculation of the virus, the symptoms in apricots and peaches are examined. Finally, the presence of the virus is analyzed by ELISADASI and RT-PCR. At least two growth cycles are required for the final conclusion (Badenes & Lláce, 2006).

Screening of apricot seedlings using molecular markers is one of the best solutions to increase the efficiency of breeding programs (Rubio et al., 2014).

5.5. Apricot rootstock

Scion adaptation, resistance to pests and diseases, adaptation to a wide range of different soil types, and climatic conditions are good plant rootstock characteristics (Cinelli & Loreti., 2004). One of the important things in apricot cultivation is the selection of the rootstock in this plant (Milošević & Milošević, 2019b).

The primary goals of rootstock selection in apricots include scion growth strength, fruit size, function, tree longevity, precocity, rootstock growth strength, graft adaptation, disease and pest resistance, tolerance to specific biological conditions (Zhebentyayeva et al., 2012).

In addition, in arid regions, such as the Mediterranean region, it is necessary to select the appropriate rootstock with the ability to withstand various types of environmental stresses to prevent further problems in the gardens and reduce cost management (Jiménez et al., 2008; Moreno et al., 2008). Suitable rootstock not only affects graft quality but also affects the long-term performance of trees, scion function, fruit size, fruit quality, tree growth, performance efficiency, nutritional characteristics, water, and nutrient absorption (Milošević & Milošević., 2019b; Milošević et al., 2012). The use of cherry plum *(Prunus cerasifera*) rootstock, apricot seedling, and 52 and 38 St. Julien rootstock, prevents grafted trees from being infected with *Phytoplasma* (Suran et al., 2020).

Choosing the suitable rootstock for each cultivar and each climatic condition is an important issue for breeders. Apricot seedlings' rootstocks are mainly used as rootstocks all over the world. However, these foundations are not suitable for heavy soils and waterlogged land (Milošević & Milošević, 2019b). Apricots, peach, and plum seedlings are also used as rootstocks in apricots (Zhebentyayeva et al., 2012). One of the cheapest apricot rootstocks is seedlings, which have acceptable physiological compatibility with scion and are resistant to sulphate and chloride salts. In addition, these rootstocks can tolerate nematodes in the soil. These factors have led to the use of this rootstock in Asia and Europe, but due to the sensitivity of these rootstocks to *Verticillium* wilt and fungal infection (Oak root fungus), mainly plum rootstocks are used commercially for this plant (Kaya et al., 2018).

In acid soils, mainly Myrobalan seedling rootstocks (*P. cerasifera* Ehrh.) and other local plum cultivars derived from *P. domestica* L. and *P. insititia* L. such as 'Belošljiva', 'Petrovača', 'Cerovačkipiskavac' are used. These rootstocks show good compatibility in grafting and have the ability to grow in these soils but they are susceptible to PPV disease (Milosevic & Milosevic, 2010). 'Marianna' (*P. cerasifera* × *P. munsoniana*), 'Myrobalan' (*P. cerasifera*), and 'Pollizo' (*P. insititia*) plum rootstocks grow in different soil conditions but show signs of incompatibility (Moreno, 2008). In some regions of Spain, the rootstocks that are used are apricot seedling rootstocks 'Real Fino' and 'Canino' cultivars and plum rootstocks 'Pollizos de Murcia' (*P. insititia*) (Hernández et al., 2010).

In the Czech Republic, apricot seedling rootstocks can also be used as rootstocks (Vachůn, 2001). In Poland, seedlings of *Prunus divaricata* Ledeb. are mainly used as rootstock (Sosna & Licznar-Małańczuk, 2012; Licznar-Małańczuk & Sosna, 2013).

One of the most important apricot rootstocks in Serbia is the suckers of local (autochthonous) plum called 'Belošljiva'. High compatibility on these rootstocks has been confirmed especially in Hungarian Best compared to 'Myrobalan' (Milošević et al., 2019a). But it should be considered that both rootstocks are not suitable for medium and high-density gardens due to vigorous growth (Milošević et al., 2015).

5.6. The impact of digitalization and mechanization equipment in modern orchards

Currently, one of the important factors in the economic growth and development of agricultural products is digital technologies and their application in the agricultural industry. Today, mechanization of fieldwork, mechanization, and use of mechanical equipment in the production process is part of it (Kovács & Husti, 2018). The use of automatic and robotic machines is associated with labor shortages and increased labor costs and is necessary to increase fruit quality with minimal dependence on manpower. Mechanized orchard management includes: pruning, thinning, spraying, and harvesting (Zhang et al., 2019). Mechanical harvesting of apricots has been introduced by various researchers since the 1960s (Erdoğan et al., 2003).

Mechanization is seen in different parts of apricot cultivation. Mechanical thinning in apricot showed satisfactory results and in addition to increasing the efficiency of thinning, reduced damage to fruits and branches (Assirelli et al., 2021). In another study, mechanical thinning removed 20.8% of flowers and 43.6% of fruits but saved 48% of the time compared to hand-thinning (Assirelli et al., 2018). Mechanical thinning of flowers in stone fruits such as apricots using tractor-mounted string thinners drastically reduces costs and saves time (Lewis, 2015).

The spraying system developed by Xiao et al. (2017) determines the rate of pesticide application using the RGB-D camera, based on leaf area density and distance-to-canopy. This issue increases the efficiency and productivity of the pesticide and reduces the waste in the pesticide spray on apricots, grapes, and peaches (Xiao et al., 2017).

CONCLUSIONS

Apricot is one of the most important stone fruits that can be eaten fresh or processed. Most research on this fruit is associated with improving the quality of the fruit, such as taste, skin color, firmness of the fruit, the amount of TSS, acidity of the fruit, and the shelf life of the fruit.

Selecting the suitable rootstock is another issue studied in most research, which is due to the climatic and soil conditions of the region. In most cases, the research has tried to select the best rootstock according to the climatic conditions of the region to improve tree growth conditions and fruit quality, resistance to diseases and biological stresses in each region, and also early and late ripening of fruit. In addition, other research on this plant has included genomic studies on commercial and local cultivars to select suitable parents to produce new hybrids.

Inbreeding of this plant, it has been tried to produce cultivars that have the best features, according to the breeding goals in each region, through hybridization and molecular markers.

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ALMOND SEED WASP, EURYTOMA AMYGDALI, A NEW FRUIT PEST IN THE ROMANIAN FAUNA

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Abstract

The almond seed wasp (Eurytoma amygdali Enderlein) is a wasp from the order Hymenoptera, Eurytomidae family. The almond wasp is an important pest for almonds in the European, the Caucasus and the Middle East almond growing countries. In Romania the almond crop has regained interest among farmers. Recent observations confirm that this insect is present also in Romania, in the Dobrogea region. Almond fruits attacked by Eurytoma amygdali have been collected from the spontaneous flora, kept in laboratory conditions and microscopically observed, before, in time and after pupation. This paper might be of interest for farmers, pest-control products companies, as well for the pheromone traps R & D industry.

***Key words**: almonds, pest control, ecologic.*

INTRODUCTION

The almond seed wasp (ASW), *Eurytoma amygdali* Enderlein, is the most important pest of almond fruits in Europe. Different authors reported damages up to 50% of the yield, in Bulgaria (Ivanov, 1960), over 70% losses (Zerova & Fursov, 1991), Cakar (1980) reported damages up to 71%, in former Yugoslavia, 35-79% in Greece (Mentjelos & Antjemis, 1970). The presence of the ASW has been reported in many countries in south-eastern Europe, Middle East and some of the Mediterranean countries. In Romania this species hasn't been reported, but Perju (2002) presumed in his book that the ASW can be found in the Dobrogea area.

Over the time, the ASW has been confused with the plum seed wasp, *Eurytoma schreineri* Schr. Schreiner reported in 1908 in Russia, in the Astrakhan region, a pest for the plum seeds, considering being the ASW. Over the years, in 1925, Ustinov finds the same plum wasp in Ukraine. In fact it was a new species, *Eurytoma schreineri* Schr., the differences between the two species, considering the morphologic characters, have been done by Nikolskaia in 1961 (Minoiu & Lefter, 1987).

The ASW is a univoltine and a monophagous species, feeding on different species of almonds, as *Prunus bucharica, Prunus dulcis, Prunus fenzliana, Prunus scoparia* (Golestaneh et al., 2013). It oviposits in March-April into the nucellar tissue of the developing seed in the almond. The larva feeds with the seed until all or almost the entire embryo is consumed. The larva then enters diapause within the intact seed integument. At that time, by the end-May-beginning-June, the fruit shrivels, and it changes from green to grey or yellowish grey (Figure 1). Most of the infested fruits remain attached to the tree past harvest time and well into the next year. Their color gradually turns dark grey to almost black, the change being caused by saprophytic fungi. The larvae pupate in March and the wasps emerge in March-April (Plaut, 1971).

The imago encloses in March-April (end of April in Romania) from the pupa within the infested fruit. It gnaws a hole through the thin parched integument of the emptied seed and through the hard shriveled pericarp of the fruit

(Figure 2), through which it then emerges (Plaut, 1971).
According to Cakar (1980) in Yugoslavia 87.5% of larvae break diapause and pupate in the first year, 12% pass into adulthood in the second year, and 0.5% of larvae remain in diapause more than two years.

Figure 1. Almond fruits shriveled, changed it's color from green to grey or yellowish grey

In laboratory conditions, Plaut (1973) showed that females were attracted to almond fruits and males were not. Fresh almond leaves had little to none, attraction for females.
Nourbakhsh (1998) found that, young almond fruits are sensitive to wasp drilling and usually fall in the first stages of growth. In contrast, the larger ones remain on the tree, and *E. amygdali* larvae feed with their kernel.
Many researchers have tried to understand the behavior of ASW females and on which criteria they choose in which fruit to oviposit. Some presume that certain chemical stimuli (kairomones) emitted from the unripe fruits of almond cultivars might influence the host finding behaviour of females. These compounds may not be present at adequate or specific ratios in all the genotypes and cultivars (Saeidi, 2021).

Flight and other activities were observed and summarized. In sunny weather the wasps concentrated in the morning on the eastern side, and in the afternoon on the western side of the trees. Males were often observed in a flying "dance", oscillating slowly in a horizontal line in a plane parallel to the periphery of the tree, at a distance of about 10 cm from the tree and at amplitude of about 6 cm, singly or in swarms of up to about 60 individuals. Males and females were observed alighting on the upper side of leaves and sitting or walking there, much more often than on the underside. Females were observed on fruits, probing or ovipositing; males were seen to alight nearby and court them. Sometimes two to four females sat on the same fruit, although there was no lack of unoccupied fruit in the vicinity. Plaut (1973) concluded that the wasps tend mainly to remain in the tree crown in which they emerged. No correlation between fruit load and amount of infestation by the almond wasp of individual trees was observed.
The almond trees are supposed to be present in Dobrogea area since ancient times. The Greek colonists, that built many fortresses in the area, on the Pontus Euxin shores, along the Danube and in land, brought with them, among other cultivated species, almonds (Cociu, 2011). The soil is dryer and calcareous, favourable to this crop and in some areas, the almond can be found in the spontaneous flora.
The almond crop is regaining in interest for the Romanian farmers. In Dobrogea, new almond orchards have been established in the last decade.
The purpose of this paper is to report the presence of the almond seed wasp (*Eurytoma amygdali* Enderlein) in Romania. This article can be of interest for farmers who intend to develop new orchards or possess one and also for the researchers in the field.

MATERIALS AND METHODS

Almond fruits containing ASW were collected from the spontaneous almonds situated in northern Dobrogea, in Greci, Tulcea County.
The fruits were collected in mid-December 2021, kept in cold conditions for two months. In February 2022, fruits have been deposited under laboratory condition at temperature

room, around 22-24°C, in petri dishes. After around 15 days the adults started to perforate the fruit and come out of it (Figure 2). Some of the fruits were cracked to evaluate the larvae and the pupae.

Figure 2. Almond perforated by the *Eurytoma amygdali*

The larva, pupae and adults of *Eurytoma amygdali* were microscopically examined at the Research Center for Studies of Food Quality and Agricultural Products, of USAMV of Bucharest.

The morphological characters of larvae and adults were compared with those presented in the dichotomic identification key of Plaut (1972) Zerova & Fursov (1991) for adults.

RESULTS AND DISCUSSIONS

The morphological identification was based on both the larvae and the adult's morphological characters. *Eurytoma amygdali* can be distinguished from other *Eurytoma* spp. of stone fruits by the following characters. Mesepisternum is without a keel at the ventral margin, the gaster of the female presents a distinctly upturned apex (Figure 5), the tergite 7 is almost twice as long as the tergite 6, the forewing has a distinctly darkened spot on the disc and the gastral petiole is distinctly longer than the hind coxa (Zerova & Fursov, 1991).

The larva (Figure 3) was described by Plaut (1972), as white (sometimes gray), except for the brown mandibulae, its body being composed by the head and 12 segments, is legless, with a tapering posteriorly; length of the neonates is 0-47 mm, and of the grown larvae, up to 10 mm.

The larvae hatch during late March and April (May in Romania), after the fruit has grown to its final size, but while the embryo is still small. After hatching, the larvae move through the nucellus and the embryo sac to feed on the embryo. Usually, the embryo grows to its normal size, supplying the larva with sufficient food for its entire development. When the larva is full grown, all that remains of the seed is the shriveled seed coat, some embryonic debris and the larval excrements. The larva then enters diapause, diminishing somewhat in size.

Although up to five eggs per fruit have been observed under natural conditions, and two eggs per fruit are common, not more than two grown larvae per seed have been found, and this very rarely.

Figure 3. Larvae of *Eurytoma amygdaly* (original)

The larva exits diapause and pupates in January-February (March-April in Romania), and adults emerge after the flowering stage of the almond in March-April. Infested fruits of almond shrivel but remain attached to the tree.

Not all larvae break diapause in the first year, and some may not emerge until two or three years after oviposition (Zerova & Fursov, 1991).
The pupa (Figure 4) changes in color from white to almost entirely black during its development. The fresh pupa is entirely white and the last larval exuvia remains attached to the tip of the abdomen. Plaut (1972) noticed that the males develop faster than the females.
The identification key of the adult ASW is described by Zerova & Fursov (1991), as it follows.
The Female (Figure 5), has the length of 7-8 mm, sometimes 5-6 mm. The body is slender, elongated, with the gaster narrowed apically. Body, antennae, coxae, hind and femora are black; fore and middle femora fuscous medially, brown basally and apically. All tibiae are reddish, only medially is slightly fuscous. The tarsi is dark yellow, apical segments are fuscous.

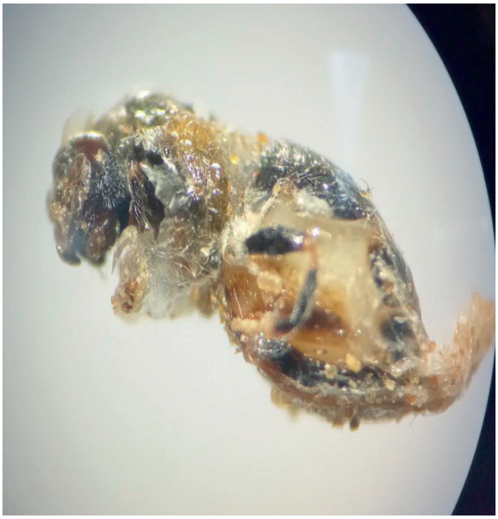

Figure 4. Pupae of *Eurytoma amygdaly* (original)

The head dorsally is slightly broader than the pronotum, in frontal view is broader than high. The eyes are small; gena distinctly longer than longitudinal diameter of eye. The clypeus is with a smooth ventral margin; the face above clypeus is with a few slender, indistinct, short carinae. The head and thorax are dorsally with a large setigerous punctures, are round, distributed sparsely and surrounded by a smaller foveae. The pubescence of the head and thorax is long, dense and silver-white. The antennae are inserted higher than the median part of the face. The scrobes are deep clearly margined. The Scape is distinctly swollen medially. The funicle is long, slender, with a short pubescence, 6 segmented. The club has 2 segmented.
The thorax is slightly convex; the pronotum 2.5 times as broad as long. The mesepisternum is slightly concave ventrally, without keel. The propodeum is widely concave medially, with a large median carina and large irregular foveae in laterally. The hind coxa is densely pubescent, with a dense foveate sculpture and a small vertical comb distally.
The gaster is slightly longer than the head plus thorax. The surface of the tergites is with indistinct punctures. The tergite 9 elongates, and is not shorter than the targite 8. The gaster is rounded, the petiole slightly longer than the hind coxae.
The forewing is with a distinct large fuscous, marking in the middle part of the disk. The veins are dark-brown.

Figure 5. Adult female of *Eurytoma amygdaly*

The male (Figure 6) has the length of 5-6 mm. Has the color and the sculpture as the female. The antennae presents 7 segmented flagellum, which is not clearly separated into the funicle and the club. The pubescence of the flagellum is short; the segmental setae are about equal in length to the width of segments.
E. amygdali it's a phytophagous species, that feed only by the fruits of *Amygdalus communis,*

A. *fenzeliana* and *A. bucharica* (the latter two are new host records for this species). This species is restricted to the genus *Amygdalus,* and records from other host plants are probably erroneous.
This species was described by Enderlein (1907) from material reared from the fruits of *A. communis*. Records of *E. amygdali* from plum most probably refer to *E. schreineri*.
Furthermore an attempt to extract the DNA barcode, by the larvae and adults had been initiated, by a DNeasy blood & tissue kit, without any success.
The differences between the ASW and the plum seed wasp are very little. Different papers (Duval & Millan, 2008; Talmaciu et al., 2006a; 2006b) point out that the same products were used to combat both of the species. Thus we can take in consideration also the measurements of control of the plum seed wasp populations.

Figure 6. Aldult male of *Eurytoma amygdaly*

It has been observed the presence of the ASW for many years in the area, and it can be presumed that it is in big numbers, as the wild almonds aren't pest treated.
For an integrated pest management insecticides based on lambda-cyhalothrine and deltramethin have been successfully applied on plum to combat *Eurytoma schreineri* (Talmaciu et al., 2006), almond (Duval & Millan, 2008). Some argue that the ASW can be successfully controlled with one treatment, if it is applied in the right moment (Katsoyannos et al., 1992).
Minoiu & Lefter (1987) are recommending to incorporate into the soil, the fruits that turn yellowish and fall from the tree, for a better combat of the plum seed wasp. This would work for the ASW as well, considering the close biology of the two species. A good way of doing this is by working the soil between plants per row, under the canopy, after the fruits fall in June.
The biological pest control of the ASW could be achieved, in certain conditions. Soil working between plants per row would be a necessity, collecting the mummified fruits remained in the trees, and applying certain foliar treatments. As some indicated, azadirachtin cannot be used to combat the plum seed wasp (Talmaciu et al., 2006a; 2006b). Some biological insecticides based on spinosad, are homologated for combating *Euritoma schreineri* in Romania. These measures could represent a very efficient control of the ASW population in the almond orchards, in Romania.
To keep the populations under control, it is recommended to place bottles with glass tube with infested almond fruits, placed in the orchards for detecting the first adult emergences in the field. One or two days after the first emerged male, it is recommended to apply foliar treatments in the orchard (Duval & Millan, 2008).
The present mention comes in the help of farmers to understand, identify and to keep under control the ASW populations in their plantations. The paper also provides new opportunities for the researchers in the field, to find out ways to better understand this species, and it's behavior, to elaborate new control strategies to keep the ASW populations under control in plantations.

CONCLUSIONS

Eurytoma amygdali has been identified as the pest found in the fruits collected from the wild almonds in Greci, Tulcea County.
Morphological observations and the behaviour of the pest, points out the ASW is the species affecting the almond fruits in the region.
The nowadays advances in technology offers methods of pest management both integrated and biologically.
Further studies are required to establish the period of emergence and the efficiency of the different products on the market.

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THE PHENOLOGY OF SOME NEW ALMOND CULTIVARS TESTED IN NORTHERN DOBROGEA

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Abstract

The almond (Prunus dulcis (Miller) D.A. Webb) crop started to regain interest for the farmers in Romania. Dobrogea is one of the most suitable regions for almond orchards. The key factor that limits the almond crop in Romania is the early flowering time, flowers and young fruits being suddenly affected by the spring frosts. In this study, the phenology of four Romanian and four foreign almond cultivars, grown in a super high-density trial orchard, in Greci, Tulcea County, is presented. The phenology study using BBCH scale was performed in 2021 and 2022 vegetative seasons, in order to determine the most suitable late and very late blooming cultivars for Dobrogea region. It resulted that Marinada and Lauranne have a very late blooming time, while the Romanian cultivars Nico and April, have an medium, medium to late blooming time. This study presents original data that could be of interest for scientists but also for farmers whom intend to plant new almond orchards.

Key words: *Prunus dulcis, climate, late blooming, early ripening.*

INTRODUCTION

The blooming time is one of the most important periods for the almond crop. Late frosts can occur during this time of the year, resulting in the freezing of flowers or young fruits. This phenomenon happens especially in Europe, in areas suitable for almond orchards. One way to avoid this phenomenon is to choose cultivars with late blooming period, adapted to the growing region. Knowing the phenology of the available cultivars on the market can help us take better decision when we establish new orchards.

Based on the phenological observations, growers could easily monitor developmental stages and schedule timely various agronomic managements such as frost protection, pollination, fruit thinning, irrigation, fertilization, pruning, pests and diseases management, and harvesting. To optimize these agronomical managements, it is advisable to take action when most of tree branches are in the same phenophase (Sakar et al., 2019).

The almond's phenological growth stages of flowering have been one of the main interests for scientists in the field, since the beginning of breeding almond cultivars in Europe. Different almond breeding programs from Europe had, among its main objectives, developing cultivars with late and very late blooming time, being a necessity to develop new cultivars more adapted to the medium conditions (Batlle et al., 2017).

During the blooming time and depending on numerous factors, like the phenological stage, temperature and exposure time, the temperatures below -1°C or -2°C, can cause severe flowers loss. Therefore, breeders were interested in delaying the flowering time of new cultivars, to bloom when the risk of late frosts are minimal (Dicenta et al., 2017).

Some researchers argue that the climate change and it's immediate implications for agricultural systems, are concerning the earlier onset of sexual reproductive development, higher or lower reproductive output, expansion of crop plants and shifts in the geographic distribution of natural populations towards higher latitudes (Hedhly et al., 2009), for the almond crop this can represent new perspectives, Romania being at its northern limit.

Over the centuries of almond growing, many attempts have been undertaken to shift its cultivation from the Mediterranean shores towards inland regions where, in most of the years, spring frosts occur during the time of the almond blooming or soon after (Socias i

Company et al., 2012). A main objective of many current almond breeding programmes is the development of cultivars adapted to cold climates, usually inland or at higher altitudes. Like other temperate trees, almond is typically without leaves and dormant during the winter. Under these conditions the almond can endure very low temperatures. In contrast, the almond tree is very susceptible to cold damage during flowering and early fruit development. At this time, temperatures below −1°C can cause crop loss. Therefore, breeders are interested in developing new cultivars possessing later flowering times in order to reduce the risk of cold damage (Martínez-Gómez et al., 2017).

The Black Sea basin was less important for the almond crop, fewer cultivars were adapted and commercially efficient here, and spring frosts were causing important damages. Since the climate changed, in the last decades, less harsh winters are encountered. In addition, the newly developed very late blooming cultivars, gained more potential for the almond crop in the Black Sea basin.

In Romania, the almond crop is regaining in popularity among farmers and scientific community. In the last decades some new medium and late blooming cultivars have been developed. In Europe different very late blooming cultivars have been released. These cultivars could present potential for growing in Romania, and can be a viable option for the farmers.

In this study is analyzed and presented the phenology of eight, medium to very late blooming time almond cultivars, for the years 2021 and 2022.

MATERIALS AND METHODS

The paper presents the phonological stages of inflorescence emerge and flowering of different almond cultivars grown in a super high-density trial orchard, located in Greci, Tulcea County. The study was realised in two years consecutively, 2021 and 2022.

Eight cultivars were taken into study, four Romanian, and four from abroad. The Romanian cultivars studied are Ana, April, Mirela and Nico, all grafted on the rootstock Tomis 1. The foreign cultivars are Lauranne, Marinada, Supernova and Vairo, all grafted on GF677. The trial orchard was planted in 2020, on a surface of 1000 m^2. It is a randomized complete block, with three samples of six trees, for each cultivar. Eighteen trees per cultivar, one hundred and forty four in total, were observed. The rows are displayed north-south. The distance of planting is four meters between rows and one and a half meters between plants per row. The canopy is formed as a vertical axe.

Ana cultivar was created at the University of Oradea by crossing Ardechoise x H 1/9-1fa, being registered in 2006 (Şcheau, 2013). It's a vigorous, self-incompatible cultivar, fruiting mainly on spurs. It is a late blooming cultivar, starting to bloom at the beginning of April, and has a medium ripening, in the third decade of August (Asănică & Hoza, 2013). The production at maturity is 1677 kg/ha in shell, the fruits are medium size (3 g). The kernel production is 823 kg/ha, with a total 49% shelling.

April was created at the University of Oradea by crossing Primorski x [(Preanâi x Crâmski], being registered in 2006 (Şcheau, 2013). It is a vigorous tree, self-incompatible, fruiting mainly on long branches. It is a medium-late blooming cultivar, starting to bloom at the end of March, and has a medium ripening that begins in the last decade of September (Braniste N., 2008). The production is 2064 kg/ha in shell at maturity, the fruits are medium-big size (4.4 g). The kernel production is 614 kg/ha, with a total 30% shelling.

Lauranne was created at INRA Bordeaux by a crossing between Ferragnes x Tuono, being registered in 1989. It is a medium-high vigor tree, self-fertile, fruiting mainly on spurs. It is a very late blooming cultivar, starting to bloom at the beginning of April, and has a medium ripening time, that begins in the last decade of August. The production is very high, the fruits are medium size, and kernel weights 1g in average. The shelling is 29-35%.

Marinada was created at IRTA Catalunia by a crossing between Lauranne x Glorieta, being registered in 2008. It is a medium vigor tree, self-fertile, fruiting mainly on spurs. It is a very late blooming cultivar, starting to bloom at the beginning of April, and has a late ripening time, that begins in the first decade of October. The production is very high, the fruits are

medium size, and kernel weights 1.3 g in average. The shelling is 32% (Vargas et al., 2008).
Mirela was created at the S.C.D.P Constanța by a free selection from a local almond population, being registered in 2016. It's a medium vigor, self-compatible cultivar, fruiting mainly on spurs. It is a medium to late blooming cultivar, starting to bloom at the beginning of April, and has a late ripening, beginning in the second decade of September. The production at maturity is 5060 kg/ha of kernels, with a 49% shelling. The kernels are big (2.5 g). (Gavăt et al., 2015).
Nico was created at the University of Oradea by a crossing between Ferragnes x (Nikitski Pozdno. x Lovrin 18), being registered in 2006. It is a medium vigor tree, self-incompatible, fruiting mainly on long branches. It is a medium blooming cultivar, starting to bloom at the half-end of March, and has a medium ripening that begins in the middle of August. The production at maturity is 2337 kg/ha in shell, the fruits are medium-big size (4.4 g). The kernel production is around 930 kg/ha, with a total 35.5% of shelling (Şcheau, 2013).
Supernova was created at ISF Roma and has been registered in 1988. It is a medium vigor tree and self-fertile. It is a late blooming cultivar, starting to bloom in the first days of April, and has a medium-late ripening time, that begins in the last decade of September. The production is very high, the fruits are big in size (6.4 g), and kernel weights 2 g in average. The shelling is 33%.
Vairo was created at IRTA Catalunia by a crossing between ('Primorskij' × 'Cristomorto') × Lauranne, being registered in 2008. It is a high vigor tree, self-fertile, fruiting mainly on spurs. It is a late to very late blooming cultivar, starting to bloom at the beginning of April, and has a medium ripening time, that begins in the last decade of August. The production is very high, the fruits are medium size, and kernel weights 1.2 g in average. The shelling is 29% (Vargas et al., 2008).
In order to characterize the flower buds development and blooming period of the cultivars studied, the growth stages from the BBCH scale of stone fruits (Meier et al., 1994) has been considered. The scale is compound by a code formed from numbers and the description of the code, as is present it next.
Principal growth stage 5: Inflorescence emergence.
51 - Inflorescence buds swelling: buds closed, light brown scales visible (Figure 1 A).
53 - Bud burst: scales separated, light green bud sections visible (Figure 1 B).
54 - Inflorescence enclosed by light green scales, if such scales are formed (not all cultivars).
55 - Single flower buds visible (still closed) borne on short stalks, green scales slightly open (Figure 1 C).
56 - Flower pedicel elongating; sepals closed; single flowers separating (Figure 1 D).
57 - Sepals open: petal tips visible; single flowers with white or pink petals (still closed) (Figure 1 E).
59 - Most flowers with petals forming a hollow ball (Figure 1 F).
Principal growth stage 6: Flowering.

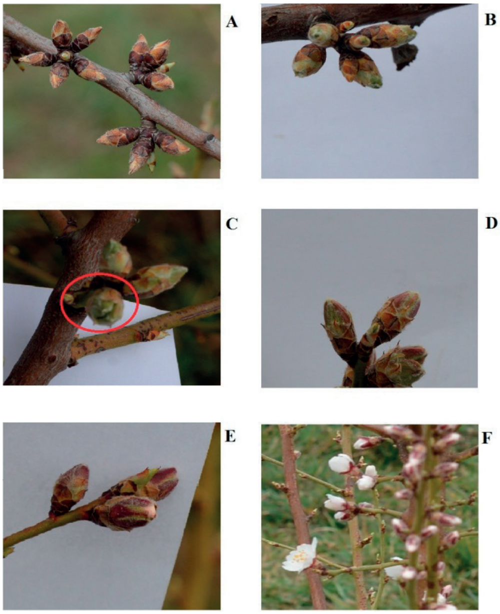

Figure 1. Principal growth stage 5: Inflorescence emergence

60 - First flowers open (Figure 2 G).
61 - Beginning of flowering: about 10% of flowers open.
62 - About 20% of flowers open.
63 - About 30% of flowers open.
64 - About 40% of flowers open.

65 - Full flowering: at least 50% of flowers open, first petals falling (Figure 2 H).
67 - Flowers fading: majority of petals fallen (Figure 2 I).
69 - End of flowering: all petals fallen (Figure 2 J).

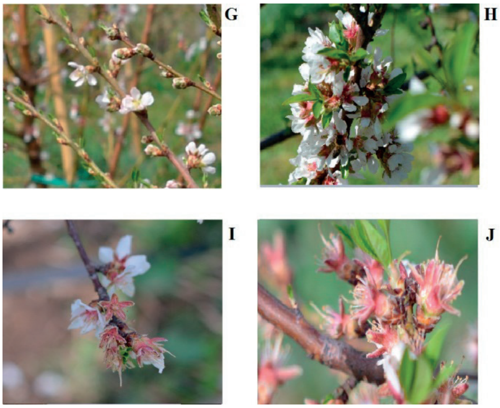

Figure 2. Principal growth stage 6: Flowering

In general, twice a week field visual assessments have been done. The observations started on 28th of February and lasted until 28th of April in 2021 and from 17th of February to 21th of April in 2022.
To characterise each cultivar, the UPOV terminology was considered, based on the guidelines for the conduct of tests for distinctness, uniformity and stability for almond.

RESULTS AND DISCUSSIONS

The almond is the first species of the genus *Prunus* to bloom (Segura et al., 2017). Due to the European breeding programs, in the last decades, many cultivars with late and very late blooming time have been created. In Romania, very late blooming time cultivars, bloom later than the apricot and close, in time, to the peach. In figure 1 are presented as thumbnails the stages of the principal growth stage 5, the inflorescence emergence. The development of flower buds is correlated with the growing degree hours and the evolution of temperatures. In the present study, inflorescence emergence lasted between 28 days (April & Nico) and 40 days (Lauranne and Marinada), in 2021, and between 33 days (Nico) and 44 days (Lauranne & Marinada) in 2022.

In Figure 2 can be observed as thumbnails the stages of the principal growth stage 6, the flowering. The flowering stage is influenced by the climatic and geographic conditions. In the present study it lasted between 17 days (April & Nico) and 23 days (Vairo), in 2021, and between 16 days (Ana, Nico & Vairo) and 20 days (Lauranne and Marinada) in 2022.
The most critical period for the almond crop is the flowering. The cultivars studied have a big diversity regarding the flowering period. In table 1 have been summarised the stages 60 - First flowers open (Figure 2 G) and 65 - Full flowering, at least 50% of flowers open, first petals falling (Figure 2 H). During this stages, the almond crop is the most sensible to spring frosts. How early this stages are attained and how fast or slowly from the stage 60 to 65 the almonds go through, shows a cultivar possibility to be affected by spring frosts or not. The cultivar first to open the flowers was Nico, in both years of study. On 18th of March in 2021 and on 25th of March in 2022. Nico has attained the full flowering on 2nd April, in 2021 respectively 29th March in 2022. It followed April cultivar on 2nd of April in 2021 and 29th March in 2022 reaching the full flowering on 5th April, in 2021 respectively 1st March in 2022.
The cultivars that had the most belated flowering were Lauranne and Marinada. Both open the first flowers on 9th April in 2021 and 1st of April in 2022, reaching the full flowering on 20th of April in 2021 and 9th of April in 2022.

Table 1. The flowering stage. The stages first flowers open and full flowering

Year		2021	2021	2022	2022
Cultivar	Stage	60	65	60	65
Ana		02-Apr	13-Apr	01-Apr	06-Apr
April		02-Apr	05-Apr	29-Mar	01-Apr
Lauranne		09-Apr	20-Apr	01-Apr	09-Apr
Marinada		09-Apr	20-Apr	01-Apr	09-Apr
Mirela		02-Apr	09-Apr	29-Mar	01-Apr
Nico		18-Mar	02-Apr	25-Mar	29-Mar
Supernova		02-Apr	09-Apr	01-Apr	06-Apr
Vairo		05-Apr	13-Apr	01-Apr	06-Apr

The evolution of the growth stages studied for the year 2021 can be observed in Figure 3. The cultivars with a medium and medium to late flowering, Nico and April, present also a shorter period of floral bud development. And cultivars with very late flowering like Marinada, Lauranne and Vairo, had a longer period for floral bud development.

The inflorescence emergence lasted 28 days for Nico and April, from 28th of February until 28th of March. 33 days for Ana, Mirela and Supernova from 28th of February until 2nd of April. 36 days for Vairo from 28th of February until 5th of April. 40 days for Lauranne and Marinada from 28th of February until 9th of April. The flowering lasted 17 days for Nico and April, from 28th of March until 13th of April. 18 days for Ana, Mirela and Supernova from 2nd of April until 20th of April. 23 days for Vairo from 5th of April until 28th of April. 20 days for Lauranne and Marinada from 9th of April until 28th of April.

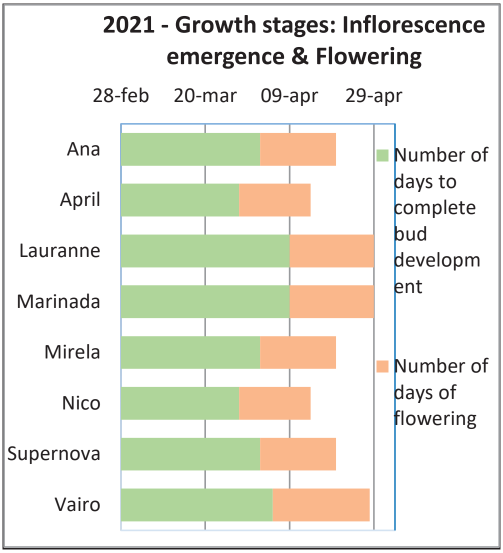

Figure 3. The evolution of the phonological stages of inflorescence emergence and flowering in 2021

In Figure 4 is presented as the evolution of the stages studied, for the year 2022. The cultivars April and Nico, as in the year of study 2021, this cultivars had a shorter period of floral bud development. Cultivars with a very late flowering like Marinada, Lauranne, also had a longer period for floral bud development in 2022.

In 2022 the inflorescence bud development for Nico lasted 33 days, from 17th of February until 21th of March. 37 days for April and Mirela, from 17th of February until 25th of March. 41 days for Vairo, Supernova and Ana, from 17th of February until 29th of March. 44 days for Lauranne and Marinada from 17th of February until 1st of April. The flowering lasted 16 days for Nico, Ana and Vairo. 17 days for April and Mirela. 18 days for Supernova. 20 days for Lauranne and Marinada.

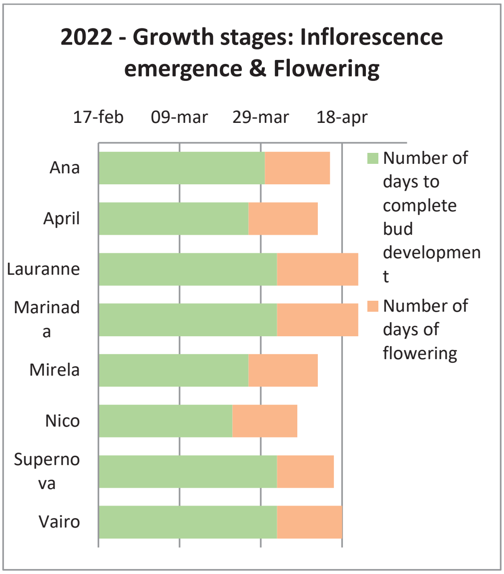

Figure 4. The evolution of the phonological stages of inflorescence emergence and flowering in 2022

In average, the number of days of flowering were 17 for Ana and April, 17.5 for Mirela, 16.5 for Nico, 16 for Supernova, 19.5 for Vairo and 20 for Lauranne and Marinada,.

In average for the years of study, the number of days to complete both stages were, 47 days for Nico, 49.5 days for April, 52.5 days for Mirela, 54 days for Ana, 55 days for Supernova, 59.5 days for Vairo and 62 day for Lauranne and Marinada.

CONCLUSIONS

Nico cultivar presents a medium time of beginning of flowering. April presents a medium to late time of beginning of flowering. Ana, Mirela and Supernova have a late time of beginning of flowering. Vairo, Lauranne and

Marinada have a late to very late time of beginning of flowering.

Nico and Supernova had the shortest period of flowering, while Lauranne and Marinada, the longest period.

To avoid the late spring frosts it is recommended to establish new plantations with cultivars that have a very late or late to very late time of beginning of flowering, and with a long period of flowering for a better pollination.

With the latest developed cultivars that bloom very late or late to very late, the spring frosts can be avoided. While this problem for the almond crop can be solved by a careful selection of cultivars, a new one had arisen. In the present experiment, the apple blossom beetle (*Epicometis hirta* Poda) became active and was very attracted by the flowers of Lauranne, Marinada and Vairo, while they were the only cultivars that had the flowers still open. This can become a new challenge for the farmers, knowing that insecticides are not recommended to be used when the almonds are in bloom.

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THE STUDY OF A FEW OLD APPLE VARIETIES AND LOCAL POPULATIONS AT POHALMA NURSERY IN LUGOJ, TIMIȘ COUNTY

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Abstract

The purpose of the current study was to analize aspects reffering to the growth vigour of the apple trees in the third year after the planting (the trunk's diameter, the height of the tree and the length of the annual growths) as well as properties of the fruit itself, including number of fruits per tree, small and large diameter, height and mass of the fruits, dry matter and sugar content. All 26 varieties are grafted on the M106 rootstock. The large diameter of the fruits varied between 42.00 mm for 'Crețesc' variety and 85.33 mm for 'Curcubătoase', the average being 63.06 mm. The mass of the fruits ranged between 63.69 g for 'Crețesc' variety and 334.96 g for 'Curcubătoase' variety, the average of the experience being 189.09 g. The biggest fruits belonged to 'Curcubătoase' and 'Jonathan', whereas 'Crețesc', 'Măr Țigănesc' and 'Florănești' had smaller fruits. In addition, a questionnaire had been created, which revealed that most people are aware of the fact that it is challenging to find truly healthy apples, but they consume these fruits regularly.

Key words: *apple trees, old varieties, Lugoj, measurements, questionnaire.*

INTRODUCTION

With the appearance of cosmopolitan apple varieties, the old local ones had started to be neglected by growers. At the moment, these varieties are only identified isolated or in certain orchards.

The value of the old varieties is given by their remarkable taste, productivity, longevity and resistance to extreme environmental circumstances. Although they possess the enumerated qualities, these varieties have some disadvantages too: due to their high growth vigour, it is impossible to establish intensive or superintensive orchards containing these specific varieties.

In the same time, it is a rustic species with high ecological plasticity, which in superior agrotechnical conditions gives significantly higher crop than the other fruit species (Chira & Pașca, 2008).

Another aspect is that the crop's value is rather high. For example, the crop from 1 ha of intensive apple orchard can be sold at the same price as 5 ha of cereals (Chira & Pașca, 2008).

Apple intake has various benefits on our bodies: eaten in the morning, the fruit helps prevent large intestine cancer, absorbing the toxins; apple is also used in infantile diarrhea treatments; it contributes to the egestion of uric acid from the organism, therefore apple is recommended in obesity cases; it is considered as a natural alternative against arterial hypertension (Chira & Pașca, 2008).

Apples are fruits eaten all over the world, they constitute a rich source of phytochemicals. Epidemiological studies found the relation between apple consumption and low risk of developing cancer, especially lung cancer, as well as cardiovascular diseases, asthma, diabetes. Laboratory analysis put in evidence the strong antioxidant property of apples, which also reduces the cholesterol level. Correlation was also made between apple consumption and weight loss. Storage does not have effect or has minimal consequence over phytochemical content, but on the other hand, processing influences the chemical composition of the fruit (Boyer & Liu, 2004).

Stored at low temperatures, the fruits can be edible up to one year (Mditshwa et al., 2018).

Recently, a decrease in apple consumption can be observed worldwide, researchers are in continous search of the explanations regarding this tendency. Konopacka et al. (2010) have come to the conclusion that apple consumption

is lower among young people, which in the future may lead to a continous decline.
Different studies have proved that one of the main factor in fruit consumption is represented by people's income: in countries like Italy, Denmark or Albania, citizens are willing to pay more in order to consume organic and local apples (Ceschi et al., 2017; Denver & Jensen, 2014; Skreli & Imami, 2012).
According to statista.com (Shahbandeh, 2021), last year the global apple production was 63.9 mil tons. In this regard, China was on the first place with 41.00 mil tons, followed by USA with 4.82 mil tons and Turkey with 3.00 mil tons.
In Romania, in 2020, apple production was 551.5 thousands tons, with 50 thousands extra than in the previous year (Brodeală et al., 2021).
With the purpose of studying and preserving the local germoplasm, a small apple plantation was established at the Pohalma Nursery in Lugoj in 2017.

MATERIALS AND METHODS

The varieties that were analyzed are the following: 'Măr Țigănesc', 'Măr Domnesc', 'Pietros', 'Curcubătoase', 'Poinic', 'Carigate', 'Mustoase', 'Florănești', 'Botu Oii Alb', 'Măr mare', 'Pătul', 'Vițate', 'Jonathan de munte', 'Măr de Jupani', 'Mari de Berini', 'Măr dulce amar', 'Măr dulce', 'Măr plăcintă Berini', 'Șovare', 'Caslere', 'Jonathan', 'Pogace', 'Aore', 'Crețesc', 'Botu Oii de Caraș', 'Pătul de Vârciorova', 'Parmen auriu'.
The trees were planted in 2017 and are all grafted on the M106 rootstock.
The measurements have been started to be carried out in early 2020, when the trees were still in resting phase and ended in October 2020.
The aims of the research were: measuring the length of the annual growths, the growth vigour of the trees and the trunk's diameter, as well as counting the fruits on each tree, then analyzing the large and small diameter, height and mass of the fruits, their dry matter and sugar content.
The data have been statistically processed and interpreted, then compared between the varieties.
Given a species that is so consumed and appreciated, it was opportune to create a questionnaire to see people's habits and preferences in eating this fruit.

RESULTS AND DISCUSSIONS

The trunk's diameter in the case of these old varieties and local populations, studied in the third year after the planting, varied between 27.16 mm for 'Mari de Berini' and 50.77 mm for 'Florănești' and 'Măr mare' varieties.
It can be observed that 16 varieties out of the 26 had values above the control's value and the other 10 below it. Only the 'Mari de Berini' variety was statistically assured, being distinctly significantly negative compared to the control (Table 1).

Table 1. The trunk's diameter

Crt. nr.	Variety	Trunk's diameter mm	Relative value (%)	Difference from the control	Semnification
1	Măr Țigănesc	40.17	94.94	-2.14	-
2	Măr Domnesc	46.30	109.44	3.99	-
3	Pietros	46.10	108.97	3.79	-
4	Curcubătoase	47.87	113.14	5.56	-
5	Poinic	47.27	111.72	4.96	-
6	Carigate	44.87	106.05	2.56	-
7	Mustoase	46.90	110.86	4.59	-
8	Florănești	50.77	120.00	8.46	-
9	Măr mare	50.77	120.00	8.46	-
10	Pătul	45.20	106.84	2.89	-
11	Vițate	43.46	102.74	1.16	-
12	Jonathan de munte	43.8	103.52	1.49	-
13	Măr de Jupani	38.06	89.97	-4.24	-
14	Mari de Berini	27.16	64.21	-15.14	00
15	Măr dulce amar	43.8	103.52	1.49	-
16	Măr dulce	47.26	111.72	4.96	-

Crt.nr.	Variety	Trunk's diameter mm	Relative value (%)	Difference from the control	Semnification
17	Măr plăcintă Berini	42.06	99.43	-0.24	-
18	Șovare	46.66	110.30	4.36	-
19	Caslere	35.63	84.22	-6.67	-
20	Jonathan	40.33	95.33	-1.97	-
21	Pogace	41.03	96.99	-1.27	-
22	Aore	46.86	110.77	4.56	-
23	Crețesc	45.00	106.36	2.69	-
24	Botu Oii de Caraș	35.4	83.67	-6.90	-
25	Pătul de Vârciorova	35.83	84.69	-6.47	-
26	Parmen auriu	34.93	82.571	-7.37	-
27	Average of the varieties	42.31	100.00	0.00	control
		DL 5% = 8.96 mm DL 1% = 12.11 mm DL 0.1% = 16.14 mm			

The height of the trees in the third year after the planting had its values between 165.0 cm for 'Mari de Berini' and 251.66 cm for 'Măr dulce' variety, the average of the experience being 204.22 cm.

Among the analyzed varieties, 13 have exceeded the control, but only 2 of them were statistically assured, 'Măr dulce' (distinctly significantly positive) and 'Măr dulce amar' (significantly positive). 'Pietros', 'Poinic', 'Domnesc' and 'Curcubătoase' varieties also had high vigour.

On the opposite pole, the lowest height was observed in case of 'Mari de Berini' and 'Caslere' varieties, both significantly negative compared to the control. A lower vigour was measured for 'Pătul de Vârciorova', 'Parmen auriu', 'Șovare', 'Jonathan' and 'Pogale', their hight being below 190.00 cm (Table 2).

Table 2. The tree's height

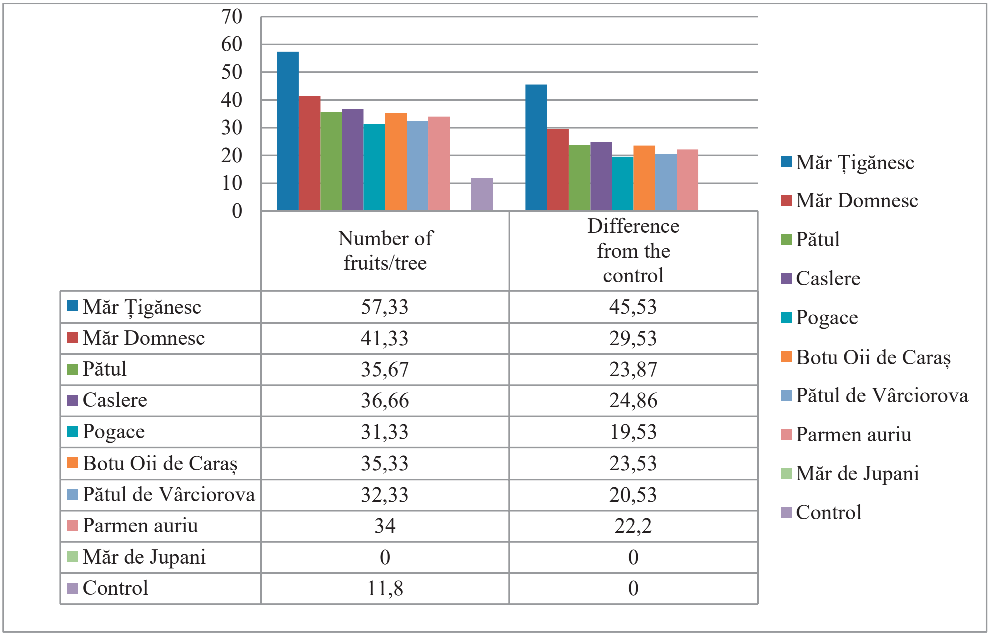
Crt. nr.	Variety	Height of the tree (cm)	Relative value (%)	Difference from the control	Semnification
1	Măr Țigănesc	210.33	103.00	6.12	-
2	Măr Domnesc	228.33	111.81	24.12	-
3	Pietros	230.33	112.79	26.12	-
4	Curcubătoase	224.67	110.01	20.45	-
5	Poinic	230.00	112.63	25.78	-
6	Carigate	207.00	101.36	2.78	-
7	Mustoase	216.67	106.10	12.45	-
8	Florănești	216.67	106.10	12.45	-
9	Măr mare	210.00	102.83	5.78	-
10	Pătul	197.00	96.47	-7.22	-
11	Vițate	215.66	105.60	11.45	-
12	Jonathan de munte	193.66	94.83	-10.55	-
13	Măr de Jupani	189.33	92.71	-14.88	-
14	Mari de Berini	165.00	80.79	-39.21	0
15	Măr dulce amar	238.33	116.70	34.11	X
16	Măr dulce	251.66	123.23	47.45	XX
17	Măr plăcintă Berini	197.33	96.62	-6.88	-
18	Șovare	186.33	91.24	-17.88	-
19	Caslere	171.00	83.73	-33.21	0
20	Jonathan	187.00	91.56	-17.21	-
21	Pogace	186.66	91.40	-17.55	-
22	Aore	208.66	102.17	4.45	-
23	Crețesc	201.33	98.58	-2.88	-
24	Botu Oii de Caraș	190.00	93.03	-14.21	-
25	Pătul de Vârciorova	176.66	86.50	-27.55	-
26	Parmen auriu	180.00	88.14	-24.21	-
27	Average of the varieties	204.22	100.00	0.00	control
		DL 5% = 32.59 cm DL 1% = 44.04 cm DL 0.1% = 58.66 cm			

The average length of the annual growths in the experiment changed between 63.44 cm for ‘Caslere’ variety and 107.55 cm for ‘Măr dulce amar’, the average being 83.56 cm.
Values above the average of the experience were obtained for ‘Măr dulce amar’ (distinctly significantly positive in comparison with the control), followed by ‘Măr dulce’ (significantly positive) and ‘Curcubătoase’, ‘Pietros’ and ‘Mustoase’ varieties, but these latter ones weren’t statistically assured.
Twigs with values under the average were found in case of ‘Caslere’ (significantly negative compared with the control) and varieties such as ‘Jonathan de munte’ and ‘Mari de Berini’ (Table 3).

Table 3. The annual growth’s average length

Crt. nr.	Variety	The annual growth’s average length (cm)	Relative value %	Difference from the control	Semnification
1	Măr Țigănesc	81.88	98.00	-1.67	-
2	Măr Domnesc	87.89	105.18	4.33	-
3	Pietros	94.97	113.66	11.42	-
4	Curcubătoase	98.77	118.21	15.22	-
5	Poinic	92.42	110.61	8.86	-
6	Carigate	83.55	100.00	0.00	-
7	Mustoase	94.66	113.29	11.11	-
8	Florănești	87.78	105.05	4.22	-
9	Măr mare	86.66	103.72	3.11	-
10	Pătul	76.22	91.22	-7.34	-
11	Vițate	90.55	108.37	6.99	-
12	Jonathan de munte	71.33	85.36	-12.22	-
13	Măr de Jupani	73.55	88.02	-10.00	-
14	Mari de Berini	70.42	84.27	-13.13	-
15	Măr dulce amar	112.77	134.96	29.21	XX
16	Măr dulce	107.55	128.71	23.99	X
17	Măr plăcintă Berini	80.44	96.26	-3.11	-
18	Șovare	79.44	95.07	-4.11	-
19	Caslere	63.44	75.92	-20.11	0
20	Jonathan	68.55	82.04	-1.00	-
21	Pogace	71.44	85.49	-12.11	-
22	Aore	84.10	100.65	0.55	-
23	Crețesc	77.55	92.81	-6.00	-
24	Botu Oii de Caraș	71.88	86.02	-11.67	-
25	Pătul de Vârciorova	72.11	86.30	-11.44	-
26	Parmen auriu	92.55	110.76	8.99	-
27	Average of the varieties	83.56	100.00	0.00	control
		DL 5% = 18.93 cm	DL 1% = 25.58 cm	DL 0.1% = 34.07 cm	

The number of fruits per tree in the third year after the planting oscillated between 0 for ‘Măr de Jupani’ and 57.33 for ‘Măr Țigănesc’ (Figure 2 and Figure 3), with an average of the experience of 11.80.
13 varieties out of the total of 26 have exceeded the control’s value. The highest value was achieved by ‘Măr Țigănesc’ (very significantly positive compared to the control), followed by ‘Măr Domnesc’, also categorized as very significantly positive.
The next varieties that were statistically assured are: ‘Caslere’, ‘Pătul’, ‘Botu Oii de Caraș’, ‘Pătul de Vârciorova’ and ‘Parmen auriu’, all of them being distinctly significantly positive compared to the control. ‘Pogace’ variety, with the average of 31.33 fruits per tree, was significantly positive (Figure 1).
Values below the average obtained 13 varieties, the lowest ones belonging to ‘Măr de Jupani’, ‘Crețesc’, ‘Măr mare’, ‘Mari de Berini’ and ‘Pietros’ varieties. None of them was statistically assured.



	Number of fruits/tree	Difference from the control
Măr Țigănesc	57,33	45,53
Măr Domnesc	41,33	29,53
Pătul	35,67	23,87
Caslere	36,66	24,86
Pogace	31,33	19,53
Botu Oii de Caraș	35,33	23,53
Pătul de Vârciorova	32,33	20,53
Parmen auriu	34	22,2
Măr de Jupani	0	0
Control	11,8	0

Figure 1. Number of fruits/tree- statistically assured varieties

Figure 2. 'Măr de Jupani' variety in august 2020

Figure 3. Closer look to the fruits of 'Măr Țigănesc' variety in august 2020

Regarding the big diameter of the fruits, it varied from 42.00 mm for 'Crețesc' variety to 85.33 mm for 'Curcubătoase', the average of the experience being 63.06 mm.

Values above the control's value have been found at 9 varieties. 'Curcubătoase', 'Jonathan', 'Aore' are very significantly positive in comparison with the control and 'Florănești', 'Jonathan de munte', 'Caslere' are significantly positive.

8 varieties had values under the control's. 4 of them ('Măr Țigănesc', 'Botu Oii Alb', 'Crețesc' and 'Parmen auriu') were registered as very significantly negative compared to the control. 'Vițate' variety was catalogued as significantly negative (Table 4).

Table 4. Big diameter of the fruits

Crt. nr.	Variety	Big diameter mm	Relative value %	Difference from the control	Semnification
1	Măr Țigănesc	43.83	69.51	-19.23	000
2	Măr Domnesc	58.00	91.97	-5.06	-
3	Curcubătoase	85.33	135.31	22.27	XXX
4	Florănești	72.00	114.17	8.94	X
5	Botu Oii Alb	50.00	79.29	-13.06	000
6	Pătul	68.33	108.36	5.27	-
7	Vițate	55.00	87.21	-8.06	0
8	Jonathan de munte	72.33	114.70	9.27	X
9	Șovare	68.00	107.83	4.94	-
10	Caslere	72.33	114.70	9.27	X
11	Jonathan	76.33	121.04	13.27	XXX
12	Pogace	66.00	104.65	2.93	-
13	Aore	77.00	122.09	13.93	XXX
14	Crețesc	42.00	66.59	-21.06	000
15	Botu Oii de Caraș	61.00	96.72	-2.06	-
16	Pătul de Vârciorova	59.33	94.08	-3.73	-
17	Parmen auriu	45.33	71.88	-17.73	000
18	Average of the varieties	63.06	100.00	0.00	control
		DL 5% = 6.97 mm DL 1% = 9.42 mm DL 0.1% = 12.55 mm			

The small diameter of the analized fruits had values between 40.00 mm for 'Crețesc' and 81.00 mm for 'Curcubătoase' variety. The average of the experience was 58.68 mm.

9 varieties exceeded the control and 8 did not reach the control's value. 'Curcubătoase' and 'Aore' varieties were very significantly positive in comparison with the control, 'Jonathan' distinctly significantly positive and 'Florănești' variety significantly positive. 'Pătul', 'Caslere', 'Pogace', 'Botu Oii de Caraș' and 'Pătul de Vârciorova' were not statistically assured.

The furthest values from the control out of the varieties that could not reach the control's values were 'Crețesc' and 'Parmen auriu' (very significantly negative compared to the control). 'Măr Țigănesc', 'Botu Oii Alb' and 'Jonathan de munte' varieties were distinctly significantly negative. 'Măr Domnesc', 'Vițate' and 'Șovare' were not statistically assured.
The height of the measured apples was changing from 29.00 mm for 'Crețesc' variety to 68.00 mm for 'Curcubătoase', the average of the experiment being 49.82 mm.
10 varieties exceeded the control, but only 2 of them were statistically assured: 'Curcubătoase' (very significantly positive) and 'Caslere' (distinctly significantly positive beside the control).
Out of the 7 varieties below the experiment's average, 2 were classified as very significantly negative beside the control: 'Crețesc' and 'Parmen auriu'.
Regarding the mass of the apples, it oscillated between 63.69 g for 'Crețesc' variety and 334.96 g for 'Curcubătoase', with an average of 189.09 g.
The biggest positive difference from the control was found in case of 'Curcubătoase', followed by 'Jonathan' and 'Florănești', all of them being very significantly positive compared to the control. 'Caslere' and 'Aore' had close values, both of them being classified as significantly positive. The rest of the varieties with higher values were not statistically assured.
On the opposite pole, 'Măr Țigănesc', 'Crețesc' and 'Parmen auriu' were very significantly negative in comparison with the control. 'Botu Oii Alb' and 'Vițate' varieties had almost identical values, and were significantly negative beside the control.
Values of the dry matter content in the fruits varied between 9.97 °Brix for 'Curcubătoase' and 18.90 °Brix for 'Măr Domnesc', with an average of 14.18 °Brix.
Above the control's value were situated 8 varieties: 'Măr Domnesc' and 'Parmen auriu' (very significantly positive in comparison with the control), 'Florănești' (significantly positive) and other varieties ('Măr Țigănesc', 'Vițate', 'Jonathan de munte', 'Șovare' and 'Botu Oii de Caraș') that were not statistically assured.
Values below the average got 9 varieties, including 3 statistically assured ones: 'Curcubătoase' (very singificantly negative beside the control, with 9.97ºBrix), 'Aore' (distinctly significantly negative, with 11.86 °Brix) and 'Pătul de Vârciorova' (significantly negative compared to the control, with 12.1 °Brix) (Table 5).

Table 5. Dry matter content of the fruits

Crt. nr.	Variety	Dry matter content °Brix	Relative value %	Difference from the control	Semnification
1	Măr Țigănesc	14.77	104.11	0.58	-
2	Măr Domnesc	18.90	133.25	4.72	XXX
3	Curcubătoase	9.97	70.27	-4.22	000
4	Florănești	16.30	114.92	2.12	X
5	Botu Oii Alb	12.90	90.95	-1.28	-
6	Pătul	12.63	89.07	-1.55	-
7	Vițate	15.40	108.58	1.22	-
8	Jonathan de munte	14.90	105.05	0.72	-
9	Șovare	15.20	107.17	1.02	-
10	Caslere	13.63	96.12	-0.55	-
11	Jonathan	13.05	92.00	-1.13	-
12	Pogace	13.65	96.23	-0.53	-
13	Aore	11.86	83.66	-2.31	00
14	Crețesc	13.10	92.36	-1.08	-
15	Botu Oii de Caraș	14.5	102.23	0.31	-
16	Pătul de Vârciorova	12.10	85.31	-2.08	0
17	Parmen auriu	18.35	129.37	4.16	XXX
18	Average of the varieties	14.18	100.00	0.00	control
		DL 5% = 1.58 °Brix	DL 1% = 2.13 °Brix	DL 0.1% = 2.84 °Brix	

The last table shows the sugar content of the fruits: the values varied between 8.06 g/l for 'Curcubătoase' and 17.58 g/l for 'Măr Domnesc' variety. The average of the experience was 12.55 g/l.

Values above the control's value have been measured in case of 8 varieties: 'Măr Domnesc' and 'Parmen auriu' were very significantly positive compared to the control; 'Florănești' distinctly significantly positive, with 14.81 g/l. The other 5 varieties ('Măr Țigănesc', 'Vițate', 'Jonathan de munte', 'Șovare' and 'Botu Oii de Caraș') had close values to the control and were not statistically assured.

Lower values were obtained at 9 varieties. 'Curcubătoase' variety was very significantly negative compared to the control. 'Aore' was distinctly significantly negative with 10.13 g/l sugar content, 'Pătul' and 'Pătul de Vârciorova' were significantly negative in comparison with the control. 'Botu Oii Alb', 'Caslere', 'Jonathan', 'Pogace' and 'Crețesc' were not statistically assured (Table 6).

Table 6. Sugar content of the fruits

Crt. nr.	Variety	Sugar content g/l	Relative value %	Diferrence from the control	Semnification
1	Măr Țigănesc	13.18	105.05	0.63	-
2	Măr Domnesc	17.58	140.12	5.03	XXX
3	Curcubătoase	8.06	64.27	-4.48	000
4	Florănești	14.81	118.04	2.26	XX
5	Botu Oii Alb	11.12	88.66	-1.42	-
6	Pătul	10.36	82.55	-2.19	0
7	Vițate	13.94	111.11	1.39	-
8	Jonathan de munte	13.32	106.19	0.78	-
9	Șovare	13.64	108.74	1.10	-
10	Caslere	12.04	95.94	-0.51	-
11	Jonathan	11.36	90.56	-1.18	-
12	Pogace	12.00	95.64	-0.54	-
13	Aore	10.13	80.79	-2.41	00
14	Crețesc	11.10	88.49	-1.44	-
15	Botu Oii de Caraș	12.9	102.81	0.35	-
16	Pătul de Vârciorova	10.79	86.02	-1.75	0
17	Parmen auriu	16.99	135.44	4.44	XXX
18	Average of the varieties	12.55	100.00	0.00	control
		DL 5% = 1.72 g/l DL 1% = 2.32 g/l DL 0.1% =3.09 g/l			

In the following, the results of the questionnaire will be presented. 93 people answered the questions.

People from all generations have been asked, based on four categories: below 18 years, 18-30 years, 31-50 years, above 50 years. Another criteria was their provenience: urban/ rural.

Apples being fruits that can be found on the market all year long, it was a curiosity to find out how people take advantage of it. Only a small percentage of those asked said that they rarely consume this fruit. Referring to age categories, individuals under 18 years and between 31 and 50 years consume apples on a weekly basis, those between 18 and 30 years a few times per month, whereas most of the persons over 50 eat apple on a daily basis.

As far as preferences regarding apple type, red apples are on the first place, followed very closely by green apples.

The sweet-and-sour flavour is appreciated the most.

About the pulp's consistency, the crunchy and hard fruits are preferred the most, but succulence is also cherished.

Fruits that look perfectly are the ones people search for the most, but from the answers it can be understood that consumers are aware of the fact that a big and flawless fruit does not necessarily guarantee good flavour or healthy food.

Another question was: "How much do you take into account the fruit's origin when buying apples?" A considerable number of consumers

choose to support local growers, but underage people do not really care about this factor.
“Do you have a favourite variety that you buy at any price or you buy whatever is on discount?” was the next question. It can be clearly seen that more than half of the respondents put more emphasis on preference than on price, which denotes a stable financial situation.
As favourite varieties, the following ones were mentioned the most: ‘Jonathan’, ‘Golden Delicious’, ‘Idared’, ‘Granny Smith’, ‘Florina’. Other mentioned varieties were: ‘Pătul’, ‘Poinic’, ‘Slav’, ‘Grushovka Moscova’, ‘Gala’, ‘Fuji’, ‘Starkrimson’, ‘Mutsu’, ‘Golden Reinders’, ‘Siculane’, ‘Papirovka’, ‘Bot de iepure’.
To the question “Are you willing to buy Bio/Eco apples, which usually cost more?” many have said that they invest in organic fruits. Lately, the population is more and more knowledgeable and healthy alimentation is becoming more popular day by day.
It is more than obvious that most people prefer the fresh fruits over the processed options.
The last question brought up for discussion the famous assertion that „An apple a day keeps the doctor away”. 61 persons agree with it, 10 persons not really or not at all, 4 agree partially. Some of the other answers are the following: „An apple does not make a difference if the rest of the food we eat is not healthy”, „This proposition is true, I have known it since I was a child”, Yes, because it provides vitamins that are good for our immune system”, „It depends. I don’t think that the simple fact of eating apples keeps you healthy. But I think it is an important fruit that contains vitamins”, „It would be nice, but I don’t believe in it. Unfortunately I don’t think there are that many vitamins in fruits anymore”, „Not necessarily, it is something that is said to encourage people to eat fruits and healthy snacks”, „It depends on their provenience. If they come from growers, and their source is safe, for sure ”.

CONCLUSIONS

By productivity stood out the most the following varieties: ‘Botu Oii de Caraș’, ‘Pătul de Vârciorova’ and ‘Parmen auriu’. However, there were a few varieties that did not produce at all in the third year after the planting: ‘Pietros’, ‘Măr de Jupani’, ‘Mari de Berini’.
The highest sugar content and dry matter content was registered in case of ‘Măr Domnesc’ and ‘Parmen auriu’.
The biggest fruits were produced by ‘Curcubătoase’, ‘Caslere’ and ‘Jonathan’ varieties, whereas the same indicators (big and small diameter, height and mass of the fruits) had the lowest values in case of ‘Crețesc’, ‘Parmen auriu’ and ‘Măr Țigănesc’.
Regarding the trunk’s diameter, the highest values were obtained in case of ‘Florănești’ and ‘Măr mare’ varieties, whereas the lowest ones for ‘Parmen auriu’, ‘Mari de Berini’ and ‘Botu Oii de Caraș’.
Regarding the height of the trees, the highest values were measured for ‘Măr dulce’, ‘Măr dulce amar’, ‘Poinic’ and ‘Pietros’ varieties. ‘Mari de Berini’, ‘Caslere’ and ‘Pătul de Vârciorova’ had the smallest vigour.
Related to the results of the questionnaire, overall it can be deducted that a very high percentage of those asked are convinced that a balanced and healthy alimentation must include these fruits too. People seem to be aware of the fact that fruits in general, especially apples, need many chemical treatments, therefore they do not believe that these are as healthy as they once used to be. This fact is the answer to why Bio apples are that expensive: it is very challenging for growers to produce fertilizer-free fruits when the number of pests and diseases is constantly growing.

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CAN KIWIFRUIT GROW IN ROMANIA? RESULTS OF THE ROMANIAN BREEDING PROGRAM AFTER 25 YEARS OF RESEARCH ON *Actinidia* spp.

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Abstract

In Romania, kiwi is a new fruit specie and the creation, testing and introduction of winter hardy genotypes, adapted to the local harsh climate conditions represent a priority. The first kiwifruit orchards with A. deliciosa and A. arguta were planted in Romania in 1993, at Ostrov (Constanța County), on the border of the Danube River. In the same year, a common Italian-Romanian kiwifruit breeding program was initiated at the Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest. Since 1993, research has been carried out to determine the best methods of propagation, growing and kiwifruit orchard management. Genotypes phenology was studied every year in comparison with the climatic data. In parallel, physical and biochemical fruit characteristics were evaluated after ripening and during the storage. After some years of observations and tests, three kiwiberry elites were selected: 'Vip Green' (R8P23), 'Vip Red' (R8P20) and 'Green Delight' (R8P1). Other intra and interspecific crossings using A. deliciosa and A. chinensis were made and from the initial hybrids, some selected genotypes as R0P13, R1P9, R1P8 and R1P12, have good fruit characteristics and yield. The new selections have to be registered as cultivars and can be successfully cultivated on commercial orchards and in private gardens. This paper presents few results of the Actinidia spp. Romanian breeding program. Some fruit quality characteristics of the new kiwi selections and hybrids as average weight, fruit shape index, soluble solids content, dry matter, acidity and ascorbic acid are detailed. After more than two decades of research, it was demonstrated that A. deliciosa and A. chinensis can be grown in Romania in peach favorable areas, while A. arguta (kiwiberry or baby kiwi) can cover larger areas, suitable for plum cultivation.

Key words: *A. arguta, A. chinensis, A. deliciosa, fruit characteristics, selection.*

INTRODUCTION

Actinidia species have been domesticated from wild populations located on Yangtze River basin (Ferguson, 1990; Young et al., 1995) and almost all the *Actinidia* species are widely distributed in Asia (Huang & Ferguson, 2007). Kiwifruit represent a recently developed crop, only within the last century started to be commercialized (Barboni et al., 2010; Ferguson & Bollard, 1990; Huang & Ferguson, 2007; Sui et al., 2013; Warrington 1990; Young et al., 1995), and it is recognized as highly nutritious and low calories fruit with the potential to deliver a range of health benefits (Burdon et al., 2004; Cangi, 2011; Drummond, 2013; Harker et al., 2007; Huang et al., 2004; Iwasawa et al., 2011; Mohammed et al., 2017; Namestnikov et al., 1989; Plekhanova, 1940; Samadi-Maybodi & Shariat, 2003; Stonehouse et al., 2013; Testolin et al., 2016; Vasile Scăețeanu et al., 2019; Yang, 2010).

Cultivation of kiwifruit spread from China in the early 20th century to New Zealand (Biao et. al., 2018; Huang et al., 2007; Meena et al., 2018). The kiwifruit history started in 1904, when Isabel Fraser, brought the first seeds from China to New Zealand (Ferguson & Bollard, 1990; Huang et al., 2007; Warrington 1990; Young et al., 1995). In 1910, Hayward Wright, obtained the first kiwi plants in New Zealand and in 1930, was selected and cultivated the first cultivar - 'Hayward' (Biao et al., 2018; Stănică et al., 2007; Stănică, 2009; Stănică & Zuccherelli, 2009). According Huang (2016) and Zhang et al. (2010), the current commercial cultivation is almost entirely based on *A. deliciosa* and *A. chinensis* species, that are naturally distributed between 25° and 45° in both northern and southern hemispheres. *A. arguta* commercial potential started to be recognized in the last years, especially in colder regions, this specie being lesser extent (Ferguson and Huang, 2007).

In Romania, *Actinidia* is a new fruit specie and the creation, testing and introduction of winter hardy kiwifruit genotypes, adapted to the local harsh climate conditions represent a priority in the national fruit growing programs (Iliescu et al., 2019a; 2019b; 2019c; Stănică et al., 2007; Stănică, 2009; Stănică & Zuccherelli, 2009).

The first kiwifruit orchards with *A. deliciosa* and *A. arguta* were planted in Romania in 1993, at Ostrov (Constanța County), on the border of the Danube River (Iliescu et al., 2019a; 2019b; 2019c; Peticilă et al., 2002; Stănică & Cepoiu, 1996a; Stănică, 2009; Zuccherelli, 1994). In the same year, a common Italian-Romanian kiwifruit breeding program was initiated between Vitroplant, Cesena and Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest (Iliescu et al., 2019a; 2019b; 2019c; Iliescu & Stănică, 2022; Iliescu et al., 2022; Stănică et al., 2003a; 2004; Stănică & Zuccherelli, 2007; Stănică et al., 2022a, 2022b).

Worldwide, the most *A. deliciosa* or *A. chinensis* cultivars were initially selected directly from the wild population (Atak et al., 2015; Ferguson, 1997; Huang et al., 1999). In time, in their breeding programs, researchers have tried to get new cultivars with different fruit characteristics, such as flesh colour, hairless skins, early maturity, better taste, high yield, long storage life, through different intraspecific and interspecific crosses techniques (Atak et al., 2015; Muggleston et al., 1998; Nicotra et al., 1999; Testolin & Ferguson, 2009; Xiao, 1999).

The aim of this paper is to present the results of the *Actinidia* spp. Romanian breeding program, after 25 years of research. Some fruit quality characteristics of the new kiwi selections and hybrids as average weight, fruit shape index, soluble solids content, dry matter, acidity and ascorbic acid are also detailed.

Romania's position is 43°37'-48°15' northern latitude, and 20°15'-29°41' eastern longitude. The climate is temperate-continental, with 8-11°C annual average temperature and 450-800 mm annual rainfall (Stănică et al., 2022a).

After more than two decades of observations, studding different morpho-productive characteristics of kiwi plants - as the main phenological stages according to the BBCH scale, plant resistance to deep frost, pest and diseases, for the most important *Actinidia* species was establish the fruit growing favorable areas where they can be cultivated with success (Stănică, 2009; Stănică & Zuccherelli, 2009; Stănică et al., 2022a; 2022b), especially due to the soil diversity (Mihalache et al., 2015; Popa et al., 2016) and quality (Schmidt et al., 2017; Vizitiu et al., 2017), and also climate condition (Bucur & Dejeu, 2016) from our country.

MATERIALS AND METHODS

Romanian breeding program objectives focused on fruit quality parameters as size, shape; fruit hairs and skin; fruit and pulp colour; maturation period and ripening indicators; fruit texture; aroma; composition in nutrients and vitamins. Plant resistance to deep frost, pest and diseases are also studied (Stănică, 2009; Stănică et al., 2022a).

Experience location and plantation description

The trial plants with kiwifruit hybrid genotypes, was established on preluvosoil in the Romanian plain, using some hybrid seedlings (Stănică & Cepoiu, 1996a; Zuccherelli, 1994). Some varieties - Hayward, Bruno etc. were also planted. Planting system used for the most trials was 4 m between the rows and 2 m between the plants. The experimental field within the Faculty of Horticulture, Bucharest - 44.4708° N and 26.0662° E (Asănică et al., 2017), was established in 1993. The climate is temperate continental, with 10.5-12°C annual average temperature and 550-600 mm annual amount rainfall, with a maximum recorded between May and July (Bălan et al., 2015). Almost 178-205 frost-free days and the vegetation period of 245 days are registered every year (Bălan et al., 2015). Air circulation is dominant from the east and north east during winter and west for the rest of the year, and the maximum wind speed is 3.5-4 m/sec (Bălan et al., 2015).

Most of the observations and analysis took place in the research orchard and laboratories of the Faculty of Horticulture and at the Research Center for Studies of Food Quality and Agricultural Products, within the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

The plants were grown under an organic orchard management, on a T-bar trellis system, represented in Figure 1. The inter row surface was covered with a mixture of perennial grasses and mowed mechanically, and along the row, the soil was kept clean (Stănică et al., 2022a). Drip irrigation and micro spray irrigation system was provided (Stănică et al., 2022a).

Figure 1. T-bar trellis system
on *A. arguta* experimental field
(Source: original, photo credit Lavinia Mihaela Iliescu)

Selection criteria for kiwifruit hybrids for all three species - *A. deliciosa*, *A. chinensis* and *A. arguta*

In order to decide if kiwifruit can grow in Romania the following selection criteria for kiwifruit hybrids, were studied:

- **different morpho-productive characteristics of kiwi plants** (habitus; the main phenological stages according to the BBCH scale and the optimal harvesting period; drought and frost resistance; resistance to water excess; plants productivity; behavior to pests and diseases; studies of polyploidy);
- flowering period, pollen viability and germination percentage were analysed, for the **hybrid male selections**;
- **some physical and chemical quality parameters of the fruits** at harvest and at consumption maturity (productivity index; average fruit weight; fruit shape index; length of the fruit peduncle; fruit flesh firmness; soluble solids content - Brix %; total dry matter content - %; fructose and glucose content - %; malic acid and citric acid content - mg/100 g; ascorbic acid content - mg/100 g), **fruits storage capacity,** initial analyzes (after harvest) and monthly analyzes in dynamics (physical and chemical fruits parameters as fruit flesh firmness; percent of fruits losses; soluble solids content - Brix %; total dry matter content - %; fructose and glucose content - %; malic acid and citric acid content - mg/100 g; ascorbic acid content - mg/100 g; polyphenol content; antioxidant capacity), **consumers perception** regarding different characteristics of the fruits (shape and size, taste, flavor and pulp colour), were analysed, for the **hybrid female selections**;
- **different fruits consumption possibilities** (fresh or processed).

Besides selection of male and female hybrids adapted to the local climate, since 1993, research has been carried out to determine the best methods of propagation, growing and kiwifruit orchard management.

RESULTS AND DISCUSSIONS

Study of morpho-productive characteristics of kiwi plants

The main phenological stages according to the BBCH scale and the optimal harvesting period, were studied every year, in comparison with the climatic data (Iliescu & Stănică, 2020).

The bud break mostly took place in the beginning to mid-March, when the temperature was higher than 3.5°C, while the flowering started in first decade of May, for Bruno, and male selections (R2P8 and R3P9).

The female selections (R0P13, R1P8, R1P9, R1P12) started to bloom in the second decade of May. The flowering period finished in the first or second decade of June (Iliescu & Stănică, 2020; Stănică et al., 2022a).

Regarding the harvesting period, this started with ‘Bruno’, R0P13, R1P8 and R1P12, in the third decade of October and continued with ‘Hayward’ and R1P9 in the first decade of November (Iliescu & Stănică, 2020; Stănică et al., 2022a).

The phenological enlargement of kiwifruit could improve the quality of fruits by providing information about evolution of the varieties and local hybrids under the environmental conditions of Southern Romanian (Iliescu & Stănică, 2020).

An accurate understanding of kiwifruit plant phenological stages it is essential for an appropriate orchard management (Iliescu & Stănică, 2020). Through this study, one proposal of principal kiwifruit phenological growth stages adapted according to the BBCH scale, was described, for all the three species – *A. deliciosa*, *A. chinensis* and *A. arguta* (Figure 2).

Figure 2. Principal kiwifruit phenological growth stages adapted according to the BBCH scale for the main *Actinidia* species
(Source: Iliescu & Stănică, 2020)

Plants productivity represents an important criterion in the selection of elites, so in every year production per plant and per hectare were studied and calculated for some hybrid, and over the time was published in different papers (Iliescu et al., 2019a; Stănică et al., 1998).

Intraspecific and interspecific crosses are frequently used in the breeding process and the genetic morphological characterization of the hybrid descendants represents an important task (Iliescu & Stănică, 2022). Because, *Actinidia* species have different numbers of chromosomes, after interspecific crosses, the new hybrids can have the same number of chromosomes as the parents or a different number. Flow cytometry has proved to be an efficient means of estimating genome size and associated ploidy level for some interspecific hybrids obtained over the years (Cotruț et al., 2013a). On the study "*Actinidia* species under microscope", the results showed how different morphological characters are influenced and defined by different species and was concluded that most of the kiwifruit interspecific hybrid genotypes *A. deliciosa* × *A. chinensis* showed similar characters to *A. deliciosa*, while the *A. chinensis* × *A. arguta* ones, to *A. arguta* (Iliescu & Stănică, 2022).

Study of kiwi hybrid genotypes for pollinator selection

No matter specie, pollination is a very important component regarding a regular and consistent production in a number of fruit crops (Costa et al., 1993; Hopping et al., 1982; Jovanovic-Cvetkovic et al., 2016; Petrisor et al., 2012; Underwood, 2001). The viability, tube growth and morphological homogeneity related to pollen quality are the most important properties in fruit plants (Iliescu et al, 2022; Petrisor et al., 2012). These properties are useful for plant breeders, geneticists, and growers (Bolat and Pirlak, 1999). Relationships between viability and pollen germination have been studied and a positive correlation between them was reported (Pearson & Harney, 1984).

After more than two decades of research, several hybrid genotypes were obtained and introduced to be tested through the Romanian breeding program (Cotruț et al., 2014; Iliescu et al., 2022; Stănică & Cepoiu, 1996b). For pollinator (male) kiwi plants, breeding programs involve the selection of elites with high pollen germination capacity and long flowering period (Cotruț et al., 2014; Iliescu et al., 2022; Stănică & Cepoiu, 1996b). In previous research (Cotruț et al., 2014) germination rate of few Romanian kiwi genotypes (*Actinidia* spp.) was evaluated after 3, 6 and 9 hours in a culture medium containing 20% sucrose, 5 ppm boric acid (H_3BO_3) and 1% agar. The results showed that in all kiwi genotypes the germination rate and pollen tube growth varied according to the incubation period and most of the studied genotypes appear to be suitable pollinators (Cotruț et al., 2014).

Studies of the male plants flowering period, pollen germination rate (Iliescu et al, 2022) and also the shape and surface of pollen grains (Iliescu & Stănică, 2022) were achieved to identify the most suitable pollinators for kiwi female selections released from the Romanian breeding program. The aim of the study was to evaluate the pollen grains quality of fifteen kiwifruit hybrids express by: shape index of viable and dead pollen grains, viability percentage (%), germination rate (%) and pollen tubes length (µm) after 4, 8, 12 and 24 hours (Iliescu et al., 2022). Regarding the study of male plants flowering period, it can be mentioned that most kiwi hybrids bloomed between the first decade of May and the first decade of June (Iliescu & Stănică, 2020; Iliescu et al, 2022). The R0P7 hybrid ensued the BBCH 60 stage in the last decade of April, with the earliest development and the longest flowering period was recorded for the R2P8 hybrid (Iliescu et al., 2022). Results showed that in all kiwi genotypes the germination rate and pollen tube growth varied according to the incubation period (Iliescu et al., 2022), confirming the previous studies accomplished in 2014 (Cotruț et al., 2014). The highest percentage of germination (93%) was recorded after 24 hours of incubation for R2P8 and R3P9 (Iliescu et al, 2022). Because the evaluation of pollen germination rate is an essential criterion for kiwi pollinator's characterization, four genotypes - R0P3, R0P6, R2P8 and R3P9 (which recorded over 90% germinability rate after 24 hours), have been selected for further field tests (Iliescu et al., 2022).

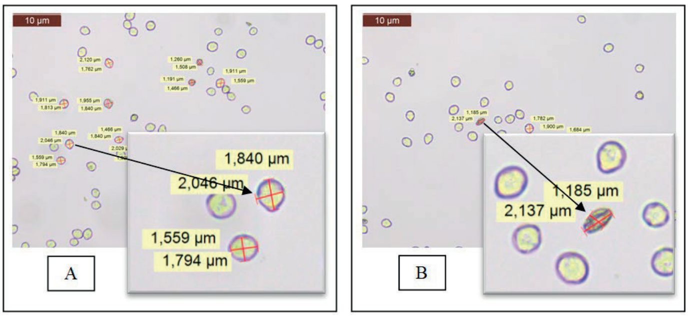

Figure 3. Viable (A) and dead (B) pollen grains of R0P7 male kiwifruit hybrid, after immerging in a 15% sucrose solution
(Source: Iliescu et al., 2022)

Study of some physical and chemical quality parameters of the fruits, at harvest and at consumption maturity, for some kiwi hybrid genotypes (*Actinidia* spp.)

Productivity index, average fruit weight, fruit shape index, length of the fruit peduncle, fruit flesh firmness, content in soluble solids (Brix %), total dry matter content (%), fructose and glucose content (%), malic acid and citric acid content (mg/100 g) and ascorbic acid content (mg/100 g), were determined on 20 fruits samples for some kiwifruit hybrids (Iliescu et al., 2019a; 2019c; Stănică et al., 2022a; 2022b; Vasile Scăețeanu et al., 2019).

Study regarding the fruit's storage capacity, initial analyzes (after harvest) and monthly analyzes in dynamics

Fruit behaviour in cold storage was evaluated in normal conditions (3°C and 95% relative humidity) and in three controlled atmosphere conditions, with 1.5% O_2, 2.0 and 5.0% CO_2, at 1-2 °C and 95% relative humidity (Cotruț et al., 2016; Iliescu et al., 2019 b; Stănică et al., 2007; Stănică et al., 2022 a, b). At harvest and then monthly, during the storage, some physical and chemical fruits parameters as fruit flesh firmness, percent of fruits losses, soluble solids content (Brix %), total dry matter content (%), fructose and glucose content (%); malic acid and citric acid content (mg/100g), ascorbic acid content (mg/100g), polyphenol content, antioxidant capacity, were determined (Cotruț et al., 2016; Iliescu et al., 2019 b; Stănică et al., 2007; Stănică et al., 2022 a, b).

Figure 4. Kiwifruits harvesting and storage possibilities (normal and controlled atmosphere conditions)
(Source: original, photo credit Lavinia Mihaela Iliescu)

Consumers perception regarding different characteristics of the fruits (shape and size, taste, flavor and pulp colour), for the hybrid female selections

Sensorial assessment was carried out in a sensorial testing laboratory by consumer panelists of different age, gender and origin (Iliescu et al., 2019c). Fruit quality was evaluated by appearance and taste (fruit size and shape, fruit pulp colour, taste and flavor) and for the results it was used a 1-5 rating Hedonic scale (Iliescu et al., 2019 c; Stănică et al., 2022a; 2022b).

Consumers education regarding local kiwi fruits production and the recommended consumption maturity

Consumer's request is focused more and more on high quality, safe and environment friendly products, as well as having a transparent traceability (Nicolae et al., 2016). In this context, Romanian consumers are more interested to consume local kiwifruits. In the framework of numerous events and fairs organized within the University of Agronomic Sciences and Veterinary Medicine of Bucharest and not only, a lot of consumers have the opportunity to test the kiwifruits produce in Romania. The purpose of this fruits testings are the educations of the Romanian consumers regarding the local production, the taste and flavor of the fruits, and also the recommended consumption maturity.

Different fruits consumption possibilities (fresh or processed)

Kiwifruits are mostly eaten fresh, although some kiwifruits are also processed into juices, alcoholic beverages (cider, liqueur, brandy), purees, candied fruit and bars, jam and marmalade, dehydrated and lyophilized products, cakes or pastries, kiwifruit leathers (Cassano et al., 2007; Guine et al., 2017; Stan et al., 2021). In the last years fruits processing starts to be more appreciate in the same way, by the consumers and also by the producers (Catană et al., 2018). The new tendency is to use local fruits and also to find innovative products using fruits waste (Catană et al., 2018).

Figure 5. Different products obtained from kiwi fruits and waste
(Source: original, photo credit Lavinia Mihaela Iliescu)

Kiwifruit propagation research in the last 25 years

According with Hartmann et al. (2011), Stănică et al. (1995), Stănică (2004) and Tanimoto (1994), the common methods of kiwifruit propagation are grafting, cuttings and micropropagation. Since 1993, Romanian researchers has been carried out a lot of studies to determine the best methods of propagation (Table 1), for all three species - *A. deliciosa*, *A. chinensis* and *A. arguta*. During the time, numerous studies have been carried out on the multiplication by grafting (Iliescu et al., 2021) or over grafting (Stănică et al., 2001), cuttings (Peticilă et al, 2015; Peticilă et al., 2016; Stănică et al., 1995; Stănică et al., 1997; Stănică et al., 2003b; 2003c) and micropropagation (Peticilă et al., 2012; Stănică et al, 1995; Stănică, 1998; Stănică & Armeanu, 2004; Stănică et al., 2004 Stănică et al., 2005). Stănică et al. (2002), in the study "Synthesis of researches regarding the kiwifruit (*Actinidia* sp.) propagation" concluded that: for **grafting** - the bark grafting method gave very good results with a rate of success between 75.7%-98.5%, waxed scions preservation at 3-4°C gave excellent result in terms of viability and aseptically and the best binding material for the grafting point was the black self-adhesive tape named Bendaflex; for **cutting** - the optimal period was between the end of January and mid-February (for *A. deliciosa* and *A. chinensis*) and beginning of August (for *A. arguta*), for obtaining a high rooting percentage a basal heating at 22-24°C and lower atmospheric temperature (15-18°C) were essential, the best results were obtained when perlite on wood compost and perlite on wood flour were used; for **micropropagation** - the best stage for the *in vitro* culture initiation is when the new shoots reach 5-10 cm length, the proper sterilization of the material was made with 0.1% mercuric chloride (HgCl2), for 15 minutes for *Actinidia deliciosa* and 10 minutes for *A. arguta*, S 2,5 medium was the best for the multiplication phase with 4 weeks duration, acclimatization of rooted explants can be made in tap water (viability 92%) or in peat + perlite substrate under mist conditions.

Latest results regarding the kiwifruit breeding

Considering that the consumers global trends are increase regarding food safety and natural products (Lelieveld, 2015); the agricultural sector is sensitive to climate change (Bucur & Dejeu, 2016; Pickering et al., 2014); the farmers started convert their cultivating methods to organic, taking into consideration the environmental protection (Koufiotis et al., 2016) and conservation agriculture without affecting crop yields, especially on soils with high initial fertility (Rusu et al., 2015); the number of trees in urban agriculture are increasing and also the diversification of species and varieties of trees and shrubs grown (Bălan et al., 2015) and farmers' desire to cultivate profitable species increased (Asănică et al., 2016), the cultivation of the *Actinidia* in Romania, can be a solution for all of this tendencies.

In time, several hybrid genotypes were obtained by free and controlled crossings between different cultivars of *A. arguta*: Francesca, Rosana, Jumbo, AA2, AA5, AA 6, AA 8 and the male ARM (Stănică et al., 2003 a; Stănică & Zuccherelli, 2007). The first flowers and fruits from *A. arguta* hybrid plants were produced in 2001 and selection has continued since then (Stănică et al., 2003a; Stănică & Zuccherelli, 2007; Stănică & Zuccherelli, 2009). After some years of observations and tests, eight elite female plant with interesting fruit characteristics were chosen for propagation and testing under commercial orchard conditions (Stănică et al., 2003a; Stănică & Zuccherelli, 2007; Stănică et al., 2007; Stănică & Zuccherelli, 2009; Stănică et al., 2022b). From this, three kiwiberry selections were registered: 'Vip Green' (R8P23), 'Vip Red' (R8P20) and 'Green Delight' (R8P1), and for male selection was proposed R9P16 (Stănică et al., 2022b).

Other intra and interspecific crossings using *A. deliciosa* and *A. chinensis* were made and from the initial hybrids, some selected genotypes as R0P13, R1P9, R1P8 and R1P12, have good fruit characteristics and yield (Iliescu et al., 2019a, 2019c; Stănică et al., 1998; Stănică et al., 2022a), good storage capacity (Cotruț et al., 2016; Iliescu et al., 2019b; Stănică et al., 2007b; Stănică et al., 2022a; 2022b) and positive appreciation from consumers (Iliescu et al., 2019c; Stănică et al., 2022a). For male selections was proposed R2P8. This hybrid selections are under the registration process as new cultivars by ISTIS (Romanian institute for testing and registration of new varieties).

Table 1. Kiwifruit propagation results in the last 25 years

Species	Propagation methods	Materials and methods	Results and conclusions	References
Actinidia spp. and hybrids	grafting	**Rootstocks:** 'Z1 Vitroplant'; **Grafted method:** whip and tongue; **Time and temperature**: April, in a cold greenhouse, where the temperature varied between 20-24°C; **Materials:** Flexiband, Arborinn.	Grafting success rate varied between 45-90%.	Iliescu et al., 2021
	cuttings	**Substrate:** sand and perlite (1:1, volumetric ratio); **Experimental variants:** V1 - control; V2 - Radistim; V3 - NAA 2000 ppm (NAA = naphthyl acetic acid); V4 - IBA 2000 ppm (IBA = beta-indolyl butyric acid); V5 - NAA + IBA 1000 ppm.	For rooting stimulation, it is recommended treatment NAA+IBA 1000 ppm.	Peticilă et al., 2016
		Treatments: alpha naphthyls acetic acid (2,000 - 3,000 ppm); **Substrates:** double layers and mixed, with: wood flour + perlite; wood compost + perlite; cotton waste + perlite; **Temperature**: at the cutting's base was maintained constantly at 22-25°C, while in the air, at 15-18°C.	The rooting percentage and the quality of formed roots were strongly influenced by specie, variety, cutting moment, substrate type used and basal and atmospheric temperature. The optimal period for cutting was between the end of January and mid-February. The best results were obtained when pearlite on wood compost and pearlite on wood flour were used. On double layer variants the root's length was higher than in one-layer variants, but the root's number per cutting was lower.	Stănică et al., 2003 b
	micropropagation	**Explant types**: roots fragments of 2 cm length; shoots' internodes; petioles; leaf blades; **Culture media:** callogenetic medium MS, supplemented with 1.0 mg/l zeatine and 0.02 mg/l ANA; organogenetic medium MS, supplemented with 0.2 mg/l ANA and 2.0 mg/l BAP.	The greatest callus production was accomplished by the petiole and leaf blade. The pH value of the culture medium radically influenced the callogenesis and indirect organogenesis processes, the best results being registered for all explant types at pH 7. The callus growing (callogenesis) alternates with the formation of a big number of shoots on that callus (indirect organogenesis).	Stănică & Armeanu, 2004
A. deliciosa	cuttings	**Substrates**: 50% manure + 20% peat + 20% fallow soil + 10% sand; 40% manure + 50% fallow soil + 10% sand.	The obtained results regarding N, P, K accumulation in kiwi leaves recommend the cultivation of Hayward cultivar on substrate based on 50% manure + 20% peat + 20% fallow soil + 10% sand, this system offering the best release of available forms of nutritive elements for plants, with not significant differences given by the fertilization system, excepting N accumulation.	Peticilă et al, 2015
A. arguta	micropropagation	**Growing media**: classic MS with unchanged components; modified MS medium with a double quantity of ammonium nitrate (2N); modified MS medium with a triple quantity of ammonium nitrate (3N).	To initiate the culture of *A. arguta*, the most successful medium for the male plant (86.6%) was MS 2N and for the female plant (66.6%) were classic MS and MS 2N.	Peticilă et al., 2012

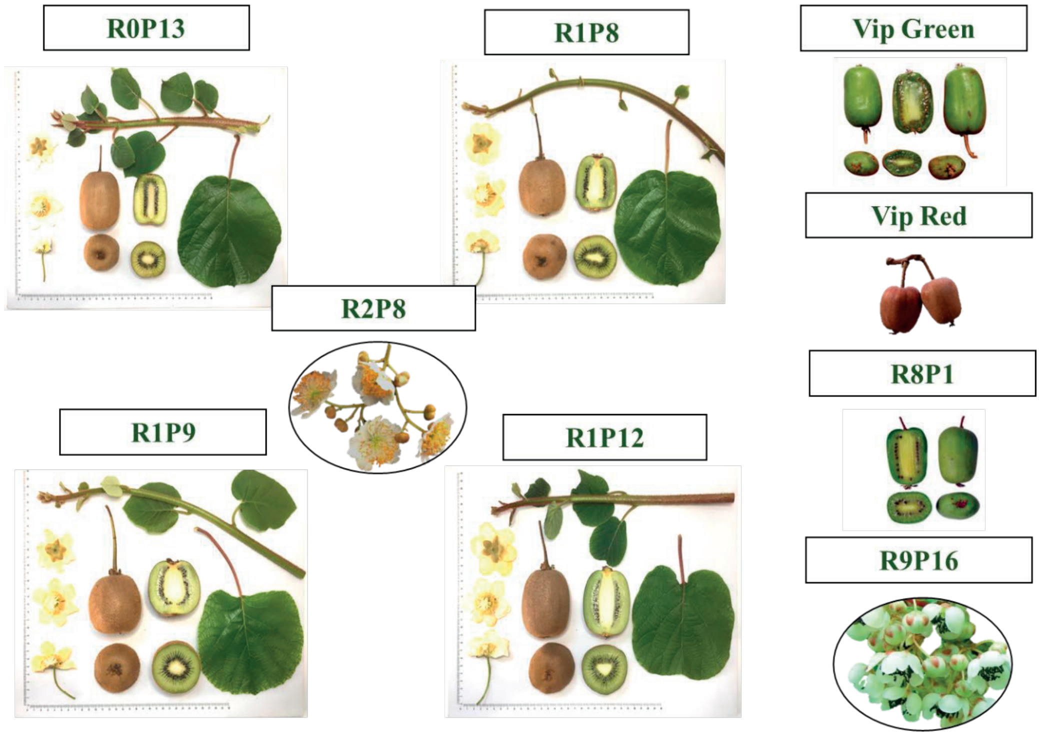

Figure 6. New released kiwifruit genotypes
(Source: original, photo credit Lavinia Mihaela Iliescu)

Actinidia arguta **Vip Green (R8P23)** CPVO: n. 2017/2828 (Figure 6) **-** it is a climbing plant of medium vigour, yielding specially on long and medium branches with determined growth; the cultivar is unisex, female with medium blooming period; the fruit is large (15.6 g), long of approximately 5.2 cm, cylindrical shape, flat dorso-ventral, with a diameter of 3.8 cm and one of 2.7 cm, olive green peel colour, shining green pulp, juicy, accentuated sweet taste, with very fine specific flavor (Stănică et al., 2022b). Contains about 14.2% dry soluble solids, balanced acidity, and 67.32 mg ascorbic acid at 100 g/fresh fruit (Stănică et al., 2022b). It starts bearing fruits in the 2nd-3rd year after planting and has a production capacity between 4.9-6.0 kg/plant (Stănică et al., 2022b).

Actinidia arguta **Vip Red (R9P20)** CPVO: n. 2017/2829 (Figure 6) **-** it is a climbing plant of medium vigour, yielding specially on long and medium branches with determined growth; the cultivar is unisex, female; with medium blooming period; the fruit is small to medium large (9.1 g), long of approximately 2.7 cm, truncated cone shape, with the largest diameter of 2.4 cm, dark red, shining red pulp, juicy, accentuated sweet taste, with very fine specific flavor (Stănică et al., 2022b). Contains about 16.65% dry soluble solids, low acidity, 76.7 mg ascorbic acid at 100 g/fresh fruit (Stănică et al., 2022 b). It starts bearing fruits in the 2nd-3rd year after planting and has a production capacity between 4.7-5.0 kg/plant (Stănică et al., 2022b).

Actinidia arguta **Green Delight (R8P1)** represented in Figure 6 **–** it is a climbing plant of medium vigour, yielding specially on long and medium branches with determined growth; the cultivar is unisex, female; with medium blooming period; the fruit is medium large (12-13 g), long of approximately 4 cm, cylindrical shape, flat dorso-ventral, olive green, shining green pulp, juicy, accentuated sweet taste, with very fine specific flavor (Stănică et al., 2022b).

Contains about 13.71% dry soluble solids, balanced acidity, 70.15 mg ascorbic acid 100 g/fresh fruit (Stănică et al., 2022b). It starts bearing fruits in the 2^{nd}-3^{rd} year after planting and has a production capacity between 4.7-5.4 kg/plant (Stănică et al., 2022b). The plants are resistant to *Pseudomonas syringae* pv. *Actinidiae* (Cotruț et al., 2013b).

For good pollination, two selected pollinators: **R9P16**, **R9P18** are recommended to be planted in the orchard in a 1:5 ratio.

Actinidia spp. **R0P13** (Figure 6) **-** it is a climbing plant; the cultivar is unisex, female with medium blooming period; the fruit is medium (65.77 ± 0.013 g), long of approximately 54.95 ± 0.812 cm, elliptical shape, with a diameter of 44.50±0.766 cm, shining green pulp, juicy, accentuated sweet taste, with specific flavor (Stănică et al., 2022a). At the consumption maturity contains about 17.56% Brix, 28.45% dry soluble solids, 1.536% citric acid, and 49.23 mg ascorbic acid at 100 g/fresh fruit (Stănică et al., 2022a). Harvesting period can start at the end of October * first decade of November (Stănică et al., 2022 a). Storage capacity is about 7 months in normal condition, respectively 10 months in controlled atmosphere (Stănică et al., 2022a).

Actinidia spp. **R1P8** (Figure 6) **-** it is a climbing plant; the cultivar is unisex, female with medium blooming period; the fruit is large (118.88 ± 8.291 g), long of approximately 57.86 ± 0.887 cm, elliptical shape, with a diameter of 57.86 ± 0.887 cm (Stănică et al., 2022 a). At the consumption maturity contains about 14.37% Brix, 16.30% dry soluble solids, 1.500% citric acid, and 38.92 mg ascorbic acid at 100 g/fresh fruit (Stănică et al., 2022a). Harvesting period can start at the end of October - first decade of November (Stănică et al., 2022a). Storage capacity is about 6 months in normal condition, respectively 7-9 months in controlled atmosphere (Stănică et al., 2022a).

Actinidia spp. **R1P9** (Figure 6) **-** it is a climbing plant; the cultivar is unisex, female with medium blooming period; the fruit is large (106.88±6.640 g), long of approximately 56.85±1.954 cm, spherical shape, with a diameter of 55.86±3.143 cm (Stănică et al., 2022 a). At the consumption maturity contains about 16.50% Brix, 32.26% dry soluble solids, 1.603% citric acid, and 77.20 mg ascorbic acid at 100 g/fresh fruit (Stănică et al., 2022a). Harvesting period can start at the beginning of November until the second decade of November (Stănică et al., 2022a). Storage capacity is about 8 months in normal condition, respectively 10-11 months in controlled atmosphere (Stănică et al., 2022a).

Actinidia spp. **R1P12** (Figure 6) **-** it is a climbing plant; the cultivar is unisex, female with medium blooming period; the fruit is very large (202.39 ± 3.479 g), long of approximately 72.91 ± 1.510 cm, oblong shape, with a diameter of 60.97 ± 1.305 cm (Stănică et al., 2022a). At the consumption maturity contains about 10.18% Brix, 13.41% dry soluble solids, 1.487% citric acid, and 125.38 mg ascorbic acid at 100 g/fresh fruit (Stănică et al., 2022a). Harvesting period can start at the end of October - first decade of November (Stănică et al., 2022a). Storage capacity is about 7 months in normal condition, respectively 8-10 months in controlled atmosphere (Stănică et al., 2022a).

For good pollination, two selected pollinators: **R2P8**, **R3P9** are recommended to be planted in the orchard in a 1:5 or 1:6 ratio. **R2P8** male plant formed between 4 and 6 or 8 flowers in the dichasium inflorescence, had over 90% pollen viability and 93.35% pollen germinability (Stănică et al., 2022a). **R3P9** male selection had between 3 to 5 flowers in inflorescence, over 97% pollen viability and 93.23% pollen germinability (Stănică et al., 2022a). The main advantage of R2P8 and R3P9 pollinators is a long flowering period, which coincides with the female selections.

CONCLUSIONS

After more than 25 years of research, it was demonstrated that the main *Actinidia* species can be cultivated in Romania with success.

Regarding *A. arguta*, **'Vip Green' (R8P23)** and **'Vip Red' (R9P20)** are two new kiwiberry cultivars in the last phases of registration at CPVO Angers. The green very large fruits (over 15 g), respectively the medium red ones (around 10 g) are very appreciated by the consumers. **Green Delight (R8P1)** and **R9P16** (male), are under the registration process as new cultivars by ISTIS (Romanian institute for testing and registration of new varieties). All cultivars showed a good adaptability to the Romanian local climate and pedological conditions, and they can be tested in other areas in order to be extended in commercial orchards. Kiwiberry or baby kiwi can cover larger areas, suitable for plums cultivation.

The new intra and interspecific kiwifruit hybrids females: **R0P13**, **R1P8**, **R1P9**, **R1P12** and male: **R2P8** selections, are under registration process as new cultivars by ISTIS (Romanian institute for testing and registration of new varieties) and can be successfully cultivated on commercial orchards and private gardens. The selected elites and other *A. deliciosa* and *A. chinensis* cultivars can be cultivated in Romania in the favorability zone for peach and apricot with some special measures for the deep frost protection of young plants and for the wind protection too.

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FUNGAL ENDOPHYTIC COMMUNITY ASSOCIATED WITH PEAR TWIGS AND BUDS

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Abstract

This study presents the first screening of the fungal endophytic community associated with pear twigs and buds. Experiments were conducted on collected biological material from the Romanian resistant-disease pear collection. Endophytic fungal community in twigs was represented by Alternaria (35%), Aureobasidium (25%), Cladosporium (16%), Penicillium (5%), Fusarium (4%), Sordaria (4%), Nigrospora (3%), Trichoderma (3%) and Botrytis (0.6%) isolates. Isolates belonging to Alternaria (56%), Aureobasidium (47%), Cladosporium (15%), Fusarium (5%), Trichoderma (4%), Epicoccum (3%), Penicillium (2%) and Nigrospora (2%) genera were detected and identified in buds. Our results highlight the presence of isolates belonging to genera Aureobasidium, Epicoccum, Sordaria, and Trichoderma, which have been described as having antagonistic properties and/or potential to promote plant growth. A better understanding of these endophytic communities in a complex network can bring information on their roles, on their interactions with pear trees and with pathogens, and their mechanism of action.

Key words: *fungal endophytes, pear trees, twigs and buds.*

INTRODUCTION

The microbial component of a plant or the microbiota has important functions in its growth and in maintaining its health (Brader et al., 2017; Lemanceau et al., 2017).
As a part of microbiota, endophytes are microorganisms that live within plant tissues without causing symptoms of disease and are important components of plant microbiomes. They interact with other core microbial groups that colonize plant tissues (Sharma et al., 2021).
As microorganisms have proven their potential in application as biostimulants or biopesticides, there is an increased interest in their integration into protection programs as an alternative to chemical products (Campant et al., 2019; Suciu et al., 2021; Stefan et al., 2021).
Isolation of endophytic fungi and their identification is the first step in studies related to signaling their possible role in the biological control of plant pathogens.
Knowing the community of endophytic microorganisms can bring information about their role, the interaction with plant pathogens and their mechanism of action.
Information about the functionality of plant-microbiome interactions as well as about the factors that are involved in establishing a microbial community can lead to a better understanding of the plant as a meta-organism and how plants benefit from their microbial partners.
The application of a consortium of microorganisms is an approach that is attracting more and more interest. Thus, microorganisms with different properties can be combined, which can be complementary or which can combine different mechanisms of action (stimulation of plant growth, biocontrol of pathogens).
In this context, the aim of this study was to detect and identify the endophytic mycobiota community of pear twigs.

MATERIALS AND METHODS

Experiments were conducted on Romanian pear disease resistant genotypes, produced at the Voineşti Research Station for Fruit Growing and Horticultural Research Station - USAMV Cluj-Napoca, and planted in the Experimental

Orchard of the Faculty of Horticulture, USAMV Bucharest.

Endophytic community associated with pear trees was assessed on pear twigs collected in march 2021 from 'Conference', 'Euras', 'Corina', 'Cristal', and 'Romcor' cultivars.

The twigs were cut into small pieces (0.5 cm), surface sterilized using 70% ethanol for 2 minutes, 4% sodium hypochlorite for 90 seconds and rinsed in water for three times (Ren et al., 2019). The tissues were blotted dried and plated on potato dextrose agar (PDA). Plates were incubated at 22°C until growing of colonies.

Fungal isolates were identified based on their macroscopic (colony color, pigmentation) and microscopic characteristics, the results being expressed as colonization rate (CR, %).

Pure culture of isolates was acquired through subsequent sub-culturing on PDA plates.

RESULTS AND DISCUSSIONS

The endophyte microbial community associated with twigs is shown in Figure 1. Of the 560 fragments analyzed, 480 were colonized, resulting in a colonization rate of 86%.

The presence of *Alternaria* isolates (35%), followed by *Aureobasidium* (25%) and *Cladosporium* (16%) was detected. Isolates of the genera *Penicillium* (5%), *Fusarium* (4%), *Sordaria* (4%), *Nigrospora* and *Trichoderma* (3%) and *Botrytis* (1%) were also detected and identified.

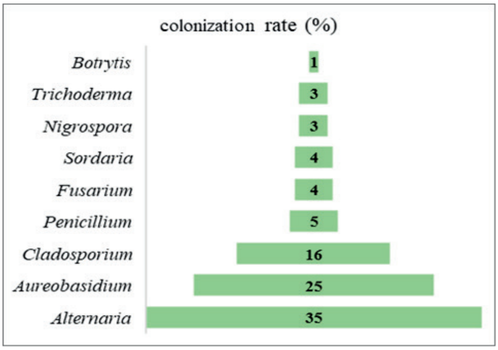

Figure 1. Endophytic community on pear twigs

Among the isolates with antagonistic potential, the presence of those from the genera *Aureobasidium*, *Sordaria*, *Nigrospora* and *Trichoderma* was detected.

In the 'Conference' variety (Figure 2), the isolates detected and identified were *Alternaria* (with the highest colonization rate, 38%), *Aureobasidium* (25%), *Penicillium* (12%), *Cladosporium* (10%) and *Fusarium* (5%). Among the isolates with antagonistic potential, we note the presence of those from the genus *Aureobasidium*.

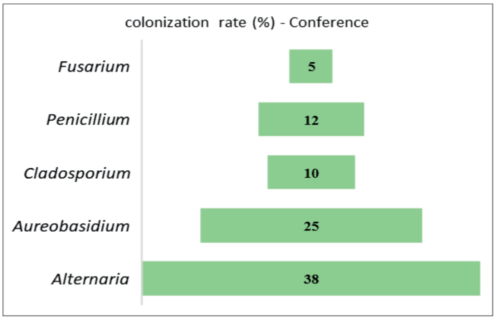

Figure 2. Endophytic community on pear twigs - 'Conference' variety

In 'Euras' variety (Figure 3), the microbial community was also mostly represented by isolates of the genus *Alternaria* (58%). *Cladosporium* (48%), *Aureobasidium* (31%) and *Fusarium* (4%) isolates were also identified.

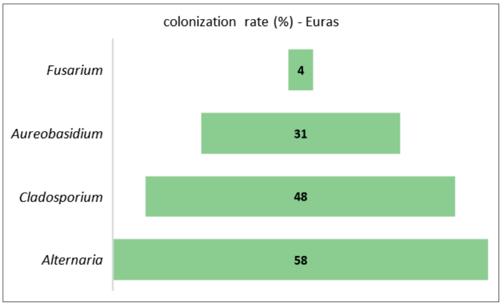

Figure 3. Endophytic community on pear twigs - 'Euras' variety

Among the isolates with antagonistic potential, the presence of those from the genus *Aureobasidium* was detected.

The microbial community identified in 'Cristal' variety is shown in Figure 4. Isolates of the genera *Alternaria* (27%), Aureobasidium (22%), *Cladosporium* (22%) and *Penicillium* (10%) were detected. Among the isolates with antagonistic potential, the presence of those from the genus *Aureobasidium* was detected.

The microbial community identified in 'Romcor' cultivar is shown in Figure 5. In this variety, the presence of *Aureobasidium* isolates with the highest colonization rate (29%) is noted. Isolates of the genera *Alternaria* (16%), *Penicillium* (13%), *Cladosporium* (5%) and *Fusarium* (5%) were also identified.

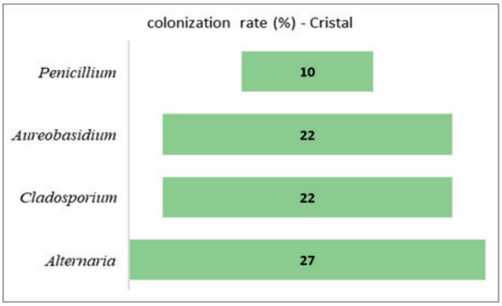

Figure 4. Endophytic community on pear twigs - 'Cristal' variety

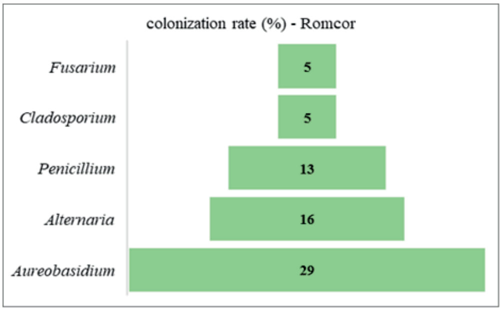

Figure 5. Endophytic community on pear twigs - 'Romcor' variety

In 'Corina' variety, the microbial community was richly represented by isolates of genus *Sordaria*, (32%), a genus known for its species with antagonistic properties (Figure 6).
Isolates of the genus *Alternaria* had a colonization rate of 23%. Isolates of the genera *Cladosporium* (2%), *Fusarium* (3%) and *Botrytis* (5%) were also detected.
Among the isolates with antagonistic potential, *Aureobasidium* (21%), *Trichoderma* (11%) and Sordaria (32%) have been also identified.
Regarding the endophyte microbial community of buds (Figure 7), our results highlight the high colonization rate of *Alternaria* isolates (56%), followed by those of the genera *Aureobasidium* (47%), *Cladosporium* (15%), *Fusarium* (5%), *Trichoderma* (4%), *Epicoccum* (3%), *Penicillium* (2%), and *Nigrospora* (2%).

All the 262 fragments analyzed (131 buds, sectioned) were colonized, resulting in a colonization rate of 100%.
A richly represented microbiota have been observed, with isolates belonging to genera known for their antagonistic potential like *Aureobasidium*, *Trichoderma*, *Epicoccum* and *Nigrospora*.
The presence of *Epicoccum* isolates was detected only at the bud level.

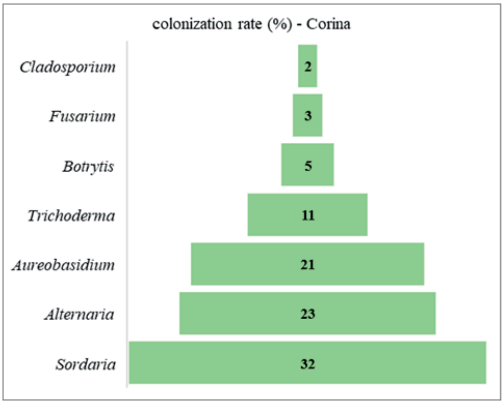

Figure 6. Endophytic community on pear twigs - 'Corina' variety

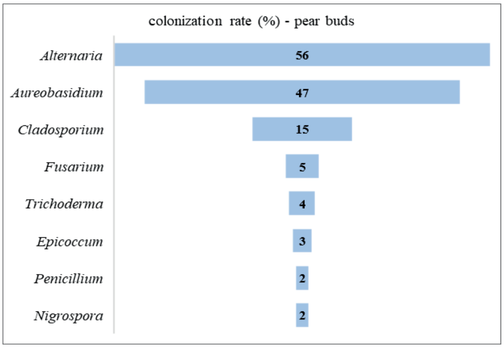

Figure 7. Endophytic community on pear buds

Aspects of the morphology of the detected and identified colonies are presented in Figures 8 and 9.
In endophytic community of pear twigs and buds our results highlight the most abundant phylum as *Ascomycota*. Among the most abundant ascomycetes we reported the *Alternaria* spp. isolates (*Pleosporaceae* family). Previous studies have shown that endophytic trees community mainly consist of Ascomycota and to a lesser extent, Basidiomycota and

Zygomycota (Muller et al., 2016; Sun & Guo, 2012).

A comprehensive view of the endophytic fungal community of pear trees was reported (Fei Ren et al., 2019). Each investigated organ (flower, leaf, fruit, stem and root) harbored a unique fungal assemblage, with *Ascomycota* the most abundant phylum.

The high abundance in all samples of *Aureobasidium* and Sordaria in twigs from 'Corina' variety may indicate an active role. Further studies are needed for the cultivation of endophytic fungal strains with biocontrol effect as well as ecological and functional roles.

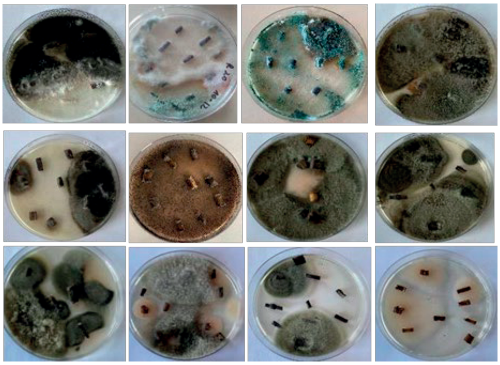

Figure 8. Colonies morphology of endophytic community on pear twigs

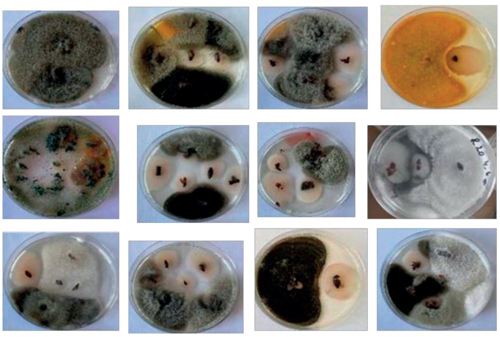

Figure 9. Colonies morphology of endophytic community on pear buds

CONCLUSIONS

This study presents the first screening of the fungal endophytic community associated with pear twigs and buds. The endophyte fungal community of twigs was represented by isolates from the genera *Alternaria*, *Aureobasidium*, *Botrytis*, *Cladosporium*, *Fusarium, Penicillium*, *Nigrospora, Sordaria*, and *Trichoderma*. Isolates belonging to the genera *Alternaria*, *Aureobasidium*, *Cladosporium*, *Fusarium*, *Trichoderma*, *Epicoccum*, *Penicillium* and *Nigrospora* were detected and identified in the buds.

Antagonistic species belonging to the genera *Aureobasidium* (*A. pullulans*), *Epicocum* (*E. purpurascens*), *Sordaria* (*S. fimicola*) and *Trichoderma* (*T. harzianum*) were detected and isolated. The presence of these communities with antagonistic properties is an advantage for plants. Knowledge of the endophytic community can bring information on their role, interactions with plant pathogens and their mechanism of action.

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SWEET CHERRY (*Prunus avium* L.) RANDOM AMPLIFICATION OF POLYMORPHIC DNA ANALYSIS OPTIMIZATION

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Abstract

Prunus avium L. is a fruit trees species belonging to the Rosaceae family, cultivated worldwide in temperate climates. This study presents the genomic DNA extraction and RAPD method optimisation using the 'Severin' sweet cherry cultivar. For genomic DNA genomic DNA extraction was optimized the method for breaking the cell wall. The parameters optimized for RAPD reaction were genomic the annealing temperature, DNA concentration and primer concentration. The best method for breaking the cell wall was Method 1, grinding the tissue with liquid nitrogen. The optimum annealing temperature was determined to be 30°C. The concentration of genomic DNA in the RAPD reaction varied between 0.05 ng/µl and 1.00 ng/µl, and that of the primer varied between 0.1 µM and 2.0 µM. The optimum concentration for the genomic DNA proved to be 0.05 ng/µl and that of the primer 2.0 µM. These results will be applied in a future experiment that will study the genetic variability of Romanian sweet cherry cultivars present in the USAMV of Bucharest orchard collection.

Key words*: genomic DNA extraction; polymerase chain reaction; RAPD optimization.*

INTRODUCTION

Even though cherries have been used by humans for more than 6000 years, domesticated by ancient Greeks and introduced to the rest of Europe by Romans in the 1st century, due to the fruits' perishability, cherry breeding started much later, during the 18th century (Blando & Oomah, 2019). Nowadays, new sweet cherry cultivars are created and evaluated for economically important traits such as fruit weight and colour, resistance to cracking, fertility, resistance to low winter temperatures (Iurea et al., 2019; 2020; Palubiatka et al., 2021).

In Romania, sweet cherry (*Prunus avium* L.) is cultivated traditionally. Local sweet cherry cultivars are the main sources of biodiversity in agriculture, so their recognition, identification and conservation is of particular importance.

To be able to implement the necessary conservation measures, it is very important to know the genetic characteristics of the varieties and their degree of relatedness (Parra-Quijano et al., 2012). Recent sequencing techniques and genome sequencing have brought to light new data that can be used for a variety of purposes, including the identification of molecular markers linked to traits of interest, and molecular marker-assisted selection (Soundararajan et al., 2019).

Since a minimum of 4-5 years are needed for cherry trees to produce the first fruits, a lot of time and space are required to develop new varieties. In this case, marker - assisted selection employing molecular markers such as RAPD, SSR, AFLP, reduces considerably both the time and space required in the breeding process, as the plants carrying the desired trait can be selected from the seedling phase (Jayasankar & Kappel, 2011). The selection at young stage can be done not only for cultivars, but also for rootstocks (Quero-Garcia et al., 2017), because cultivar/rootstock combination impacts the growth and development of the plants, affecting economically important traits such as yield and fruit size (Asănică et al., 2013).

Random amplification of polymorphic DNA (RAPD) is a PCR technique that uses an arbitrary single oligonucleotide primer that can anneal to template DNA sequences on both DNA strands and amplify sequences between the annealing sites under low stringency conditions, creating a “fingerprint” of a particular genome (Babu et al., 2021). RAPD has been used in studies of genetic diversity, plant breeding, germplasm management, cultivar and hybrid verification, taxonomic studies, phylogenetic studies, study of genetic marker–trait association, etc. (Antić et al., 2020; Babu et al., 2021; I. V. Berindean et al., 2016; Khadivi-Khub, 2014; Sharma et al., 2012; Zarei et al., 2017).

RAPD techniques has a high versatility, low cost, but a low reproducibility due to low quality of DNA template, and variations in template DNA concentration (Babu et al., 2021). Therefore, optimization of genomic DNA extraction, as well as optimization of template DNA concentration, RAPD primer concentration, and primer annealing temperature are three parameters that should be optimised to get the best results in a RAPD reaction.

The objective of this study was to optimise the genomic DNA extraction conditions as well as the RAPD reaction conditions before proceeding to the genetic variability study of sweet cherry cultivars present in the USAMV of Bucharest orchard collection.

MATERIALS AND METHODS

Plant material

Leaves from sweet cherry cultivar ‘Severin’ were used for this study. The leaves were harvested from the University of Agronomic Sciences and Veterinary Medicine of Bucharest orchard.

Genomic DNA extraction optimization

Young leaves, harvested from the top of the shoots were washed with distilled water, weighed, placed in 50 ml tubes, and stored at -70°C.

Cell wall breaking was performed using the InnuPREP Plant DNA I KIT IPC 16 (Analitik Jena) by three different methods: 1. Grinding the tissue with liquid nitrogen using a mortar and pestle, then adding SLS lysis solution and proteinase K; 2. Grinding the tissue with a micro pestle in a 1.5 ml tube containing the SLS lysis solution and proteinase K; 3. Tissue frozen at –70°C for 24 hours in 2 ml tubes containing steel balls was homogenized using the SpeedMILL PLUS homogenizer, then SLS lysis solution and proteinase K were added. Thereafter, all three protocols continued with the external lysis of the cells, according to the InnuPREP Plant DNA I KIT IPC 16 manufacturer instructions. Briefly, samples were homogenized, then incubated at 65°C for one hour. Thereafter, the samples were filtered to remove tissue debris, treated with RNase A, and transferred to plate for automated DNA extraction. Automatic extraction was performed using InnuPURE C16 System with the Ext_Lysis_200_C16_04 program. Following extraction, samples were stored at –20°C.

Measurement of DNA quantity and quality

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

RAPD optimization

Polymerase chain reaction (PCR) was done with the Platinum™ II Hot-Start PCR Master Mix (2X) (Invitrogen) according to the manufacturer’s instructions regarding the extension temperature and the amounts of PCR master mix and Platinum GC enhancer used. PCR setup was done according to Table 1.

Table 1. PCR reaction setup

Component	Volume	Final concentration
Nuclease-free water	to 10 µl	-
Platinum™ II Hot-Start PCR Master Mix (2X)	5 µl	-
10 µM Primer P59	0.1-2 µl	0.1-2.0 µM
0.5-10 ng/µl Template DNA	1 µl	0.05-1 ng/µl
Platinum GC Enhancer	2 µl	-
Total	10 µl	-

The sequence of primer P59 was 5’-GTTGGTGGCT-3’. For genomic DNA, the concentration varied between 0.5 ng/µl and 10 ng/µl. The concentration of the primer varied between 0.1 µM and 2 µM, and the annealing temperature gradient was between 30°C and 34°C. PCR program is visible in Table 2.

The online tool *Oligo Calc: Oligonucleotide Properties Calculator* was used to determine the range of annealing temperature to be tested

(Kibbe, 2007). The basic Melting Temperature of the primer was calculated using the formula Tm= (wA+xT) * 2 + (yG+zC) * 4, where w, x, y, z are the number of the bases A, T, G, C in the sequence, respectively, since the length of the primer is below 14 bp (Marmur & Doty, 1962).

Table 2. PCR program used for RAPD reactions

Temperature	Time	Cycles
94°C	2 min	1
94°C	15 sec	40
30-34°C	15 sec	
68°C	30 sec	
68°C	2 min	1
4°C	HOLD	

The PCR products were visualized using Molecular Imager® PharosFX™ and PharosFX™ Plus Systems after migration in a 2% agarose gel at 100 V for 45 min.

Data analysis

Data for genomic DNA extraction optimization was analysed using the Excel for Microsoft 365 software.

RESULTS AND DISCUSSIONS

Genomic DNA extraction optimization

The three parameters considered to obtain the optimum method of genomic DNA extraction were the yield (DNA concentration), and the A260/A280 and A260/A230 ratios.

DNA yield

The genomic DNA yield varied between 30.9 ng/µl (Method 2) and 40.3 ng/µl (Method 3) (Figure 1). The DNA yield obtained by Method 1 (39.51 ng/µl) was similar to the one obtained by Method 3.

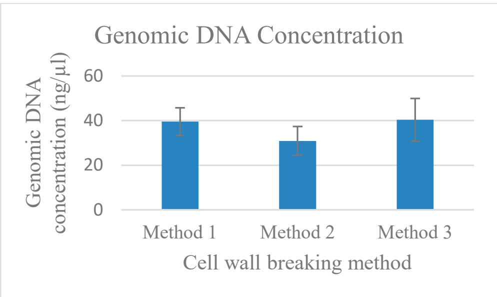

Figure 1. Genomic DNA concentration obtained with three different methods for breaking the cell wall. Error bars represent standard error of the mean

DNA purity

Genomic DNA purity was assessed based on the A260/A280 and A260/A230 ratios. For genomic DNA, an ideal A260/A280 ratio is ~1.8, and A260/A230 ratio should be between 2.0-2.2 (Matlock, 2015).

Methods 1, using liquid nitrogen, and Method 3, using prior deep freezing of the tissue before homogenization with SpeedMill Plus, yielded A260/A280 absorbance ratio values over 1.5 (1.72 for Method 1 and 1.63 for Method 3), whereas when using Method 2, the A260/A280 absorbance ratio was much lower, 1.14 (Figure 2).

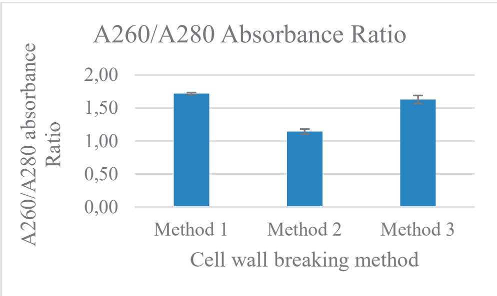

Figure 2. DNA purity measured using the A260/A280 absorbance ratio for the three different methods used to break the cell wall. Error bars represent standard error of the mean

In a study to optimize genomic DNA extraction protocol for twelve rosaceous species, Antanaviciute et al. (2015) observed an A260/A280 ratio of 1.53 for sweet cherry, one of the lowest ratios noted among the species studied. The low ratios indicate contaminants absorbing at 280 nm or less (Matlock, 2015).

When looking at A260/A230 ration, all samples have low values, indicating carbohydrate carryover (Matlock, 2015), however the best results were observed for Method 1 with an almost double value when compared to the other two methods (Figure 3).

Taking into consideration the results obtained, the optimum Method that was used to extract genomic DNA to be used further in the RAPD reaction optimization was Method 1, breaking of the cell walls using liquid nitrogen, with the highest yield and the purest genomic DNA extracted.

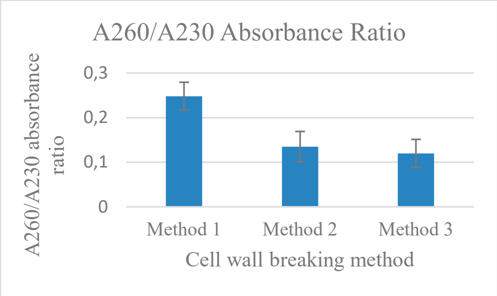

Figure 3. DNA purity measured using the A260/A230 absorbance ratio for the three different methods used to break the cell wall. Error bars represent standard error of the mean

RAPD reaction optimization

The three parameters optimised for the RAPD analysis were DNA template concentration, RAPD primer concentration, and primer annealing temperature.

Annealing temperature optimization

Based on the basic melting temperature of the primer of 32°C, the range of annealing temperatures tested was between 30.0°C and 34.0°C. The clearest amplification of the PCR products was observed at 30°C (Figure 4). The low value of the annealing temperature is due to the short length of the primer, thus in RAPD reactions annealing temperatures are usually below 40 °C (Antić et al., 2020; I. V. Berindean et al., 2016; Guan et al., 2014; Sevindik et al., 2020; Sharma et al., 2012; Thakur et al., 2018).

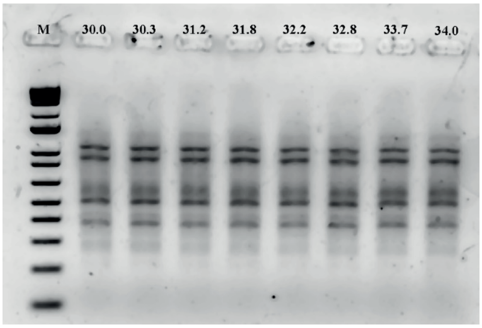

Figure 4. Effect of annealing temperature optimization on RAPD amplification patterns. M-1Kb Plus DNA Ladder (Invitrogen). Numbers represent the annealing temperature in °C

Genomic DNA concentration optimization

The range of genomic DNA concentrations tested was between 0.5 ng/µl and 10.0 ng/µl. The best results were obtained with the initial concentration of 0.5 ng/µl (final concentration of 0.05 ng/µl) for template DNA (Figure 5). Williams et al., who developed the RAPD technique in 1990, used a template DNA concentration of 1 ng/µl, however they recommended reducing the template DNA concentration to obtain distinct bands as opposed to a smear (Williams et al., 1990).

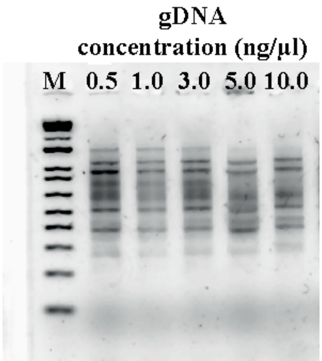

Figure 5. Effect of genomic DNA concentration on RAPD amplification pattern. Numbers represent the initial concentrations of genomic DNA

Primer concentration optimization

The range of final concentrations for the analysed primer P59 was between 0.1 µM and 2.0 µM and it was noticed that the optimum concentration was 2.0 µM, (Figure 6). Final primer concentration in RAPD reactions are usually below 1 µM (Antić et al., 2020; Berindean & Itu, 2019; Eimert et al., 2012; 2016; Zamani et al., 2012)

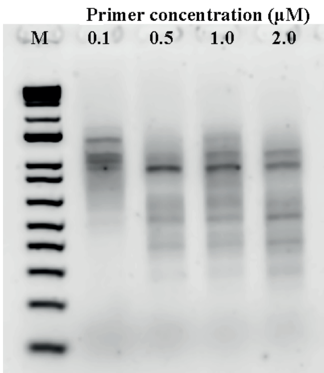

Figure 6. Effect of primer concentration on RAPD amplification pattern. Numbers represent the final concentrations of the primer in the reaction

The results obtained in this study will be applied in a future experiment that will study the genetic variability of cherry varieties present in the USAMV Bucharest orchard.

CONCLUSIONS

In conclusion, in the present study were optimised the method for breaking the cell wall prior to genomic DNA extraction and three RAPD reaction parameters as follows:

1. The best method for breaking the cell walls was Method 1, grinding the tissue with liquid nitrogen with a mortar and pestle, as it gave the best DNA yield and the purest DNA.
2. Optimum annealing temperature was 30°C.
3. Optimal concentration of genomic DNA was 0.05 ng/µl.
4. Optimum primer concentration was 2.0 µM.

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GENETIC RELATIONSHIPS BETWEEN SEVERAL ROMANIAN PLUM VARIETIES USING RAPD MOLECULAR MARKERS

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Abstract

Plum (Prunus domestica L.) is the dominant fruit tree species in Romania and has an ancient tradition in growing all over the country. The assortment of the plum varieties has known a continuous increase in time, enhancing its genetic variability. In the current work, RAPD analysis was carried out in order to assess the genetic relationships between seven Romanian plum cultivars ('Brumării de Voinești', Record', 'Gemenea', 'Elena', 'Centenar', 'Silvia', and 'Pescăruș') existent in the germplasm collection of the Faculty of Horticulture, USAMV Bucharest, Romania. Five random decamer primers identified 28 polymorphic and 11 monomorphic RAPD loci. The constructed UPGMA dendrogram associated the cultivars studied into 2 clusters, one cluster with the varieties 'Record' and 'Gemenea', and another cluster grouping the rest of the varieties. The genetic fingerprints obtained following amplification with the RAPD markers, are specific for each variety, and may be used for molecular identification of the varieties in the germplasm collection.

Key words: *European plum, genetic variability, plum breeding, plum germplasm, genetic fingerprint.*

INTRODUCTION

European plum, (*Prunus domestica* L.), a hexaploid species (2n = 48), is the dominant fruit tree species in Romania and has an ancient tradition in growing all over the country. By the end of 2021, Romania ranked 6th in the European Search Catalogue for Plant Genetic Resources (EURISCO) Catalogue, based on the existing number of European plum accessions, after Switzerland, Russian Federation, Spain, Germany and United Kingdom (Sottile et al., 2022). In an effort to identify, conserve and evaluate plum genetic resources in Romania, two research centers host plum germplasm collections with over 1000 accessions: wild species, local populations, named cultivars, breeder's selections and rootstocks (Butac et al., 2019).

Current climate changes, resulting especially in drought and salinization of farmlands, are calling for development of new cultivars that are drought and salt resistant through faster breeding using molecular methods to assist classical breeding (Vicente, 2022).

Recent developments in sequencing techniques have made whole genome sequencing more affordable, but still there are challenges to be met for polyploid species. Since the European plum is a hexaploidy species, in the National Center for Biotechnology Information (NCBI) database no genome or genome assembly has been deposited as yet, and there are only 130 genes registered for *Prunus domestica* L. A draft genome assembly is available on Genome Database for Rosaceae (GDR) database (Callahan et al., 2021), so in the future more specific molecular markers such as Simple Sequence Repeats (SSRs) can be discovered. However, the assessment of genetic diversity can still be done with Random Amplification of Polymorphic DNA (RAPD) molecular markers (Iordachescu et al., 2021; Udriște & Bădulescu, 2019).

RAPD is a PCR technique developed in 1990 (Williams et al., 1990), that uses a single random decamer primer that will anneal to both DNA strands, and will amplify the sequences between the annealing sites (Babu et al., 2021). RAPD technique has multiple applications besides the study of genetic diversity among cultivars, such as checking the genetic stability of plants grown in vitro, study of genetic relationships among cultivars, and cultivar identification within collections (Athanasiadis et al., 2013; Ben Tamarzizt et al., 2015; Iancu & Chivu, 2021; Li et al., 2022; Thakur et al., 2018; 2021; Yu et al., 2013).

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

MATERIALS AND METHODS

Materials

Seven Romanian plum accessions enclosed in the collection of University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania, 'Brumării de Voinești', Record', 'Gemenea', 'Elena', 'Centenar', 'Silvia', and 'Pescăruș', were studied in the present research.

Genomic DNA extraction

Extraction of genomic DNA from young leaves was performed using the Innu PREP Plant DNA II KIT IPC 16 Kit (Analytik Jena) according to the manufacturer instructions. Frozen tissue was grounded to powder with liquid nitrogen. The extraction was performed with InnuPREP Plant DNA I KIT IPC 16 (Analytik Jena), according to the manufacturer's instructions.

Briefly, for each sample, approximately 100 mg of powder was transferred to 1.5 ml tubes, and then 600 µl lysis solution SLS and 20 µl Proteinase K were added to the sample. Thereafter, the samples were incubated for 1 hour at 65°C, centrifuged for 5 min at 10000 x g, and then the supernatant transferred to prefilters fitted to collection tubes. After an additional centrifugation for 2 min at 10000 x g, 2 µl of RNase A (10 mg/ml) were added and samples were incubated for 5 min at room temperature. After this step of external lysis, samples were transferred to the reagent plates and into the InnuPure C16 (Analitik Jena) apparatus for automatic genomic DNA extraction, using the Ext_Lysis_200_C16_04 program. DNA quality and quantity were checked with Nanodrop 1000 (Biorad).

RAPD

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

Table 1. PCR reaction setup

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

Annealing temperature optimization (between 30 and 35°C) was done for all primers. PCR program consisted of an initial denaturation step of 2 min at 94°C, followed by 40 cycles of denaturation 15 sec at 94°C, annealing 15 sec at 30°C, and extension 30 sec at 68°C, and a final extension step of 2 min at 68°C. The nucleotide sequences of the primers used are presented in Table 2.

Table 2. Decamers' nucleotide sequences

Decamer	Nucleotide sequence
P59	5'-GTTGGTGGCT-3'
P60	5'-GGGAACGTGT-3'
P61	5'-CCGTGACTCA-3'
P62	5'-CTTCCGCAGT-3'
P63	5'TGCCGAGCTG-3'

DNA amplicons from the RAPD reactions were separated on 1.5% agarose gel, visualized with the Pharox FX system (BioRad), and were measured using the Quantity One software (Version 4.6.9., BioRad).

Data analysis

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

RESULTS AND DISCUSSIONS

Initially, the annealing step was optimised in a gradient temperature PCR reaction, using 30.0, 30.9, 32.2, 34.1 and 35.0°C (Figure 1). All bands amplified were below 2000 bp. As the best clear bands were observed for all primers used at 30°C, further RAPD analysis of selected cultivars was done with this temperature at the annealing step. Yu et al. (2013) used for the annealing step a temperature of 44°C, however in their study the primers were 11 nucleotides long, so it is expected that the annealing temperature is higher.

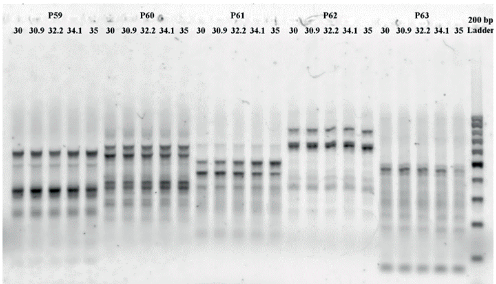

Figure 1. Effect of annealing temperature on RAPD amplification patterns for each primer used. On the right is present the 200 bp Ladder (Invitrogen). Numbers represent the annealing temperature in °C

The five random decamer primers (Table 3) identified a total of 27 polymorphic and 12 monomorphic RAPD loci.

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

Decamer	Polymorphic loci	Monomorphic loci	Amplified fragments sizes (bp)*
P59	4	2	1200, 1150,1100, 900, **620, 430**
P60	5	2	2000, **1200**, 1050, 950, 700, **630**, 520
P61	1	2	**950, 800**, 450
P62	8	2	1500, 1450, **1230**, 1220, 1050, 900, **820**, 700, 620, 550
P63	9	4	1200, **1000, 900**, 800, 750,650, 550,540,**450**, 350, 250, 200, **150**

*Fragments written with bold letters represent monomorphic loci.

P61 decamer is the least desirable decamer to be used, as it amplified only three DNA fragments, out of which only one was polymorphic. The P62 and P63 decamers amplified 10, respectively 13 DNA fragments with 8, respectively 9 polymorphic loci, making them the best candidates from this set of markers for further fingerprinting studies. The number of loci amplified by each primer was lower than those obtain by Athanasiadis et al. (2013), however in that study was observed the polymorphism of three different plum species, *P. domestica, P. insititia* and *P. cerasifera*.

The markers used have provided specific "fingerprints" for each genotype, allowing for molecular identification.

NAME	Brumării de Voinești	Record	Gemenea	Elena	Centenar	Silvia	Pescăruș
Brumării de Voinești	0.00	0.69	0.77	0.58	0.72	0.69	0.64
Record	0.69	0.00	0.58	0.67	0.75	0.61	0.67
Gemenea	0.77	0.58	0.00	0.79	0.72	0.79	0.69
Elena	0.58	0.67	0.79	0.00	0.64	0.47	0.61
Centenar	0.72	0.75	0.72	0.64	0.00	0.69	0.75
Silvia	0.69	0.61	0.79	0.47	0.69	0.00	0.54
Pescăruș	0.64	0.67	0.69	0.61	0.75	0.54	0.00

Figure 2. Roger distances calculated with the Bio-R software. The shortest Roger's genetic distance between cultivars is marked with green, and the longest is marked with blue

The calculated Roger's genetic distances are represented in Figure 2. The most closely related are the cultivars 'Silvia' and 'Elena', whereas the most distantly related are the accessions 'Silvia' and 'Gemenea' and 'Elena' and 'Gemenea', as it can be also observed from the dendrogram presented in Figure 3.

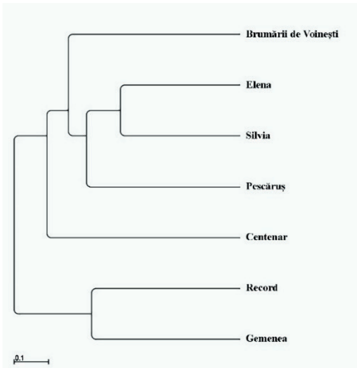

Figure 3. Dendrogram based on the RAPD data generated with the Bio-R software

The constructed UPGMA dendrogram (Figure 3) associated the accessions studied into two clusters, one cluster with the genotypes 'Record' and 'Gemenea', and another cluster grouping the rest of the cultivars. 'Record' is a cultivar resulted from the free pollination of 'Renclod violet' at the SCDP Voinești Research Station (Table 4). Since 'Gemenea' is a local population present in the same area, so it is possible that ‘Gemenea’ or another closely related population to be the pollen donor.

Interestingly, the most closely related are the genotypes ‘Silvia’ and ‘Elena’, even though they have different genitors. Further studies with additional markers and eventually whole genome sequencing may unravel the similarity between these two accessions.

Table 4. Accession’s origin

Accession	Origin
Elena	Tuleu gras x Stanlay, SCDP Bistrița*
Silvia	Renclod Althan x Early Rivers, SEA Mărculești*
Pescăruș	Renclod Althan x Wilhemina Spath, SEA Mărculești*
Centenar	Tuleu gras x Early Rivers, SEA Mărculești*
Record	Natural pollination Renclod violet - SCDP Voinești*
Brumării de Voinești	Local population - Voinești region
Gemenea	Local population - Voinești region

*(Ștefan et al., 2018)

CONCLUSIONS

RAPD analysis of the seven accessions from the USAMV of Bucharest revealed that:

- All random decamer primers identified both polymorphic and monomorphic RAPD loci.
- P62 and P63 are the most suitable markers to be used in discriminating the accessions.
- The genetic fingerprints obtained following amplification with the RAPD markers, are specific for each variety, and may be used for molecular identification of the varieties in the germplasm collection.
- The RAPD analysis using five decamers provided enough data to construct a UPGMA dendrogram, demonstrating the genetic variability among the cultivars studied.

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RAPD, ISSR AND SSR MOLECULAR MARKERS APPLICATIONS IN *Vaccinium* spp.

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Abstract

The consumption of berries on a global level, either from wild or cultivated species, is on an ascending trend, being linked to the fruits' high nutraceutical qualities. Therefore, the demand to create robust cultivars adapted to the various environmental conditions worldwide is becoming higher. In order to reduce the time needed to obtain new cultivars, breeders have started to use more and more molecular methods and techniques. Molecular markers, such as RAPD, ISSR and SSR, specific DNA regions linked to genes responsible for various traits such as colour, shape, taste, firmness, tolerance to biotic and abiotic stresses, are some of the molecular tools used in genotype-assisted breeding programs. The current review presents data related to the use of RAPD, ISSR and SSR molecular markers in Vaccinium species.

***Key words**: Vaccinium corymbosum, Vaccinium myrtillus, Vaccinium macrocarpon, Vaccinium ashei, Vaccinium angustifolium, genetic diversity, breeding.*

INTRODUCTION

Nowadays, the consumption of cultivated and wild berries is on the rise due to their high nutraceutical qualities and organoleptic properties (Asănică, 2018; Mudd et al., 2013). Numerous studies demonstrated the effect of berries in treating or preventing various diseases, such as high blood pressure, diabetes and cancer (Afrin et al., 2016; Bouyahya et al., 2022; Golovinskaia & Wang, 2021; Hameed et al., 2020; Wang et al., 2021).

Among the numerous berry fruits, genus *Vaccinium* from Ericaceae Family is very well represented, with 450 species and a worldwide distribution, covering the Globe from the arctic/subarctic area to the tropics (Edger et al., 2022; Kloet & Avery, 2010).

With the evident climate changes, the pressure to faster create new *Vaccinium* cultivars adapted to the present environment conditions is higher, so marker assisted breeding nowadays is a must, as it greatly increases the selection efficiency and reduces the time needed for cultivar release (Iwata et al., 2016; Lobos & Hancock, 2015). Breeding strategies to create new cultivars are greatly supported by the molecular techniques to reduce the time, space and biological materials used in the breeding programs. Currently, molecular markers such as RAPD, ISSR and SSR are some of the molecular tools used for plant breeding (MAS - marker assisted selection), and multiple other purposes in various fields: genetic variability studies, accession identification in collections, germplasm management, checking genetic stability after micropropagation, etc.

Preserving and increasing the genetic variability of the possible genitors' pool is extremely important, as it gives a better chance to find genitor combinations that would ensure the production of new cultivars with traits adapted to the environmental changes and customers' demands. One way to increase this pool is to look into the wild relatives of the cultivated species (Migicovsky & Myles, 2017). Considering the fact that the abundance of some wild relatives of the cultivated blueberry species has declined or become more variable (Hupp et al., 2015; Vega-Polo et al., 2020), is important to preserve these genetic resources *in situ* and *ex situ*, in collections.

Plant collections management also makes use of molecular markers, as they can be utilised to identify duplicates and mislabelled accessions, especially when there are little differences at morphological level.

Micropropagation technique is used for clonal mass propagation of genotypes. Berry crops are well suited for this technique, as they are heterozygous, thus their genetic characteristics are preserved using vegetative reproduction. However, plants propagated in vitro could still be the object of somatic mutations, so the genetic stability of micropropagated plants can be checked using molecular markers (Debnath et al., 2012).

Current review presents recent data on the use of three types of molecular markers, RAPD, ISSR and SSR for species belonging to *Vaccinium* genus.

RAPD, ISSR and SSR marker development and uses in *Vaccinium*

RAPD markers were first developed to assess DNA polymorphism based on the amplification of random DNA fragments with a single, short (~10 bp) primer with an arbitrary nucleotide sequence (Williams et al., 1990). The technique is simple, cost effective, it does not need prior knowledge of the genome studied, and it can be used for a variety of purposes: estimation of genetic diversity, genetic mapping, germplasm management, monitoring of genetic erosion, cultivar identification, hybrid verification, genetic fidelity testing for *in vitro* grown plants, etc. (Babu et al., 2021). A summary of the RAPD marker uses in *Vaccinium* spp. is presented in Table 1.

Table 1. RAPD marker uses in *Vaccinium* spp.

Species	Use of RAPD marker	Reference
V. macrocarpon	Identification of varietal misclassification; genetic diversity	(Novy et al., 1994)
V. darrowi; V. elliottii	Linkage map	(Rowland & Levi, 1994)
V. ashei	Cultivar identification	(Aruna et al., 1995)
V.darrowi; V. corymbosum	Inheritance mode in interspecific hybrids	(Qu & Hancock, 1995)
V. macrocarpon	Genetic variability	(Stewart & Nilsen, 1995)
V. macrocarpon	Genetic variability	(Stewart Jr. & Excoffier, 1996)
V. corymbosum, V. ashei, V. darrowi	Cultivar identification	(Levi & Rowland, 1997)
V.darrowi; V. corymbosum	Linkage map	(Qu & Hancock, 1997)
V. stamineum	Genetic variability	(Kreher et al., 2000)
V. hiepii	New taxon discovery	(Vander Kloet & Paterson, 2000)
V. cilindraceum	Genetic variability	(Martin-Clemente et al., 2001)
V. vitis-idaea	Genetic variability	(Persson & Gustavsson, 2001)
V. myrtillus	Genetic variability	(Albert et al., 2003)
V. myrtillus	Genetic variability	(Albert et al., 2004)
V. macrocarpon; V. angustifolium; V. vitis-idaea	Cultivar identification	(Debnath, 2005)
V. vitis-idaea	Genetic diversity; Selection for ex-situ conservation	(Garkava-Gustavsson et al., 2005)
V. oxycoccos	Genetic variability	(Areškevičiūtė et al., 2006)
V. macrocarpon	Genetic variability	(Debnath, 2007)
Vaccinium oxycoccos	Genetic variability	(Cesoniene et al., 2013)
V. bracteatum; V. corymbosum	Hybrid confirmation	(Tsuda et al., 2013)
V. corymbosum	Cultivar identification	(Carvalho et al., 2014)
V. padifolium; V. corymbosum	Hybrid confirmation	(Ehlenfeldt & Polashock, 2014)
V. myrtillus; V. uliginosum; V. vitis-idaea	Genetic variability; population dynamics	(Bjedov et al., 2015)
V. corymbosum	Genetic variability	(Wach et al., 2016)
V. corymbosum	Genetic variability	(Gawroński et al., 2017)
V. corymbosum	Genetic stability	(Nowakowska & Pacholczak, 2017)
V. myrtillus	Genetic variability	(Giordani et al., 2018)
V. corymbosum	Genetic stability	(Clapa et al., 2019)
V. myrtillus	Genetic variability	(Nin et al., 2019)

One of the most common uses of the **RAPD technique** is the study of ***genetic diversity*** among cultivars, or within populations in the case of wild species.

Genetic variation in the case of cultivated cranberry (*Vaccinium macrocarpon*) was studied in United States among samples picked from sites in Massachusetts, New Jersey, and Wisconsin, North Carolina, Tennessee, West Virginia, New York, Michigan, USA (Novy et al., 1994; Stewart & Nilsen, 1995; Stewart Jr. & Excoffier, 1996). In Canada, a genetic diversity assessment of 43 wild cranberry clones and 5 cultivars from 4 Canadian provinces was done using the RAPD technique (Debnath, 2007).

Genetic variability of highbush blueberry, *Vaccinium corymbosum*, was assessed in cultivars grown in Poland (Gawroński et al., 2017; Wach et al., 2016).

For wild *Vaccinium* species, genetic variation was studied on local populations of lingonberry, *Vaccinium vitis-idaea,* in Sweden (Garkava-Gustavsson et al., 2005; Persson & Gustavsson, 2001), and Central Balkans (Bjedov et al., 2015), on wild cranberry, *Vaccinium oxycoccos*, in Lithuania (Areškevičiūtė et al., 2006; Cesoniene et al., 2013), on bilberry, *Vaccinium* *myrtillus*, in Belgium (Albert et al., 2003, 2004), in Tuscan Apennines, Italy (Giordani et al., 2018), and in Central Balkans (Bjedov et al., 2015), on *Vaccinium stamineum* L. in USA (Kreher et al., 2000), on Azores archipelago endemic *Vaccinium cylindraceum* Smith (Martin-Clemente et al., 2001) and on *Vaccinium uliginosum* L. on Central Balkans (Bjedov et al., 2015).

A genetic diversity assessment study was done also on Tuscan Apennines wild bilberry seedlings to check the preservation of variability following micropropagation, to aid in the species conservation (Nin et al., 2019).

Cultivar identification is another benefit of using RAPD technique. In USA, using 15 rabbiteye blueberry (*Vaccinium ashei* Reade) cultivars and 4 wild selections, Aruna et al. (1995) developed a cultivar key based on 11 RAPD markers, and Levi and Rowland (1997) used RAPD and SSR-anchored primers to identify highbush and rabbiteye blueberry cultivars. In Canada, Debnath (2005) used 22 decamer primers to differentiate genotypes of three *Vaccinium* species: cranberry, lowbush blueberry (*V. angustifolium* Ait), and lingonberry. Carvalho et al. (2014) differentiated northern cultivars types from the southern types of highbush blueberry using RAPD and ISSR markers from fruits and leaves. Going beyond the scope of simply cultivar identification for its own purpose, Tsuda et al. (2013) used RAPD and CAPS markers to confirm the hybrid nature of plants resulted from the crosses of *Vaccinium bracteatum* (♀) and *Vaccinium corymbosum* (♂), and Ehlenfeldt & Polashock (2014), used RAPD markers to confirm the hybrid nature of plants resulted from the crosses of *Vaccinium padifolium* (♀) and *V. corymbosum* (♂). Also, in an earlier study, Qu and Hancock (1995) used the RAPD markers to determine the mode of inheritance and the level of heterozygosity transmitted by 2n gametes in the hybrid plants US75 resulted from crosses of *Vaccinium darrowi* (Florida 4B) and *V. corymbosum* (cultivar 'Bluecrop').

Linkage map construction based on molecular markers is useful to indicate gene loci linked to useful traits such as fruit quality indicators, disease tolerance or abiotic stress resistance. Based on RAPD markers, linkage maps were constructed from a cross between an F1 interspecific hybrid, *Vaccinium darrowi* Camp x *V. elliottii* Chapm, and a *Vaccinium darrowi* plant (Rowland & Levi, 1994), and from a cross between hybrid US75 and *V. corymbosum* cultivar 'Bluetta' (Qu and Hancock, 1997).

Genetic stability of plants propagated ex vitro was assessed on microcuttings of *V. corymbosum*, cultivars 'Bluecrop' and 'Duke'. Genetic stability of the cuttings was not affected regardless of the type of rooting enhancer used (0.2% Goteo, 50 mg/l auxin indole-3-butyric acid IIBA), or Rhizopon AA containing 1% IBA) (Nowakowska & Pacholczak, 2017). In another study, genetic stability of cuttings of *V. corymbosum* cultivars 'Aurora', 'Draper' and 'Liberty,' micropropagated in vitro for 10 subcultures, was tested using RAPD and SRAP markers, revealing no genetic variations (Clapa et al., 2019).

Last but not least, Vander Kloet & Paterson (2000) reported the **discovery of a new taxon**, *Vaccinium hiepii* vander Kloet, sp. nov., following RAPD and morphological assessment.

Microsatellites, or simple sequence repeats markers consisting of repetitions of 1-6 bp DNA sequences, have been used for several decades for assessment of genetic diversity, QTL discovery, marker assisted selection for desired traits (MAS), cultivar DNA fingerprinting, germplasm characterization, genome organization, etc. (Nybom & Lācis, 2021; Taheri et al., 2018).

SSR technique is based on amplifying DNA sequences containing simple sequence repeats by using primer pairs designed from the conserved flanking sequences (Gupta et al., 1996).

If initially the discovery and development of microsatellite loci has been cumbersome, next generation sequencing (NGS) techniques that allowed faster whole genome sequencing and resequencing, greatly increased the easyness of detecting SSRs in plants (Zalapa et al., 2012).

Presently there are four reference genomes publicly available in the National Center for Biotechnology Information (NCBI) database, that can be mined for molecular markers (Table 2).

Table 2. *Vaccinium* reference genomes published to date publicly available in the NCBI database

Species	Genome coverage	Sequencing technology	Reference
V. macrocarpon cv. 'Ben Lear'	100.0x	Oxford Nanopore GridION; Illumina NovaSeq	(Kawash et al., 2022)
V. darrowii F1 hybrid NJ 8807/NJ 8810	64.0x	Illumina; PacBio	(Yu et al., 2021)
V. corymbosum cv. "W8520"	40.0x	454	Direct submission to NCBI
V. myrtillus ecotype "North-Norwegian"	100.0x	Illumina; Oxford Nanopore	Direct submission to NCBI

After sequencing *de novo* the cranberry genome, *Vaccinium macrocarpon*, cultivar 'HyRed', over 100000 SSR loci were detected, with the dinucleotide AG being the most frequent repeat detected (34% of the total SSRs). From the 96 loci tested in 25 cranberry genotypes, 48 proved to be polymorphic (Zhu et al., 2012). Another study on *V. macrocarpon* identified ~700 polymorphic loci located in transcribed and genomic regions, and suggested ~500 loci for genetic diversity and segregation analyses (Schlautman et al., 2015).

For non-model plants in which reference genomes are not yet available, SSR mining in transcriptomes is a viable option (Taheri et al., 2018).

In *Vaccinium corymbosum*, cultivar 'Bluecrop', almost 16000 EST-SSR loci were identified from the leaf, developing fruit, and flower buds at different stages of cold acclimation transcriptomes. Based on these loci, 100 primer pairs were tested for amplification and polymorphism, the results being a 68% amplification rate and a 43% polymorphism rate. Among the SSRs discovered, AG repeats accounted for 38% of the total SSRs (Rowland et al., 2012).

A genetic variability study of 24 populations of *V. macrocarpon* and 21 populations *V. oxycoccos* from United States National Forests using 32 SSRs, revealed over 600 for the first and almost 900 highly heterozygous alleles for the second, identifying a unique population of *V. macrocarpon* outside its native range, and helping decide conservation actions priorities (Rodriguez-Bonilla et al., 2020). A study with a similar purpose was done for the Andean blueberry, *Vaccinium floribundum* Kunth., using 16 SSR to characterize 100 individuals from 27 collection sites from ten provinces in the Ecuadorian Highlands, the analysis yielding 4 genetic cluster, distributed according to their geographic location (Vega-Polo et al., 2020).

Another use for SSR markers is genetic "fingerprinting". Two sets, one with 5 and the other with 10 SSRs containing three nucleotide repeats, were enough to genotype 367 Vaccinium samples from National Clonal Germplasm Repository (NCGR) (Corvalis, Oregon, USA), and confirm the accession identities by detecting true-to-type cultivars, homonyms and synonyms (Bassil et al., 2020).

ISSRs, inter simple sequence repeats, are DNA sequences located between two identical SSRs. **ISSR technique** also uses microsatellites, however, as opposed to SSR technique it is not specific, as it uses for amplification a single primer – the microsatellite itself, usually with a length of 16-25 bp (Pradeep Reddy et al., 2002). The technique is used similarly to RAPD technique, and it has the additional advantage of higher reproducibility, due to the longer primer's size (Grover & Sharma, 2016).

Intra and inter-population genetic diversity of 32 bilberry individuals belonging to populations from Iceland, Norway, Sweden, Finland and Germany, were studied using four ISSR primers (UBC-825, UBC-857, UBC-873 and UBC-881), that amplified 127 polymorphic loci, permitting the identification of 85% of the **genetic variation** within these populations (Zoratti et al., 2015).

(Debnath & An, 2019) used ISSR markers together with EST-SSR and EST-PCR markers to **study biodiversity** within a group of 75 wild cranberry clones, in an attempt to correlate biochemical (antioxidant properties) and genetic clustering. However, clustering differed, probably due to markers' degree of genomic coverage. A similar study, this time of blueberry cultivars and hybrids using three types of SSR markers (EST-SSR, G-SSR and EST-PCR), confirmed the poor correlation between genetic and biochemical data, however some of the markers proved to be associated with antioxidant properties (Bhatt & Debnath, 2021). EST-PCR, EST-SSR and ISSR markers have also been used to monitor and **confirm clonal fidelity** of micropropagated lingonberry plants (Arigundam et al., 2020). ISSR markers have been used as well **for confirming the hybrid nature** of interspecific hybrids of *V. uliginosum* × *(V. corymbosum* × *V. angustifolium*) propagated *in vitro* (Erst et al., 2021).

CONCLUSIONS

Molecular markers such as RAPD and microsatellites have been employed for decades to facilitate plant breeding, wild species conservation efforts, plant collections management, micropropagation industry, and much more. The advent of next generation sequencing made easier the discovery and development of novel markers based on microsatellites, especially in the light of affordable resequencing of genotypes for which there are reference genomes available, and this is the case in the present for four *Vaccinium* species. However, for those species that are not yet sequenced, RAPD markers are still an option, as they are easy to use and relatively not expensive.

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STUDY OF THE PRODUCTION OF SOME STONE FRUITS IN THE COUNTRIES ON THE BALKAN PENINSULA THROUGH MATHEMATICAL APPROACHES

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Abstract

This is a study of the yields of stone fruits (apricots, cherries, peaches, nectarines and plums) on the territory of the Balkan countries for the period 2000-2016. A comparative assessment of the countries according to this indicator was made. Hierarchical cluster analysis and single factor analysis of variance were applied. For the period under study, the highest yield of apricots, cherries and plums is in Slovenia (157293.82 hg/ha, 313841.82 hg/ha and 1580446.53 hg/ha respectively). Greece (185991.47 hg/ha) and Italy (183474.12 hg/ha) have the highest yields of peaches and nectarines. The lowest yields of apricots are proven in Croatia (17966.59 hg/ha), followed by Bosnia and Herzegovina (21697.47 hg/ha). Bosnia and Herzegovina also has the lowest yields of cherries, peaches and nectarines (45717.29 hg/ha and 19491.18 hg/ha respectively). Slovenia has the highest proven instability of the yields of all crops. They are the most stable in Bosnia and Herzegovina.

Key words: *fruits, Balkan countries, cluster analysis*

INTRODUCTION

Fruit growing is a traditional subsector of plant growing not only in Bulgaria but also in many Balkan countries. In Bulgaria, the most favorable factors are the natural conditions, on the one hand, and the generations' traditions, on the other.

Fruits are known for their rich content of vitamins, minerals, fiber, folic acid, beta-carotene, etc. This makes them an important component of the everyday nutrition of every person who is striving for a healthy lifestyle.

There are many scientific developments in the field of fruit growing aimed at increasing crop yields (Trivedi, 2015: Ali et al., 2016; Bass et al., 2016; Dongnan Li et al., 2016; Sestraş et al., 2007).

Tanasescu and Paltineanu (2004) show the effects of various irrigation methods on the distribution of roots in the Golden Delicious apple cultivar grafted on MM 106 rootstock under the specific conditions of the hilly region of Pitesti - Maracineni, Southern Romania. The results obtained here showed that a higher influence was induced by the different irrigation treatments to the active tree root cross-sectional area (TRCSA) versus the total TRCSA. A direct, linear and distinctly significant correlation was found between the sum of the total TRCSA and the fruit yield on the one hand, and between the total TRCSA and the annual growth in tree trunk cross-sectional area on the other hand.

Radunic et al. (2015) evaluate the physical and chemical properties of eight pomegranate accessions (seven cultivars and one wild genotype) collected from the Mediterranean region of Croatia. Accessions showed high variability in fruit weight and size, calyx and peel properties, number of arils per fruit, total aril weight, and aril and juice yield. Variables that define sweet taste, such as low total acidity (TA; 0.37-0.59%), high total soluble solids content (TSS; 12.5-15.0%) and their ratio (TSS/TA) were evaluated, and results generally aligned with sweetness classifications of the fruit. Pomegranate fruit had high variability in total phenolic content (1985.6-2948.7 mg/L). HPLC-MALDI-TOF/MS analysis showed that accessions with dark red arils had the highest total anthocyanin content, with cyanidin 3-glucoside as the most abundant compound. Principal component analysis revealed great differences in fruit physical characteristics and

chemical composition among pomegranate accessions.
Pouyesh et al. (2017) studies the possibilities for hybridization of seven melon varieties, taking into account indicators such as yield, number of fruit from a plant, carotenoid and chlorophyll content. They found that combining the genotype Garmak × Rish-baba with other forms would result in plants with maximum yield and high concentration of chlorophyll. However, the authors recommend that parenting lines be improved before being used in future hybridizations.
Asmai Ozturk (2005) studies different types of apricots according to the stochastic, phenological parameters and yields. They prove that among these genotypes there are sensitive differences in the yield, total acidity, stone mass, etc. They find different correlations between the surveyed indicators, from which they conclude that the Levent variety is suitable for future selection research.
Diaa et al. (2016) evaluate varieties of watermelon according to the yields over a three-year period and the geographical location of their cultivation. They analyze the stability of yields and determine the correlations in the base of experimental data. They demonstrate the strong effect of genotype-environment interaction on watermelon production. The hybrid forms Big Crimson and Legacy stand out both with high yield and with considerable stability.
The present study aims to make a comparative assessment of the countries on the Balkan Peninsula according to the average yields of some stone fruit for the period 2000-2016. Based on proven differences in production quantities, the countries are grouped into clusters and the factors that influence the clustering are analyzed.

MATERIALS AND METHODS

The present work analyzes data connected with the average yields of the following stone fruit: apricots, cherries, peaches and nectarines and plums in the Balkan countries, namely: Bulgaria, Albania, Bosnia and Herzegovina, Serbia, Croatia, Greece, Romania, Turkey, Italy and Slovenia from 2000 to 2016. Montenegro is the only Balkan country that is not included in this study because of a lack of database for some of the indicators surveyed. The grouping of the countries from the Balkan Peninsula in clusters according to a similarity in the average yields of apricots, cherries, peaches and nectarines throughout the period was performed by hierarchical cluster analysis. Clustering has been done according to various agglomeration methods, but the intragroup binding method has a maximum contingency coefficient. This determined its application in the study. A measure of similarity is the square of the Euclidean distance. This method calculates all possible distances between all points of the two clusters A and B, i.e. the distances between all nA and nB points of the same cluster by the formula:

$$D(A,B)\frac{1}{(n_A+n_B)(n_A+n_B-1)}\sum_{i,j} d(x_i,x_j)$$

where the sum changes in all points x_i from A and x_j from B. Here

$$d(x_i,x_j)=\sum_{m=1}^{p}\left(x_{i_m}-x_{j_m}\right)^2$$

is the square of a Euclidean distance between two vectors.
The comparative assessment of the countries according to the average yields of the respective stone fruits was carried out using single-factor analysis of variance (ANOVA) and a Duncan test. The statistical data match Levene's test for equality of variances, which in turn means that the countries can be compared according to their respective indicators.
In order to study the trends in the production of each of the plants, graphs have been built that allow the countries to be compared according to the production quantities of each fruit.
The data in this publication are obtained from the FAOSTAT database. Their mathematical processing was performed using the statistical program product IBM Statistics SPSS 23 (Wendler and Grottrup, 2016).

RESULTS AND DISCUSSIONS

After a cluster analysis, it was found that the Balkan countries could be grouped into four clusters due to the similarity in the average yields of apricots, cherries, peaches and nectarines and plums from 2000 to 2016. The dendrogram in Figure 1 presents the result of the clustering procedure. The first cluster includes the countries with the lowest yields of apricots, cherries and peaches and nectarines: Bulgaria, Serbia, Bosnia and Herzegovina and Croatia. The countries with the highest yields of peaches and nectarines are Greece and Italy, which form a second cluster. The third one includes Albania, Romania and Turkey, and the fourth cluster is single. It consists of Slovenia, the country with the highest yields of apricots, cherries and plums for the entire study period, significantly higher than those of the other European countries surveyed.

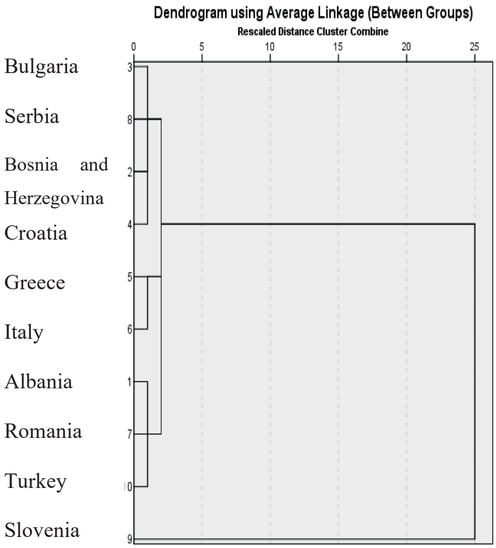

Figure 1. Dendogram showing the result of the clustering of the countries from the Balkan Peninsula according to the similarity in the average yields (hg/ha) of some stone fruit for the period 2000-2016

The comparative assessment of the countries according to the average yields of the respective stone fruit provides a more detailed qualitative description of the formed clusters (Table 1). After applying a single-factor analysis of variance and a Duncan test, it was found that for the period of study Slovenia (157293.82 hg/ha, 313841.82 hg/ha and 1580446.53 hg/ha respectively) has the highest yield of apricots, cherries and plums ha). Greece (185991,47 hg/ha) and Italy (183474.12 hg/ha) have the highest yields of peaches and nectarines. The lowest yields of apricots are proven in Croatia (17966.59 hg/ha), followed by Bosnia and Herzegovina (21697.47 hg/ha). Bosnia and Herzegovina has again minimum yields of cherries and peaches and nectarines (45717.29 hg/ha and 19491.18 hg/ha respectively). The standard deviation values in Table 1 give information on the degree of stability of the yields of each fruit for the country concerned. The most stable, albeit low, are the yields of all the studied crops in Bosnia and Herzegovina. The largest variation in the production of these crops is found in Slovenia.

Figures 2-5 show the trends in the change of the average yields of the studied fruits in each Balkan country.

There was an increase in apricot production in Albania in 2007, which remains until 2016 (Figure 2). For Slovenia, the whole period is full of peaks and falls in apricot production, which shows the yield instability throughout the period. For the other countries, there is comparative sustainability in the quantities of apricots produced. The whole period of the survey is characterized by the comparative stability of apricot yields in Bulgaria. No sharp fluctuations are detected, and the trend is towards increasing the production of this crop.

Figure 3 shows the change in cherry production. Slovenia still has the highest yields, but we can observe analogical fluctuations in the quantities over the years. However, it exceeds the yields in other countries several times. According to the Agro-Statistical Directory of the Republic of Slovenia, cherry yields in 2017 increased by 25% compared to the previous year and of apricots by 41%. This fact proves the wavy trend in the production of cherries in this country. In Bulgaria we have again stable yields for the whole period.

Analyzing the graph in Figure 4, peaches and nectarines can be said to be the crops with the highest degree of instability in the Balkans. There are periods of increase and decrease of their production in Greece, Albania, Italy, Slovenia, and they are overlapping over time. In 2001-2003 there were declines in Greece, Italy, Albania, Croatia and a rise in Romania and Slovenia. In Bulgaria, Serbia, Slovenia, Albania, there is a trend towards increasing the production of peaches and nectarines after 2012, which remains until the end of 2016.

As the quantity of plums produced in Slovenia exceeds the quantity in the rest of the Balkan countries, a further coordinate axis (at the right), which shows the yields in this country, is introduced on the graph in Figure 5. The left coordinate axis shows the production in the other countries. This allows for better visualization of the trends in the changes of the yields of the respective crop. It turns out that in most countries there are no sensitive peaks or drops in the production of plums. A significant increase occurred in 2009 in Albania, which continued until 2012, followed by a period of relative stability. The most unstable is the yield of plums in Slovenia, although this is the country with maximum quantities. The whole period is characterized by peaks and falls, and the smallest quantity for the seventeen years of research is produced in 2016. Low variations are observed for Bulgaria, wherein the period after 2014 there is a trend of increasing plum yields.

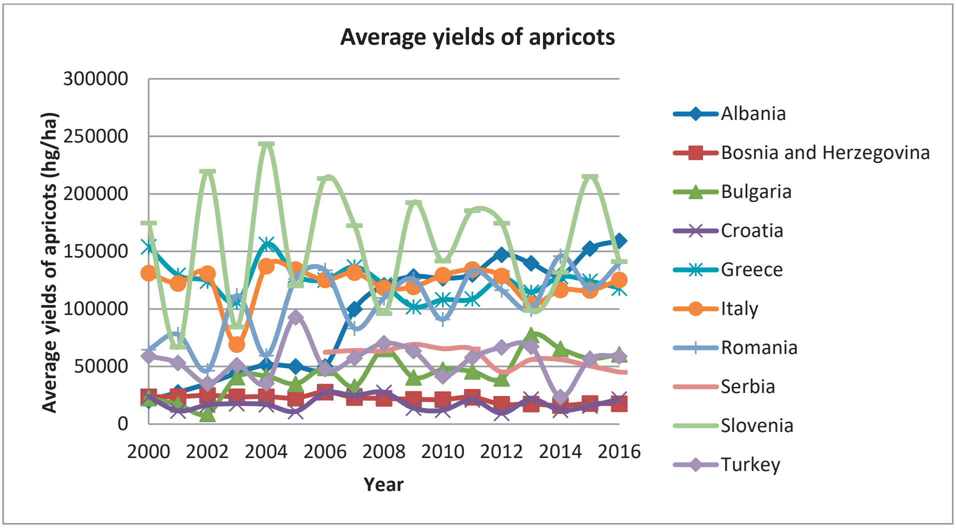

Figure 2. Changes the in average yields of apricots in the Balkan countries for the period 2000-2016

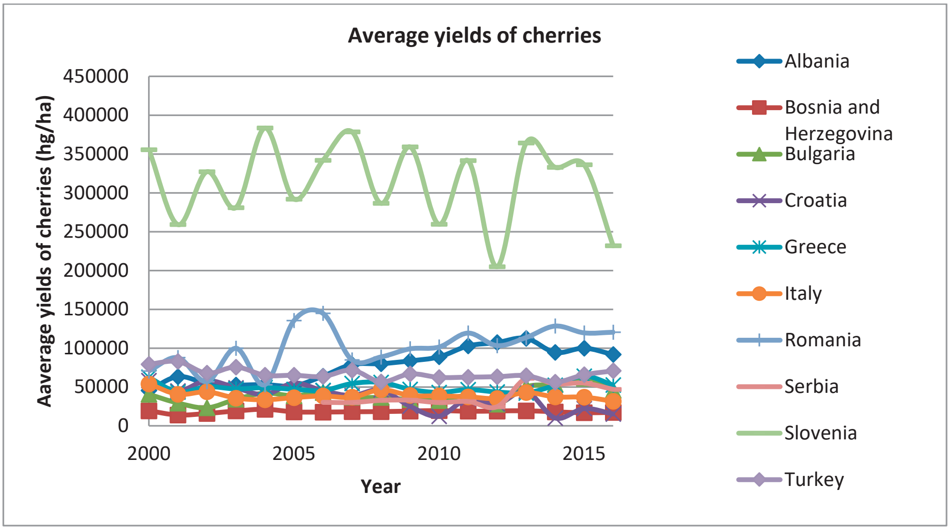

Figure 3. Changes in the average yields of cherries in the Balkan countries for the period 2000-2016

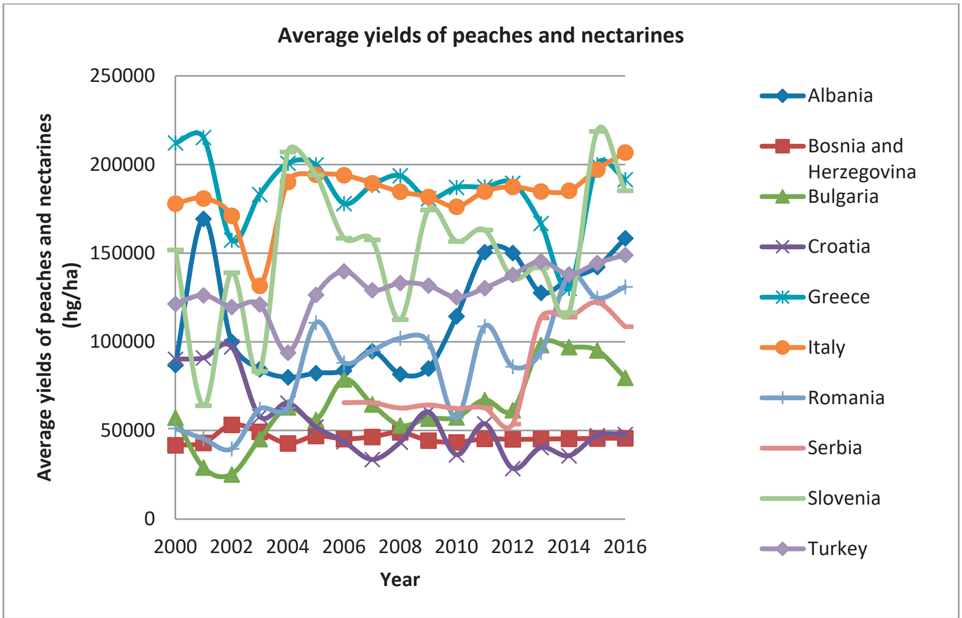

Figure 4. Changes in the average yields of peaches and nectarines in the Balkan countries for the period 2000-2016

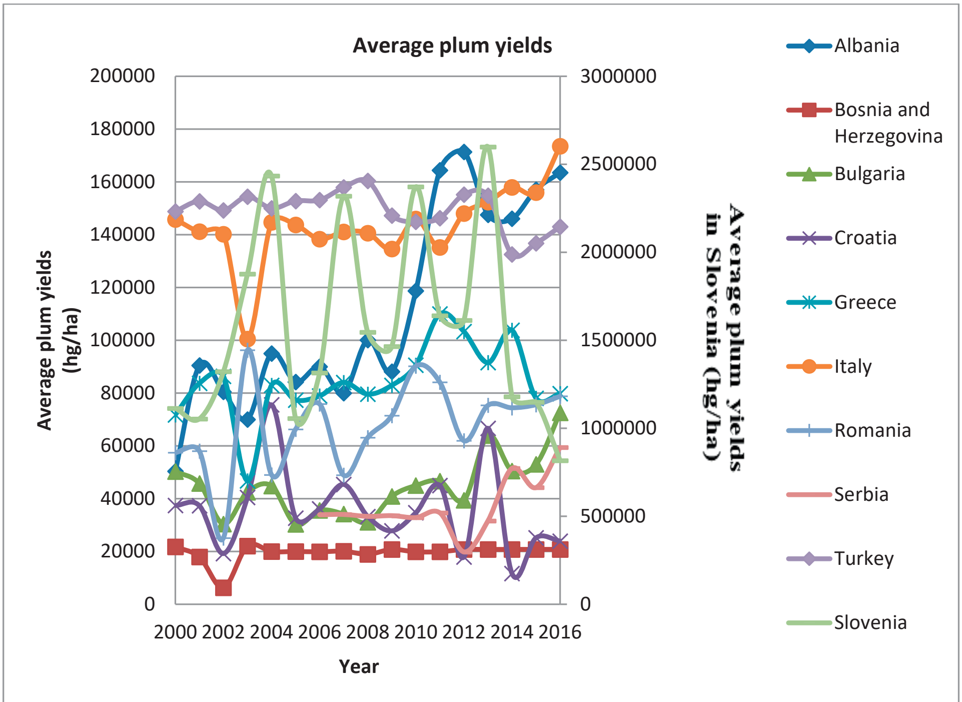

Figure 5. Change in the average plum yields in the Balkan countries for the period 2000-2016

Table 1. Comparative assessment of the countries on the Balkan Peninsula according to the average yield of some stone fruits for the period 2000-2016

Cluster №	Country	Apricots Duncan		Apricots Standard deviation	Cherries Duncan		Cherries Standard deviation
1	Bulgaria	43967.29	d	18015.14	39249.18	d	9571.19
	Serbia	58480.36	d	8444.62	39008.45	d	12682.46
	Bosnia and Herzegovina	21697.47	e	3288.28	18225.71	e	1659.25
	Croatia	17966.59	e	5911.72	37007.82	d	15612.35
2	Greece	124313.00	bc	15030.00	49824.35	d	6464.96
	Italy	122050.35	bc	15983.41	39089.00	d	5326.31
3	Albania	94864.12	c	49732.97	77943.12	c	22022.70
	Romania	104894.53	bc	29885.87	101616.71	b	26034.59
	Turkey	55226.29	d	15958.28	67075.35	c	7137.50
4	Slovenia	157293.82	a	52357.49	313841.82	a	52939.83

Cluster №	Country	Peaches and nectarines Duncan		Peaches and nectarines Standard deviation	Plums Duncan		Plums Standard deviation
1	Bulgaria	63858.94	ef	21099.00	44513.94	b	11508.25
	Serbia	81404.91	d	26699.74	37166.36	b	10747.56
	Bosnia and Herzegovina	45717.29	f	2752.03	19491.18	b	3545.91
	Croatia	54407.65	ef	20817.13	35908.53	b	16294.25
2	Greece	185991.47	a	20399.33	84216.71	b	14192.70
	Italy	183474.12	a	15810.75	143507.82	b	14646.78
3	Albania	113389.29	c	31862.02	111618.82	b	38506.71
	Romania	88026.65	d	30421.08	67760.88	b	17191.49
	Turkey	130188.06	c	12872.06	149422.00	b	7224.80
4	Slovenia	150628.47	b	40986.74	1580446.53	a	552359.92

a, b, ..., f - level of statistical significance at $\alpha = 0.05$

CONCLUSIONS

As a result of the analyses, it has been proven that in the Balkans Slovenia is the country with the highest but at the same time the most unstable yields of all stone fruit included in the survey. At the other pole are Bosnia and Herzegovina and Croatia. Bulgaria has a low yield of stone fruit and therefore occupies one of the last places based on this indicator. Considering that the yields of all crops in the respective countries are largely overlapping, the reasons for the low production could be sought in the geographic, soil and climatic characteristics of the individual Balkan countries.

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COMPARATIVE PERFORMANCE OF LOCAL CHERRY CULTIVARS IN PLOVDIV, BULGARIA

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Abstract

In the conditions of global climate change and invasion of new pests, the utilisation of the local genepool is a vital step towards sustainable agriculture. Local cultivars and forms are generally considered better adapted to the conditions of the originating region than a widely spread cultivars bred elsewhere. In the current study, two old local cherry cultivars 'Kuklenska belitsa' and 'Ranna cherna edra' and two newly selected hybrids 'Asparukh' (El.17-90) and 'Tzvetina' (El.17-37) are compared to commercial cultivars 'Bigarreau Burlat', 'Van', 'Sunburst' and the FGI-Plovdiv cultivar 'Kossara' in order to assess their performance in terms of fruit characteristics (biometry and fruit chemical composition) and resistance to pests.

Key words: *cherry, local cultivars, hybrids, genetic resources.*

INTRODUCTION

Globally, it is recognised that global trade and competition has led to the use of a limited number of cultivars, often of a similar origin, which reinforces the pressure from the changing climate and the multiplication and transfer of pathogens from one country to another. The lack of genetic diversity can lead to vulnerability (susceptibility) to diseases and pests, as well as the emergence of new resistant races and pressures on the environment (Scott & Lawrence, 1975). According to Shelef et al. (2017), most food crops are currently produced, transported and consumed long distances from their country of origin. At the same time, research and practices are mainly focused on improving the productivity of a small number of existing crops that form the cornerstone of the global food economy, rather than increasing crop diversity. The result is a loss of agrobiological diversity, which leads to unsustainable practices in the food industry, and it is more susceptible to abiotic and biotic stressors and is at risk of catastrophic losses. People cultivate only around 150 of approximately 30 000 edible plant species globally, with only 30 plant species constituting the majority of our diets.

Plant Genetic Resources (PGR) are of fundamental importance for the agricultural science and in particular for fruit growing. They have a direct relationship with breeding programs and researches in the field of integrated plant protection and organic farming, as they are the basis for creating genetic diversity, bearing valuable biological, including agricultural qualities and resistance to pests and diseases (Haussmann et al., 2004; Nass et al., 2012).

In order to enrich the variety of cultivars grown in the country with well adapted sustainable and productive, it is obligatory in the breeding programs besides the newly introduced promising cultivars to include traditional local forms as donors of these desirable qualities. Additionally, it is necessary to preserve certain local foods or authentic tastes by preserving traditional cultivars associated with certain regions or even individual settlements (Altieri & Merrick, 1987).

During the development of project "Innovations and traditions in the conservation and use of old and local genetic resources in fruit growing and viticulture" funded by the National Science Fund of the Bulgarian Ministry of Education and Science, old and local genetic resources in fruit growing and viticulture were sought, preserved and studied, using modern methods and techniques in the field of fruit growing, viticulture, phytopathology, entomology, chemistry and biotechnology. This study presents an analysis of local cultivars, both old and newly selected, compared to widely used commercial cultivars. The objectives set in this paper would contribute to both the conservation of local genetic resources and enhancement of the sweet cherry breeding programme in the Fruit Growing Institute - Plovdiv, Bulgaria (FGI).

MATERIALS AND METHODS

During the development of Project KP-06-PN46/3, many scientific expeditions were carried out resulting in a collection of specimens from different fruit crops including cherries. In this study two preserved old local cherry cultivars ‘Kuklenska belitsa’ (point of origin 42°00'47.8"N 24°48'24.6"E) and ‘Ranna cherna edra’ (point of origin 42°01'37.5"N 24°44'42.1"E) and two newly selected hybrids ‘Asparukh’ (El.17-90) and ‘Tzvetina’ (El.17-37) were compared to commercial cultivars ‘Bigarreau Burlat’, ‘Van’, ‘Sunburst’ and the FGI-Plovdiv cultivar ‘Kossara’ in order to assess their performance in terms of fruit characteristics (biometry and fruit chemical composition) and susceptibility to cherry fruit fly *Rhagoletis cerasi* (L.) (Diptera: Tephritidae).
The biometric indicators were measured according to the standard methodology used in FGI-Plovdiv “Methodology for the study of plant resources in fruit plants” (Nedev et al., 1979) and updated in 2016 (Malchev, 2016).
The chemical analyses were carried out in the chemical laboratory of the Fruit Growing Institute - Plovdiv. Average samples were formed randomly at a time of consumptive maturity of the fruits. Determined are the chemical parameters: soluble solids content - refractometrically by Brix; sugars (inverted, sucrose and total) content - by Schoorl-Regenbogen; total acid content - titrimetrically, active acidity (pH) - potentiometrically.
The beginning and dynamics of the cherry fruit fly's flight were determined with the help of visual traps and attractants. Yellow visual traps were used - type Pherocon "AM" *Rhagoletis* sp. and attractant of the company CSALOMON®. Two traps are placed in the selection cherry orchard.
To account for the worminess of the cherry fly fruit, 100 fruits were taken for each cultivar immediately before harvest. The fruits were picked from different heights and parts of the canopy and are examined by opening the fruit flesh and visual inspection for the presence of larvae in them. Samples were also taken 10 days after the full ripening of the other fruits on the trees.
The results were subjected to statistical analysis using the methods of Multiple Range Test and correlation analysis using the software products “MS Excel 365 Analysis ToolPak Add-Ins” (https://support.office.com) and “R-4.1.2” in combination with “RStudio Desktop 2021.09.1+372” and installed package “agricolae 1.3 -5”(Mendiburu, 2021).

RESULTS AND DISCUSSIONS

The time of ripening differs between the cultivars covering the end of May and the beginning of June (Table 1).
Although fruit taste is the most important to the consumers, on the market they make their choice according to the fruit characteristics, appearance and attractiveness (Nesheva & Bozhkova, 2018). For the tested cultivars and hybrids, the predominant form of the fruit is reniform. Other forms are cordate in ‘Ranna cherna edra’ and ‘Kuklenska belitsa’ and oblate for ‘Kossara’. The colour of fruit skin and juice cover a variety of consumer preferences (Bujdosó et al., 2020) (Table 1).
An important quality characteristic of the cherry fruit is the firmness of the flesh and the mass of the fruit. The highest firmness of the flesh was measured in ‘Asparukh’ (El.17-90) - 7.71 kgf/cm^2, ‘Tzvetina’ (El.17-37) and ‘Sunburst’ - all derived from the cultivar ‘Van’ (Zhivondov, 2008; Quero-García et al., 2017). The cultivar ‘Kossara’ with 3.65 kgf/cm^2 has soft fruit flesh in between its parents ‘Bigarreau Burlat’ (4.13 kgf/cm^2) and ‘Ranna cherna edra’ (3.30 kgf/cm^2) (Table 1).
The biometric analysis reveals that the largest fruit size and weight (10.83 g) is of the cultivar ‘Sunburst’. It is followed by the other ‘Van’ progeny, ‘Asparukh’ (El.17-90) with 27.42 mm diameter and fruit weight of 10.01g. ‘Kossara’ is of medium size and relatively high fruit weight for the early ripening group, surpassing

its parents ‘Ranna cherna edra’ x ‘Bigarreau Burlat’ (Zhivondov & Gercheva, 2009). With the smallest fruit weight is the old local cultivar ‘Kuklenska belitsa’ with 5.04g. The two cultivars with the longest stalk are ‘Kuklenska belitsa’ and ‘Ranna cherna edra’. That could be explained by the modern consumer tendencies in Bulgaria, namely the preference of shorter fruit stalk (Bujdosó et al., 2020) (Table 2).

‘Sunburst’ and the new Bulgarian cultivars and hybrids have more favourable fruit/stone weight ratio compared to the old local cultivars ‘Kuklenska belitsa’ and ‘Ranna cherna edra’ (Table 3). Despite having softer fruits (Table 1), the old local cultivars ‘Kuklenska belitsa’ and ‘Ranna cherna edra’ have the highest soluble solids content - 19.30 Brix % and 19.20 Brix %, respectively (Table 4).

Table 1. Pomological characteristics of cherry fruits

Cultivar	Ripening time	Fruit shape	Fruit skin colour	Fruit flesh colour	Juice colour	Fruit firmness (kgf/cm^2)	Stone shape in ventral view
‘Asparukh’ (El.17-90)	09-June	Reniform	Dark red	Medium red	Red	7.71	Medium elliptic
‘Bigarreau Burlat’	26-May	Reniform	Dark red	Dark red	Purple	4.13	Broad elliptic
‘Kossara’	25-May	Oblate	Dark red	Medium red	Red	3.65	Medium elliptic
‘Kuklenska belitsa’	21-May	Cordate	Yellow with blush	Cream	Colorless	3.89	Medium elliptic
‘Ranna cherna edra’	23-May	Cordate	Blackish	Dark red	Purple	3.30	Medium elliptic
‘Sunburst’	14-June	Reniform	Red	Pink	Pink	6.31	Circular
‘Tzvetina’ (El.17-37)	14-June	Reniform	Dark red	Medium red	Red	6.72	Broad elliptic
‘Van’	14-June	Reniform	Dark red	Dark red	Red	5.84	Circular

Table 2. Biometric characteristics of cherry fruits

Cultivar	Fruit height [mm]		Fruit diameter (cheeks) [mm]		Fruit diameter (suture) [mm]		Fruit weight [g]		Length of fruit stalk (mm)	
‘Asparukh’ (El.17-90)	23.26	c	27.42	b	22.37	bc	10.01	b	24.13	ef
‘Bigarreau Burlat’	22.52	d	23.27	e	19.61	e	6.98	e	24.63	e
‘Kossara’	24.13	b	25.77	d	21.28	d	8.47	d	34.55	c
‘Kuklenska belitsa’	22.64	d	20.23	g	17.28	f	5.04	g	40.30	b
‘Ranna cherna edra’	21.86	e	22.82	f	19.44	e	5.98	f	42.93	a
‘Sunburst’	25.44	a	28.12	a	24.02	a	10.83	a	31.90	d
‘Tzvetina’ (El.17-37)	22.79	d	26.93	c	22.57	b	9.35	c	21.72	g
‘Van’	24.24	b	26.92	c	22.04	c	9.59	c	22.23	fg

*Different letters in the same column means significant difference at P=0.05

Table 3. Biometric characteristics of cherry stones

Cultivar	Stone height [mm]		Stone width [mm]		Stone thickness [mm]		Stone weight [%]		Fruit / Stone weight ratio [%]	
‘Asparukh’ (El.17-90)	10.36	d	7.08	a	8.99	b	0.34	a	3.40	d
‘Bigarreau Burlat’	11.88	b	6.79	bc	8.65	c	0.33	ab	4.68	c
‘Kossara’	11.01	c	6.88	b	8.98	b	0.30	cd	3.54	d
‘Kuklenska belitsa’	12.98	a	6.28	d	8.51	cd	0.33	ab	6.52	a
‘Ranna cherna edra’	11.07	c	6.78	bc	8.55	c	0.31	bc	5.15	b
‘Sunburst’	9.97	e	6.66	c	9.26	a	0.31	bc	2.86	e
‘Tzvetina’ (El.17-37)	9.81	e	6.77	bc	8.35	d	0.28	d	2.98	e
‘Van’	9.70	e	7.07	a	9.03	b	0.34	a	3.56	d

*Different letters in the same column means significant difference at P=0.05

Table 4. Chemical composition of cherry fruits

Cultivar	Soluble Solids content Brix %	Sugars content %			Acids content %	Active acidity (pH)
		Total	Inverted	Sucrose		
'Asparukh' (El.17-90)	15.30	12.44	12.44	0.000	0.663	3.04
'Bigarreau Burlat'	18.60	13.26	13.26	0.000	0.714	3.57
'Kossara'	12.90	9.12	9.12	0.000	0.454	4.11
'Kuklenska belitsa'	19.30	na[1]	na[1]	na[1]	na[1]	na[1]
'Ranna cherna edra'	19.20	16.56	16.64	0.000	0.629	3.19
'Sunburst'	16.00	13.18	12.74	0.418	0.697	3.19
'Tzvetina' (El.17-37)	19.20	12.66	16.48	0.000	0.765	3.09
'Van'	17.60	13.56	12.88	0.646	0.834	3.13

[1]na- data not available

Figure 1. European cherry fruit fly, *Rhagoletis cerasi* (L.) (Diptera: Tephritidae)

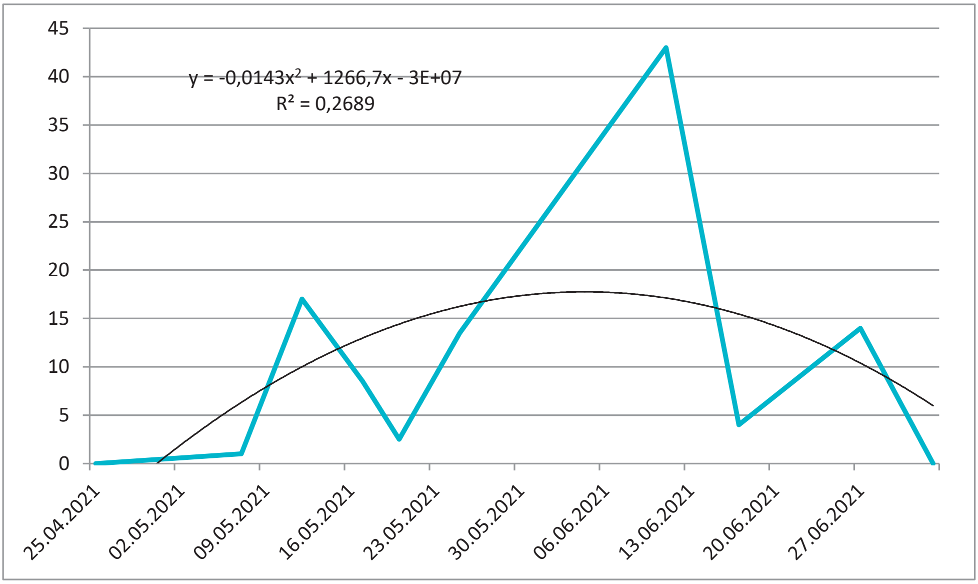

Figure 2. Dynamics of the cherry fruit fly's flight

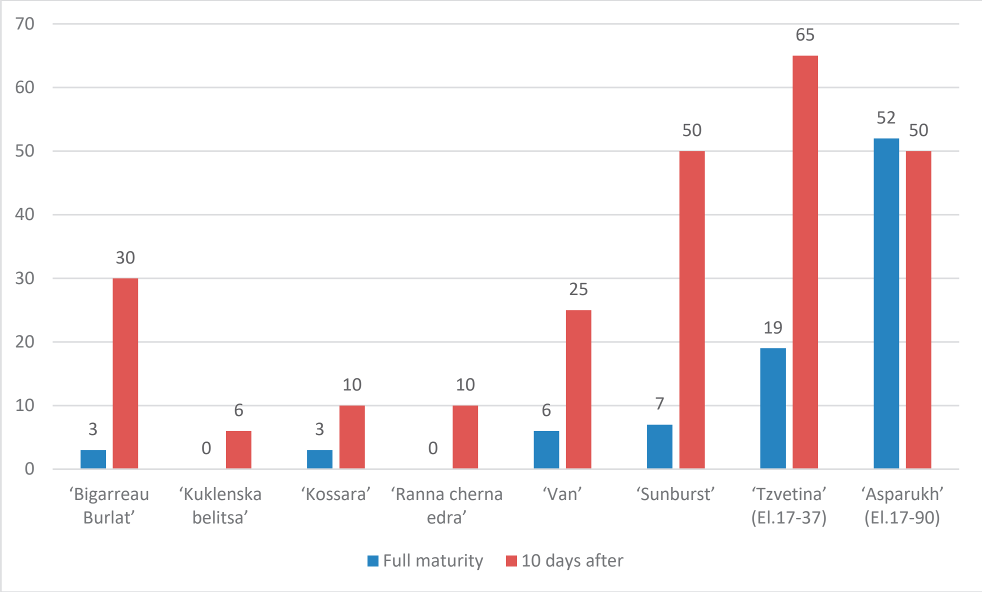

Figure 3. Rate of damage from the cherry fly at harvest and 10 days after

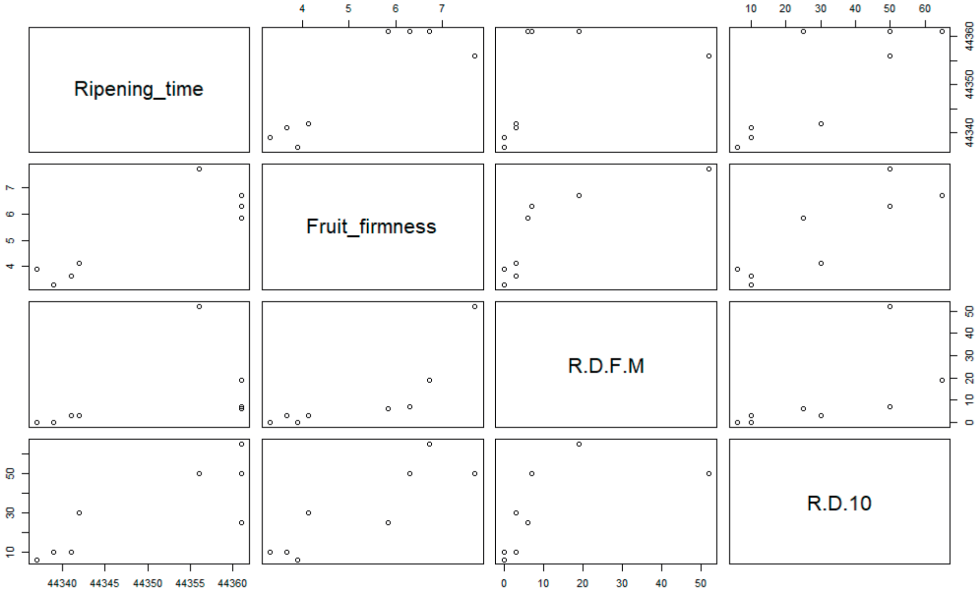

Figure 4. Correlation between ripening time, fruit firmness and rate of damage (R.D.) at full maturity and 10 days after

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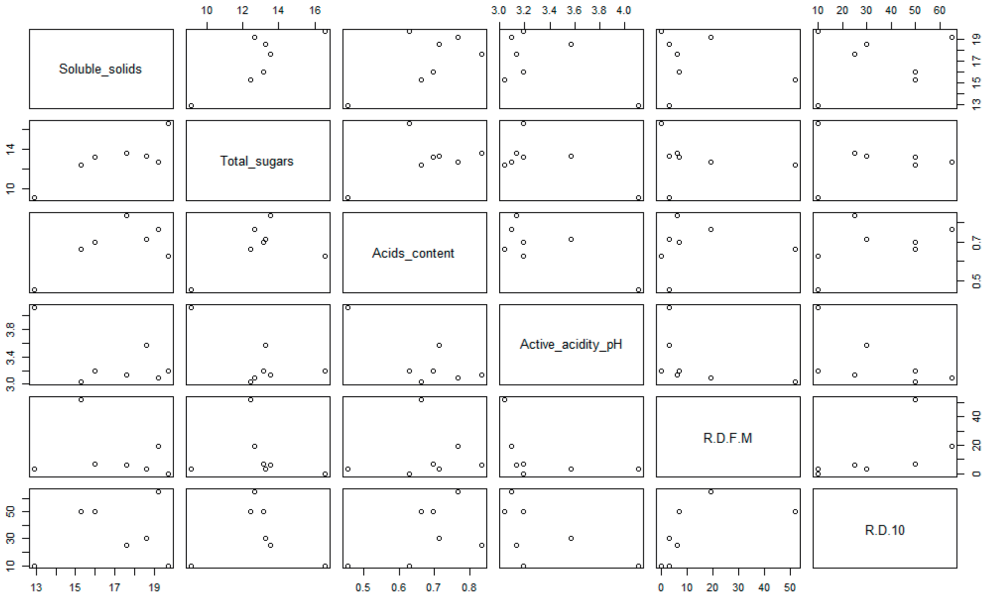

Figure 5. Correlation between fruit chemical characteristics and rate of damage (R.D.) at full maturity and 10 days after

The European cherry fruit fly, *Rhagoletis cerasi* (L.) (Diptera: Tephritidae) is the most important pest of sweet cherries in Europe affecting the fruit quality (Figure 1). According to CABI, 2021 it is reported in Asia (8 countries), Europe (34 countries) and North America. In unmanaged orchards and solitary trees, the infestation rate can reach 100%, whereas infestation rates usually remain below 20% in commercial production (Stamenkovic et al., 2012). However, fruit fly infested fruit cannot be sorted out, therefore the whole lot is rejected if tolerance levels are exceeded (Daniel & Grunder, 2012). The level of infestation mainly is influenced by the ripening time of cherry cultivar: very early ripening cultivars are not affected, whereas later ripening cultivars show high infestation rates (Bandzo et al., 2012). Warm and sunny weather conditions during the oviposition period lead to higher infestation levels.

The cherry fly is a monovoltine species, but in the general dynamics of its flight in 2021, there are three distinguishable peaks. In our opinion, this is probably due to several reasons. The first is the unfavourable meteorological factors for the flight, which depress the activity of the cherry fly until the flight stops. The second reason is translocation, respectively concentrations of flies on late varieties after ripening and harvesting of medium-early varieties of cherries. The beginning of the flight was observed on 07.05.2021. Maximum of the first peak was reported on 16.05., the second peak on 13.06., and the third - 30.06.2021 (Figure 2).

Tracking the percentage of worming in individual cultivars confirms the opinion of several authors that the rate of damage from the cherry fly depends on the ripening period of the fruits. The first reporting of the percentage of worming in the early varieties was made on 26.05.2021. and for later ripening cultivars of 09.06.2021. For the cultivars ‘Bigarreau Burlat’ and ‘Kossara’ the percentage were 3%, while ‘Ranna cherna edra’ and ‘Kuklenska belitsa’ were - 0%. The two newly selected hybrids ‘Asparukh’ (El.17-90) and ‘Tzvetina’ (El.17-37) show the highest percentage, respectively 52% and 19%. For comparison the commercial cultivars, ‘Van’ and ‘Sunburst’ have a percentage of worming 6% and 7% respectively (Figure 3).

The results of the re-testing of the percentage of cherry fly infestation, 10 days after the period of full maturity, show a significant increase in values. The two hybrids 'Asparukh' (El.17-90) and 'Tzvetina' (El.17-37) have the highest percentage again. A significant increase in the percentage is observed in the early cultivar 'Bigarreau Burlat' - 30% and the commercial cultivar 'Sunburst' - 50% (Figure 3).

The results of the re-testing confirm the conclusion made by Sredkov (2000) that the cherry fly lays in ripe fruits as well.

Analysing the correlation data between the rate of damage at full maturity and 10 days after, ripening time, fruit firmness and the fruit chemical characteristics (Figures 4 and 5), a tendency that needs further investigation was noticed. There is a correlation between the rate of damage and fruit firmness - stronger at full maturity and as time passes on the tenth day after full maturity - acid content plays a role for the increase in damage.

CONCLUSIONS

The fruit size, colour of fruit skin and juice cover a variety of consumer preferences, enriching sweet cherry breeding programme in the Fruit Growing Institute - Plovdiv, Bulgaria (FGI).

The level of infestation is influenced by the ripening time of cherry cultivar: very early ripening cultivars are not affected, whereas later ripening later ripening cultivars show high infestation rates. Re-testing confirm that the cherry fly lays in ripe fruits as well.

A tendency is noticeable for correlation between the rate of damage from European fruit fly, fruit firmness and acid content that needs further investigation.

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EDIBLE CLIMBING ROSE DISEASES MANAGEMENT IN THE ORGANIC SYSTEM

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Abstract

This study presents the results of the organic diseases management strategy applied to the climbing edible rose plantation. The research was carried out on the Experimental Field of the Faculty of Horticulture - USAMV Bucharest established in 2015 with three climbing cultivars from the David Austin collection: Crown Princess Margareta, Falstaff, and Brother Cadfael. Two main pathogens which affect susceptible rose cultivars worldwide were detected: Podosphaera pannosa var. rosae (powdery mildew) and Diplocarpon rosae (black spot). Our management protection scheme was based on environmentally friendly practices and organic products to prevent the pathogens severity and/or as natural resistance inducers. Both black spot and powdery mildew were present with a very low degree of attack (below 1%). These results could be explained by the efficacy of our organic scheme in preventing and limiting both pathogens incidence and severity. The influence of varieties and their position in the field on disease occurrence were analysed.

***Key words**: organic edible climbing rose, powdery mildew, black spot, integrated management.*

INTRODUCTION

The rose has been cultivated for centuries, both for ornamental and therapeutic purposes, for its beauty and fragrance, or food purposes like the special product obtained from its petals, and also cosmetically for its essential oils and not only (Lambraki, 2001; Hessayon, 2005; Milică et al, 2010; Bojor & Răducanu, 2010; Lia et al, 2014; Park et al, 2016; Sengul et al, 2017; Dong et al, 2017; Fernandes et al, 2017; Pires et al, 2017).

Organic farming systems include production methods that combine traditional knowledge with scientific advances in agricultural fields. The main objectives of this culture system are both to obtain healthy and quality plants, respectively products, and to protect the biosphere and the planet's resources.

One of the principles of this system is based on the minimization of economic and ecological risks and the maximum utilization of local resources.

The organic production method differs from the conventional one by avoiding the use of chemical fertilizers and synthetic pesticides. The use of this production system determines a lower risk of contamination of horticultural products, which are healthier and safer for human consumption (Wagner, 2010; Wagner, 2012; Dinis et al., 2015; Tsanaktsidis et al., 2015; Commission Regulation (EC) No 889/2008; Regulation (EU) 2018/848; Boiu-Sicuia & Cornea, 2020; Ciotea et al., 2021; Stan et al., 2022).

Rose is susceptible to a large number of diseases, some of them being more severe and having an important negative impact on yield quantity and quality (Chalova et al., 2017).

Diplocarpon rosae (black spot) and *Podosphaera pannosa* (powdery mildew) are the most common and damaging fungal pathogens in roses (Debener & Byrne, 2014; Byrne et al., 2019), affecting cultivars worldwide (Gachomo et al., 2006; Munnenkhoff et al., 2017)

This study aimed to evaluate the effectiveness of the organic diseases management strategy applied to a climbing edible rose plantation in limiting the attack of the main pathogens. Special attention was paid to the influence of the plant position in the plantation correlated to the influence of the windbreak.

MATERIALS AND METHODS

Experiments were conducted on the climbing edible roses experimental plot of the Faculty of

Horticulture, in the Phytopathology Laboratory of the Faculty of Agriculture - Department of Plant Sciences, and in the Fruit Growing Laboratory from the Research Center for Studies of Food Quality and Agricultural Products, within the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

The experimental plot, established in 2015, includes three edible climbing roses varieties: 'Falstaff', 'Brother Cadfael', and 'Crown Princess Margareta' (Figure 1). The plantation has a windbreak planted with *Populus balsamifera*.

Figure 1. Edible climbing roses ('Brother Cadfael', 'Crown Princess Margareta', 'Falstaff')

The plantation is in a super-intensive system, the planting distances being 2m between rows and 1 m per row (Figure 2) (Butcaru & Stănică, 2018).

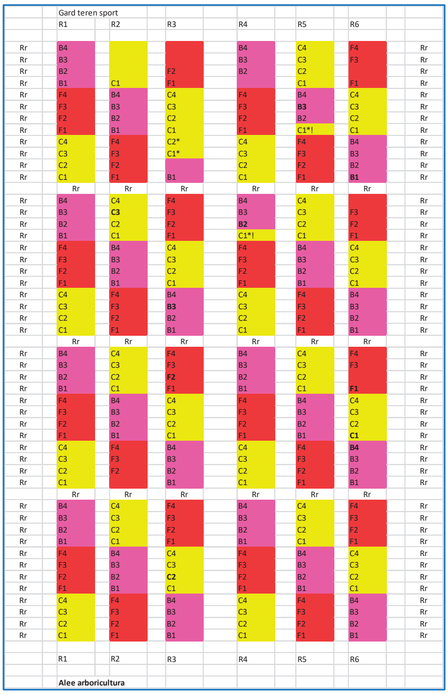

Figure 2. Rose planting scheme (B - 'Brother Cadfael', C - 'Crown Princess Margareta', F - 'Falstaff')

An organic technology was applied including pruning, trellising, weeds management, irrigation, harvesting, fertilization, and pest and disease scheme application.

During the experiment, fertilization applied consisted of an algae product, "Super fifty" (0.5%), and a new Romanian patented product, "Folarex" (0.45%), obtained from animal manure.

Pest and disease organic fertilization scheme consisted of cupric and sulphury based products, sodium bicarbonate, raw caw milk, neem oil, garlic extract, paraffin oil, chitosan, orange oil, propolis tincture-based products, home-made or commercial.

For disease monitoring and evaluation, field monitoring was carried out to determine the degree of black spot and powdery mildew infection in the plantation.

To estimate the attack of the black spot, three shoots per plant from each side of the plantation and a group inside it were analyzed. On the shoots taken under observation, 15 mature leaves, starting from the tip to the base were analyzed. To estimate the severity of the attack, a 6-class grading scale was used (Figure 3).

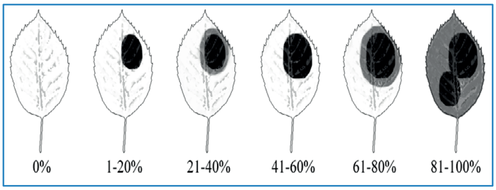

Figure 3. Illustrated scale for estimating the severity of black leaf spot attack in rose (Yasin et al., 2016)

To estimate the powdery mildew attack, three shoots from each side of the plantation and a group inside it, were analyzed. On the observed shoots, 10 mature leaves, starting from the tip to the base were analyzed. The 6-class scoring scale was used to estimate the severity of symptoms (Yan, 2006).

The first monitoring took place in July, and the second in October 2020. For each variety, its behavior to pathogen attack was analyzed, on each side of the lot, respectively North, South, West, and East. Groups of plants from inside the plot were also analyzed.

Based on primary data, disease incidence (DI, %), disease severity (DS, %), and attack degree (AD, %) were calculated.

Disease incidence, DI (%) = leaves with disease symptoms/total leaves observed x 100

Disease severity, DS (%) = black spot and powdery mildew severity were rated on 1 to 6 classes.

DS (%) = $\sum(ixf)/n$, where i = class or disease severity (%); f = number of leaves observed in this category; n = total number of leaves with symptoms.

The degree of attack, AD (%), was calculated according to the formula: AD (%) = DI x DS/100.

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

RESULTS AND DISCUSSIONS

Following the monitoring of the plantation, the presence of powdery mildew and black spot attack was identified during the study period.

Black spot attack was observed, in the 'Brother Cadfael' variety, in both monitoring, the plants on the West side (0.21%) in July and the South, respectively inside parts (0.11%) in October, presented a higher degree of attack than those on the eastern side (0.03% in July and 0.02% in October) (Figure 4).

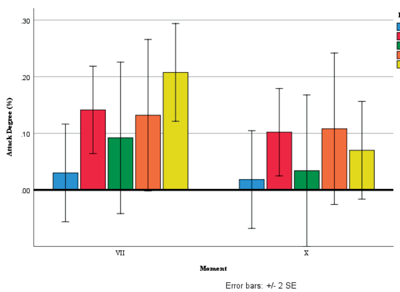

Figure 4. The influence of the location and moment on the black spot attack degree at 'Brother Cadfael' cultivar

At the 'Crown Princess Margareta' variety, a similar behavior to the 'Brother Cadfael' variety was observed regarding the black spot attack. A significant influence of the location was observed only at the second moment of the evaluation. For the attack degree, values ranged from 0.03% (North and East sides) to 0.16% (Southside) in July, respectively 0.11% (East side) to 0.07% (South and inside locations) in October (Figure 5). Combining the two experimental factors, location and moment, there was not a significant influence at this variety on the disease attack degree.

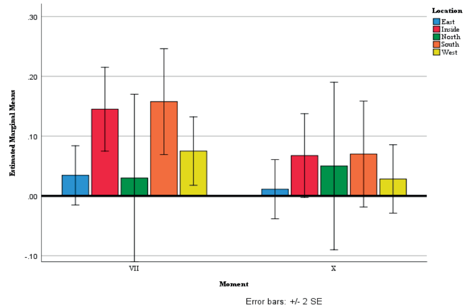

Figure 5. The influence of the location and moment on the black spot attack degree at 'Crown Princess Margareta' variety

Susceptibility to black spot attack was observed in the 'Falstaff' variety. The degree of attack increased at the second monitoring, compared to the first, but similar to the other varieties, a very small value was observed on the eastern side (0.03% July and 0.04% October) (Figure 6). An increased rate was observed inside the plantation, between the two moments, followed by the southern and western locations.

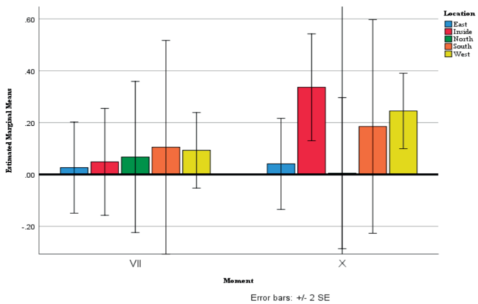

Figure 6. The influence of the location and moment on the black spot attack degree at 'Falstaff' cultivar

When all three cultivars were analysed, a significant influence of the location on the attack degree of the black spot was observed in July (figure 7). A slight decrease of the attach was observed to 'Brother Cadfael' and 'Falstaff' varieties, most caused by the summer pruning and protection scheme.

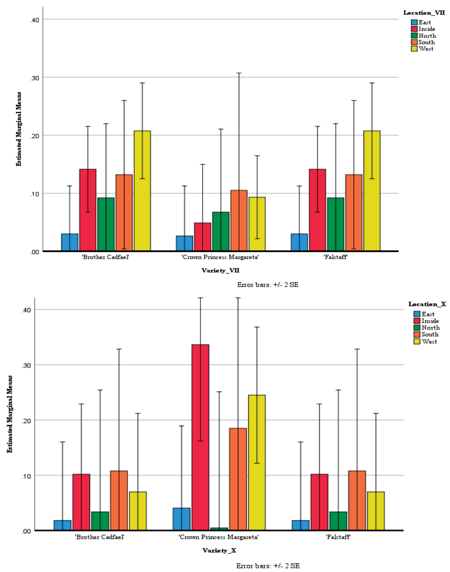

Figure 7. The influence of the variety, location, and moment on the black spot attack degree

Regarding the powdery mildew attack, in the first moment of evaluation, for all three varieties, only traces were identified. Due to favourable climatic conditions, specific for the *Podosphaera pannosa*, at the second evaluation plants affected were observed. In October, the powdery mildew attack registered a degree of attack between 0.06% in the 'Crown Princess Margareta' variety and 0.97% in the 'Falstaff variety'. The analysed varieties behaved similarly, with the values of the degree of attack being reduced.

At the 'Brother Cadfael' variety, the highest values were observed at the inside location (0.13%) and the lowest at the eastern part (0.01%) (Figure 8).

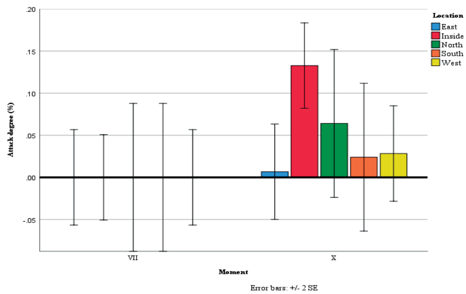

Figure 8. The influence of the location and moment on the powdery mildew attack degree at 'Brother Cadfael' variety

The 'Crown Princess Margareta' variety showed a higher degree of attack on the southern side (0.65%) while at the 'Falstaff' variety, the degree of attack varied between 0.03% (inside the plantation) and 1.91% (at the plants on the western side) (Figure 9).

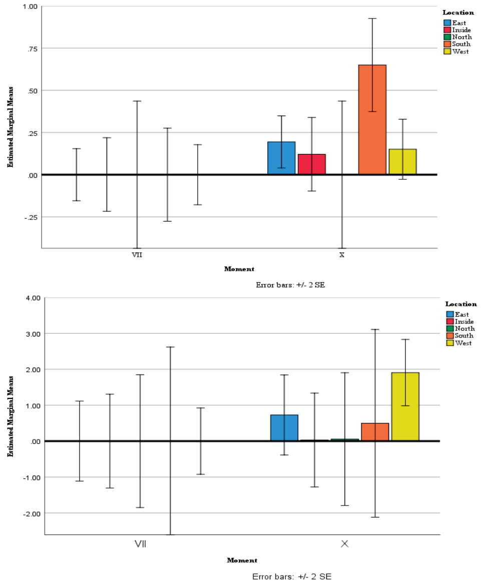

Figure 9. The influence of the location and moment on the powdery mildew attack degree at 'Crown Princess Margareta' (up) and 'Falstaff' (down) varieties

These reduced values of the degree of attack, both black spot and powdery mildew, can be considered the result of the application of organic technology, against the climatic background of the year in which the observations were made. They are similar to previous observations made (Butcaru & Stănică, 2018). The positive effect of neem, algae, or sodium bicarbonate is in accordance with Stangarlin et al. (2011), Plaza et al. (2004), Palou et al. (2002), Oliver et al. (1998), Palmer et al. (1997).

CONCLUSIONS

In the edible climbing rose plantation, managed in an organic system, the presence of the attack of black spot (*Diplocarpon rosae*) and powdery mildew (*Podosphaera pannosa*), two of the diseases known for the damage produced by this crop, was detected.

Black spot and powdery mildew infections occurred naturally. The protection treatment scheme reduced the attack to low levels (below 1%). Phytotoxicity, chlorosis, defoliation, discoloration, or stunting were not observed in any of the treated plants.

The products that have been integrated into the protection program ("Bouille bordelaise", "Pre-vam", "Garex", "Altosan") as well as for fertilization ("Folarex", "Super fifty") are known for their preventive action and have proven their efficacity.

The hypothesis regarding the significant influence of the location of plants on the disease attack degree, correlated to the effect of the windbreak was partially demonstrated, specific to some varieties and moments. Further and extended research can be valuable on this topic, important in the specificity of the newest climate change conditions and rose plant particularities.

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THE BEHAVIOR OF SOME FIG (*Ficus carica* L.) GENOTYPES IN NORTHEN AREA OF BUCHAREST

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Abstract

At the Faculty of Horticulture in Bucharest, an important collection of fig (Ficus carica L.) genotypes found in Romania and some introduced from Italy, was started in 2015 and since then its evaluation is in process. The paper presents the results regarding the behaviour of some fig genotypes grown in the climatic conditions of Northern area of Bucharest, using an organic crop technology. Fruits measurements in accordance with IPGRI descriptors are presented. Fruit biochemical characteristics as soluble solids, total solids, acidity, glucose and fructose content are also presented. All the analyzed parameters were influenced by the genotype. The results showed that some of the local and foreign genotypes had a late harvesting or very small fruits. The genotypes that produced good results concerning fruit quality, such as: size, biochemical composition and cracking resistance, will be studied in the next years in order to observe their productivity, earliness and resistance to biotic and abiotic stresses.

Key words: *Ficus carica, vigour, yield, fruit composition.*

INTRODUCTION

In Romania, the socio-economic importance of figs in this moment is very low, but in recent years we are witnessing an increased interest of farmers in cultivating these fruit species. Thus, it is necessary to identify some fig genotypes that are adapted to the conditions in our country and have high pomological value.

Although the climatic conditions in our country are quite restrictive for the fig culture, there are areas where we can find favourable conditions such as: Mehedinți (Șvinița), Dolj, Dobrogea, and Southwest part of the country (Stănică and Braniște, 2011). In Dobrogea, the fig trees can be found in a semi-spontaneous state, and in Șvinița there is a compact surface of 120 ha with fig plantations, being unique in Europe in terms of surface area. The fig is also found in the Timișoara area, between Buziaș and Drobeta Turnu-Severin, in Câmpia Română (Tomescu, 2014), and in Bucharest it was frequently cultivated in people's gardens (Stănică, 2017).

The fig belongs to the *Moraceae* family. It is assumed to be the first plant cultivated by man, before millet and wheat (Kislev, 2006), which attracted researchers to study the genetic variability of this species. There are over 1000 genotypes, of which, due to their superior taste qualities, over 600 were studied by the American horticulturist Condit (1947).

The Swedish botanist De Candolle, based on the hypothesis that the frequent appearance of these species in the wild in certain areas is a determining criterion, considers the area of origin Caria, Syria and the Mediterranean Basin. Fig cultivation is widespread in this area because it is a species that adapts well to different soil and climate conditions (Mars, 2003). It is considered a xerophilous plant (Minonne et al., 2011), does not withstand temperatures below -16°C, the maturity of fruit consumption is achieved at temperatures of 20-21°C (Chira, 2009; Cimpoieș, 2018).

It is a unisexual dioecious species, being represented by the common fig tree that has female flowers and the caprifig with functional male flowers. Their spread in the wild is found in a similar proportion (Valdeyron and Lloyd, 1979). The cultivated fig tree is of 3 types: a) Adriatic type (common) that develops parthenocarpic fruits, b) Smyrna type that bears fruit by pollination and c) San Pedro type that bears fruit at the first harvest (breba) parthenocarpic and at the second harvest (main crop) requires pollination (Giraldo et al., 2008). In our country 10-12-15 kg/plant are obtained (Hoza, 2001), in Provance-France 33 kg/plant (8 t/238 plants) (Vidaud, 1997), while in areas favorable to fig fruiting 40-80-100 kg/plant (Accorsi and Beldi, 2011). The fruit is a sicone (Hoza, 2001; Hoza, 2003; Berg, 2003). The average weight of the fruits from the main crop varies between 12.6 g 'Celeste', 18.6 g 'LSU Purple', 28.1 g 'Florentine' and 43.6 g 'LSU Gold' (O'Rourke et al., 2004); 20-25 g 'Marsellaise' and 50-70 g 'Bourjassotte' (Vidaud, 1997); 22 g 'Zimica', 24 g 'Rezavica' and 69 g 'Zeleni Matalon', 77 g 'Crna Bruzetka' (Bandelj Mavsar et al., 2008).

Glucose and fructose are the main sugars found in figs, representing 51% and 44% of total sugars, respectively, along with sucrose (4.5%) and maltose (0.6%) but in much smaller quantities (Bandelj Mavsar et al., 2008). Fresh fruits contain between 13-25% carbohydrates, vitamins B1, B2, B6, C, and Pp, fiber (Hoza, 2000), significant amounts of Ca, Zn, Fe, K, Mg, Na but they vary depending on the cultivation area (Lo Turco et al., 2020).

Leaves and fruits of the fig trees have nutraceutical characteristics, being recommended in alleviating diseases such as: anemia, cough, urinary tract inflammation, senescence (Gherman, 2013), cancer (Abdel-Rahman et al., 2021), diabetes (Wojdylo et al., 2016).

The fig usually multiplies clonally through cuttings, causing the misidentification of genotypes by the appearance of synonyms and homonyms in their name (do Val et al., 2013).

The fig has a rich genetic diversity because it has not undergone intensive breeding programs, but requires the correct identification and classify-cation of genotypes (Perez-Jiménez et al., 2012). Some of pomological characterizations or identification of fig genotypes studies were performed in France (Vidaud, 1997), Morocco (Khadari et al., 2008), Tunisia (Aljiane et Ferchichi, 2008), Istria (Bandelj Mavsar et al., 2008), Albania (Koka, 2008), Iran (Khadivi et all., 2018), Canary Islands (Gil et al., 2008), and in Romania, at the Faculty of Horticulture - USAMV of Bucharest, it was conducted the first research of this kind by Stănică (2017), Ahmad et al. (2017).

In order to preserve the genetic diversity of the local fig genotypes, a collection of germplasm was made at the Faculty of Horticulture in Bucharest. A good management and conservation of the local varieties implies a prior morphological evaluation of them. This study characterizes the morphological variability of some fig genotypes from Romania and Italy. The objective of the study is to find those genotypes that have some special agronomic traits that make them interesting for farmers to have a fig orchard.

MATERIALS AND METHODS

The area where the research is carried out is in the Northern part of Bucharest, in the experimental field of the Faculty of Horticulture, Bucharest. The geographical coordinates are 44°28'10" N lat. and 26°04'00" E long., and the altitude is 78 m above the sea level. The climate is characterized by hot, dry summers, cold winters, 10.5°C annual temperatures and the annual average of precipitations sums up to 550-600 mm.

The vegetal material is represented by the fig trees, on their own roots, come through cuttings from the figs grown in Romania and Italy. The cuttings were rooted in the greenhouse of the Faculty of Horticulture.

The genotypes studied are from the southern part of the country: 'Galben mare' from Braniștea, GR; 'Brazi' from Brazi, PH; 'Stork', '1 Mai', 'Piața Obor', 'Dr. Constantinescu' from Bucharest, 'Smochin negru' and 'Ploiești nr.1' from Ploiești, PH, 'Negoiești 01' from Negoiești, PH; 'Viscool' from Mărăcineni, AG; from the western part: 'Oli Timișoara' from Timișoara, TM; 'Săvârșin' from Săvârșin, AR; from south-western part: 'Rot negru' from Șvinița, CS; and from Italy: 'Awitato', 'Bianco Etna', 'Passulana nera', 'Cilento nero', 'Fig primizia'.

On these fig genotypes we made determinations and measurements, such as: harvest period, tree vigour and average fruit weight. Biochemical analyses, such as: soluble solids, glucose and fructose, total dry matter and titrable acidity (g/kg citric acid).

Determinations were performed in the Researcher Centre for Study of Food Quality and Agricultural Products, USAMV of Bucharest. The period analyzed in this study was 2020 and 2021 years.

The sugar content, expressed in %Brix, was determined using the Milwaukee MA871 digital refractometer, the glucose content using the Milwaukee MA872 digital refractometer, and the fructose content using the Milwaukee MA873 digital refractometer.

The acidity of the figs was determined by the titrimetric method. 10 fruits from the same sample have been mashed, with the Retsch GM 200 grinding mill, until a homogeneous paste resulted from which 5g of sample were weighed on the analytical balance over which 25ml of double-distilled water were added. The sample was homogenized using a magnet by inserting a Teflon-coated iron bar into the suspension. The pHmeter of the automatic titrator was rinsed with double-distilled water and swabbed with a paper towel after which it was placed in the sample. It was automatically titrated with 0.1 N NaOH to pH 8.1 or 8.2 depending on the sample. The initial and final pH were read, as well as how many ml of 0.1 N NaOH were consumed to the desired pH. The device used to determine acidity was the TitroLine easy automatic titrator. The acidity was expressed in citric acid (g/kg). Determinations were performed in triplicate.

The results were calculated according to the following formula and expressed as a percentage:

$$\text{Titratable acidity (g/kg citric acid)} = (V \times N \times C \times 100) / m$$

V - volume of NaOH consumed (ml), N - normality of NaOH, C - equivalent citric acid (0.0064), m - mass of sample (g)

The titratable acidity was determined according to the official method AOAC 942.15 and Saad et al (2014).

The determination of the total dry matter was carried out by introducing the fig paste into the Memmert oven, model 200, at 105°C for a period of 24 hours. Determinations were performed in triplicate.

RESULTS AND DISCUSSIONS

Total yield

Regarding the production, it was noticed that the best results in terms of yield were obtained by the genotypes of Romanian origin: 'Stork' (17.73 t/ha), 'Galben mare' (12.33 t/ha) and 'Rot negru' (8.55 t/ha), whilst the smallest was been recorded for the Italian 'Bianco Etna' genotype. Although it is a genotype that produces a lot of fruits, these cannot be marked because they do not reach maturity (ripen), 'Bianco Etna' being the latest genotype among those analyzed by us (Figure 1).

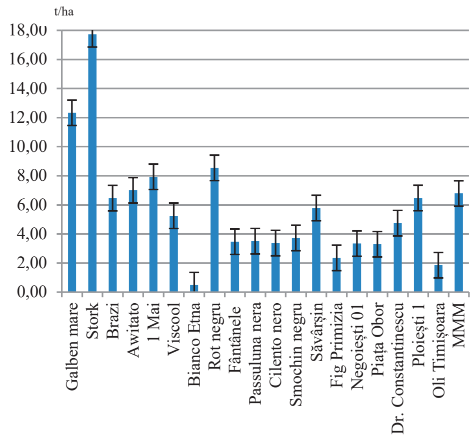

Figure 1. Total yield in 2021 year (t/ha)

Here there are charts of harvesting dynamic of genotypes with a similar period of fruit ripening in 2021. The genotypes were divided into the earliest, middle and latest ones. It is noticed that the best results for the earliest genotypes for the fourth year plants were registered between 9 - 16 September 2021 and around 7 October 2021 (Figure 2), whilst for the third year plants around 9, 16, 27 September 2021 (Figure 3).

For the middle and latest genotypes we had the best results, in terms of yield, around 17 September, 29 September, 7 October and late October, for the fourth year plants (Figure 4), whilst for those of third year between 16-26 September and around 8 and 20 October 2021 (Figure 5).

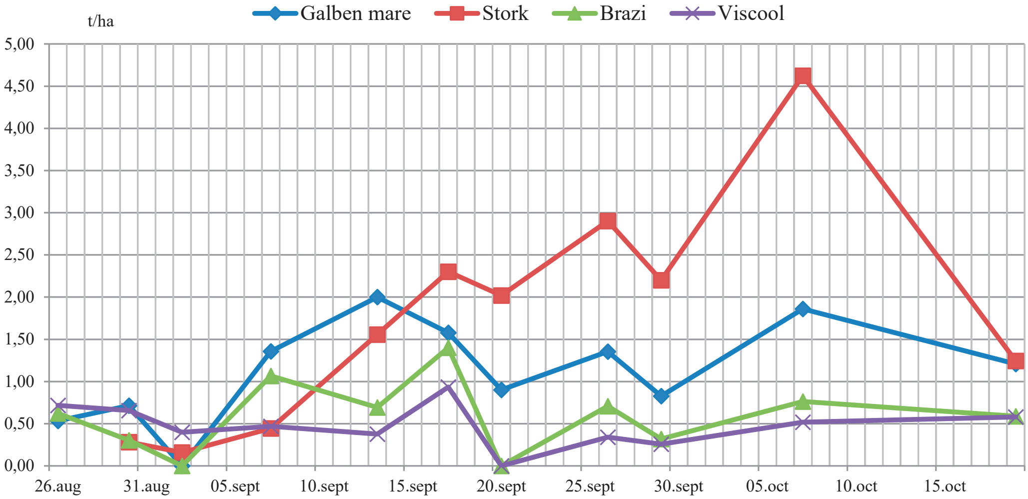

Figure 2. Yield dynamic (t/ha) of the earliest genotypes for the fourth year plants. The thickened points represent the days on which the harvest was carried out and the quantities of figs that were harvested on those days

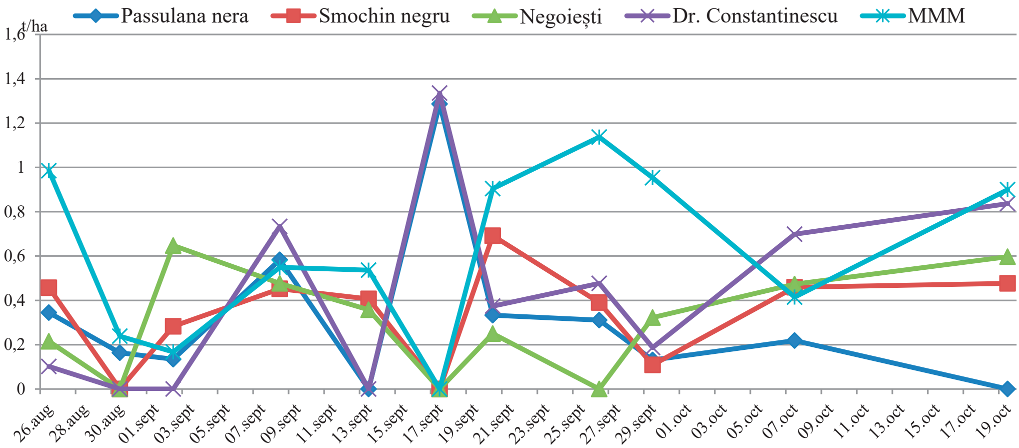

Figure 3. Yield dynamic (t/ha) of the earliest genotypes for the third year plants. The thickened points represent the days on which the harvest was carried out and the quantities of figs that were harvested on those days

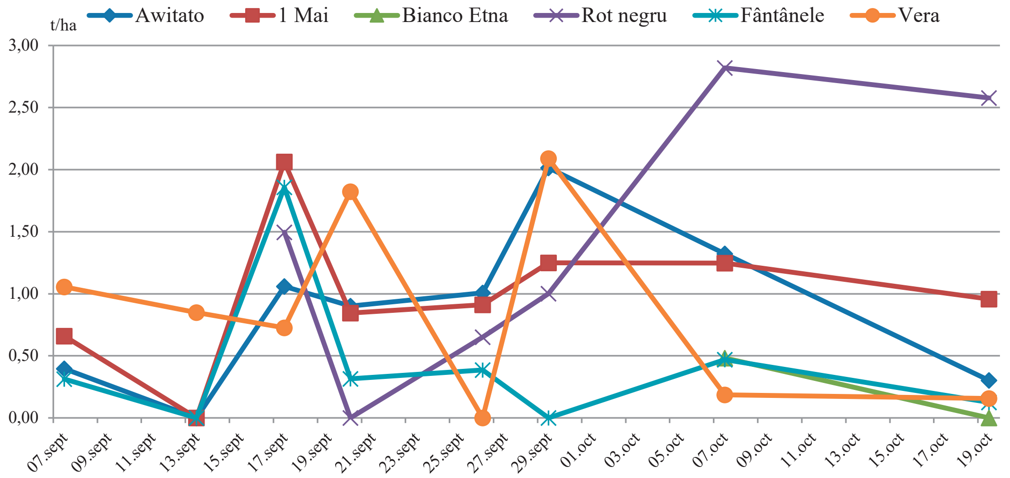

Figure 4. Yield dynamic (t/ha) of the middle and the latest genotypes for the fourth year plants.The thickened points represent the days on which the harvest was carried out and the quantities of figs that were harvested on those days

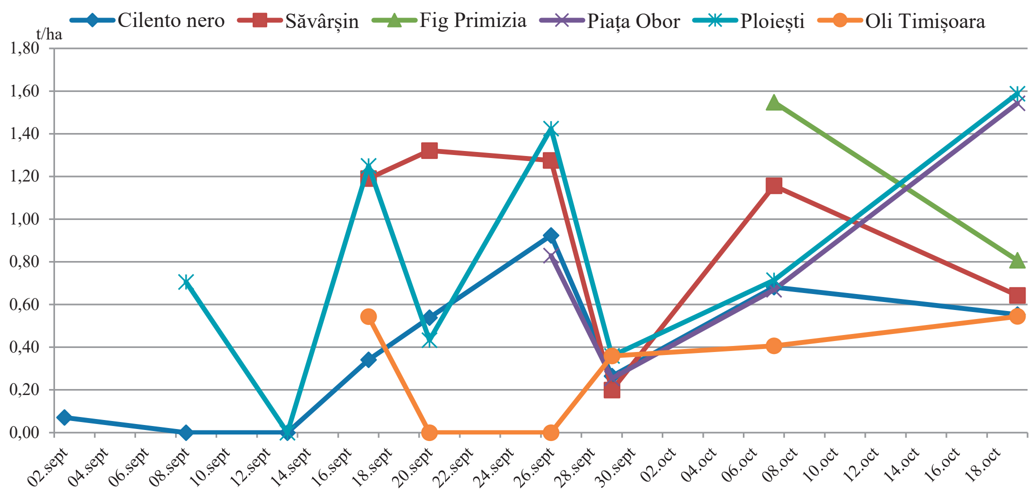

Figure 5. Yield dynamic (t/ha) of the middle and the latest genotypres for the third year plants. The thickened points represent the days on which the harvest was carried out and the quantities of figs that were harvested on those days

Harvesting time

For the studied genotypes, the second harvest (main crop) was analyzed and the results showed different harvesting period.

According to the harvest timeline for the two years 2020 and 2021, the earliest genotypes are: 'Galben mare', 'Brazi' and 'Viscool'.

We notice that in the case of earliest and latest genotypes, we have a decade or two delay in fruit ripening due to the late accumulation of useful temperatures in 2021.

This condition has little impact on genotypes with a middle maturity (Table1).

Table 1. Harvest schedule

Month		August		September			October		
		Decade		Decade			Decade		
Genotype	Year	2	3	1	2	3	1	2	3
‘Galben mare’	2020								
	2021								
‘Stork’	2020								
	2021								
‘Brazi’	2020								
	2021								
‘Awitato’	2020								
	2021								
‘1 Mai’	2020								
	2021								
‘Viscool’	2020								
	2021								
‘Bianco Etna’	2020								
	2021								
‘Rot negru’	2020								
	2021								
‘Fântânele’	2020								
	2021								
‘Passulana nera’	2020								
	2021								
‘Cilento nero’	2020								
	2021								
‘Smochin negru’	2020								
	2021								
‘Săvârșin’	2020								
	2021								
‘Fig primizia’	2020								
	2021								
‘Negoiești’	2020								
	2021								
‘Piața Obor’	2020								
	2021								
‘Dr. Constantin ’	2020								
	2021								
‘Ploiești’	2020								
	2021								
‘Oli Timișoara’	2020								
	2021								
MMM	2020								
	2021								

Towards the end of the vegetation period, from some genotypes (’Stork’ 3.35 t/ha; ‘Rot negru’ 3.02 t/ha; ‘Galben mare’ 0.83 t/ha) significant quantities of fruit in the veraison state were harvested, but which due to unfavorable temperatures no longer reach the maturity of consumption. These fruits are smaller in size (‘Rot negru’ 17 g; ’Galben mare’ 14.96 g; ‘Stork’ 14.92 g) and can be used successfully in the preparation of jams, caramelized figs or pickles.

Plant vigour

The comparison of the growth vigour was performed, as well (Figure 6). The plants on row R4 were planted in 2017, those on row R3 in 2018.The planting distances are slightly bigger on row R4. Comparing the vigour of the plants at the same age (three years old) we notice that those on R4 (the blue ones) were slightly more vigouros than those on row R3 (the red ones). The most vigouros plants were: ‘Stork’, ‘Bianco Etna’, ‘Galben mare’ (all of them are on row R4). The lowest growth vigour have had the following genotypes: MMM, ‘Oli Timișoara’ and ‘Fig primizia’ on row R3, ‘Fântânele’ and ‘Brazi’ on row R4.

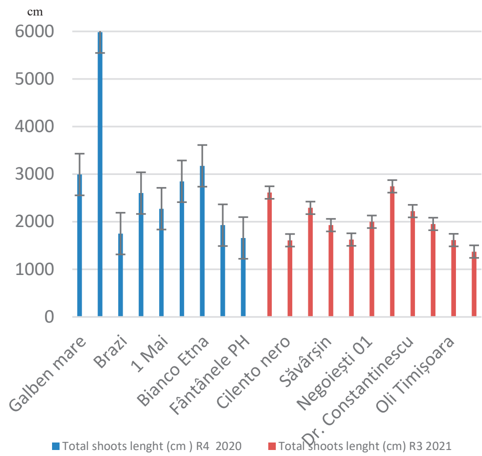

Figure 6. Total shoots length (cm)

Fruit characteristics

The average fruit weight was between 11.34 g/fruit (‘Smochin negru’), and 39.57 g/fruit (‘Săvârșin’) in 2021 (Figure 7) and it was lower than in 2020 (Figure 8) when this was between 17.16 g/fruit (‘Negoiești 01’) and 54.11 g/fruit (‘Săvârșin’), but the sugar content was higher, which indicates that the sugar concentration increased in 2021 compared to 2020.

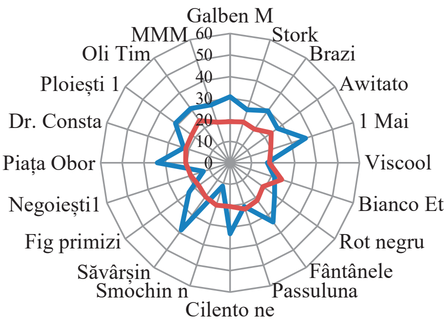

Figure 7. Average fruit weight (g) and Sugar content (% Brix) in 2021

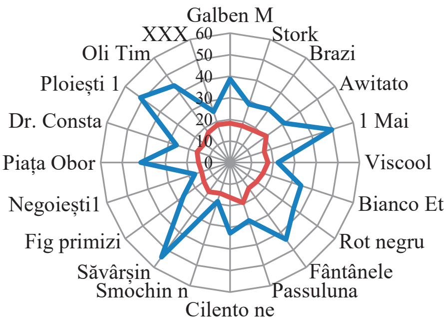

Figure 8. Average fruit weight (g) and Sugar content (% Brix) in 2020

We have had the same situation in case of glucose and fructose, their values being higher in 2021 than in 2020 (Figure 9).

We have noticed that the genotypes with the highest dry matter content (Figure 10) were the Italian ones: 'Bianco Etna' (25.32%), 'Awitato' (25.12%) and 'Passulana nera' (23.78%), this being in accordance with the soluble substances content.

Figs are alkaline fruits with an extremely low titrable acidity (g/kg citric acid), the highest values being observed in the genotypes 'Stork' (0.26 g/kg), 'Smochin negru' and 'Negoiești 01' (0.21 g/kg) (Figure 11).

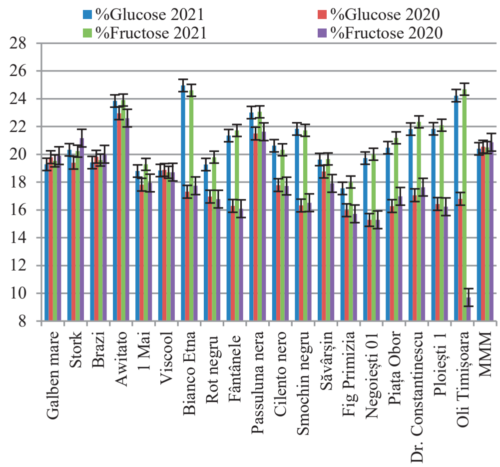

Figure 9. Glucose and fructose content (%)

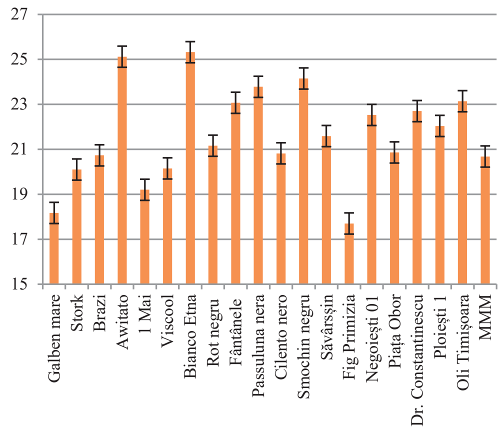

Figure 10. Total dry matter (%)

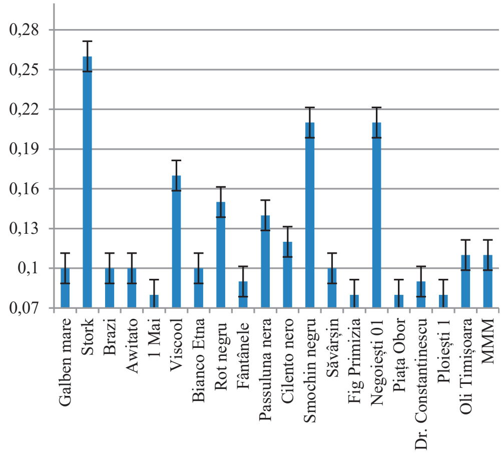

Figure 11. Acidity (citric acid g/kg)

CONCLUSIONS

The Romanian genotypes are the best adapted to the climatic conditions in Romania, being the earliest genotypes and, also, the ones which have had the highest yields per hectare.

Also, for some genotypes, significant quantities of fruit in the veraison state, that due to unfavorable conditions do not reach maturity for consumption, can be harvested and used to produce products such as jam, caramelized figs or pickles.

The best productions were registered in the second decade of September and the second decade of October.

The plants of italian origin, such as 'Bianco Etna', 'Awitato', 'Passulana nera' and 'Cilento nero' have had a good evolution, beeing the genotypes with the highest sugar and dry matter content, a very tasty fruits and good cracking resistance.

A high morphological variability was found, so that in the future new determinations will be made for a more precise characterization of the genotypes from the germplasm collection.

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STORAGE CONDITIONS INFLUENCE ON STANLEY AND BLUEFREE ORGANIC PLUMS QUALITY

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Abstract

The paper presents the influence of storage conditions on two varieties of plums: 'Stanley' and 'Bluefree'. In order to assess the influence of different factors, several indicators were taken into consideration: physiological parameters, quality indicators and bioactive compounds variations. Both 'Stanley' and 'Bluefree' plums varieties were harvested at the end of August 2021 from an organic orchard located in Prahova County and stored in three different conditions: 1) normal atmosphere (NA) with 1°C and 95% relative humidity (RH), 2) controlled atmosphere (CA) conditions with 1°C, 95% RH, 3% O_2 and 5% CO_2, and 3) CA conditions with 1°C, 95% RH, 1.5% O_2, and 10% CO_2. The samples were evaluated in seven different moments: initially (at harvest), after 2, 4, 6, 8, 10, and 12 weeks of storage. For both 'Stanley' and 'Bluefree' varieties, the storage period was shorter in NA than for those stored in CA conditions (2 weeks shorter for 'Stanley' variety and 3 weeks shorter for 'Bluefree' samples). The results showed that total titratable acidity and dry matter content registered similar variation trend during storage period for both samples, stored in all conditions. The obtained results suggest that the plums stored in controlled atmosphere conditions, kept their qualities better and for longer than those stored in NA.

***Key words**: antioxidant activity; controlled atmosphere; normal atmosphere; polyphenols; storage.*

INTRODUCTION

Plums are classified in Rosaceae family, like many other stone fruits and they are divided into two groups: Japanese plums and European plums (Okie et al., 2008). It is one of the most cultivated fruit trees in the world, with around 2000 cultivars (Ramming, 1991). Fruits have nutritional value similar to peaches and apricots. These are sources of minerals (Ca, Fe, P, Mg, K) and vitamins (vitamin A, riboflavin and niacin from the group of B vitamins, vitamin C and folic acid - folacin) (Ramming & Cociu, 1991).

European plum it`s the most cultivated plum in Europe, because of the adaptability in cooler areas (Hartmann and Neumüller, 2009). Some of the proprieties does make European plums so popular are the size, shape and color of the fruits, but also the flesh of the fruits and it`s proprieties, along with its high content of flavonoids, anthocyanins and other phenolic compounds (Hartmann and Neumüller, 2009). Plums can be consumed fresh or as dried fruits, juices and jams.

According to FAOSTAT, in Romania, in 2020, plums were cultivated at an area of 67,010 ha.

Plum in Romania is the number one cultivated specie in the fruit growing sector because of the good adaptability to climatic conditions and soils (Butac et al., 2019).

'Bluefree' is a variety of plum with an early fruiting and high productivity. The fruits have a dark blue skin, with a light waxy bloom. The fleshy part has a greenish-yellow color that turns yellow when it is ripe for consumption. Also, these are very aromatic, have a slightly low firmness and an unmistakable pleasant taste. 'Stanley' is a variety of plums that ripens in late summer or early fall; the fruits are large, have dark blue skin with greenish-yellow pulp. They are sweet, juicy and suitable for consumption as such or for preservation (Asanica and Hoza, 2013).

In order to reduce the losses and to extend the postharvest life of organic plums, the controlled atmosphere conditions as postharvest technologies are more used (Peano et al., 2010).

The aim of this paper is to evaluate quality indicators of two plums varieties in three different storage conditions.

MATERIALS AND METHODS

Samples

Two varieties of organic plums, 'Stanley' (Figure 1) and 'Bluefree' (Figure 2), were harvested in August 2021 from an orchard located in Prahova County. After the harvest, the fruits were transported to Postharvest Technologies Laboratory from Research Center for Studies of Food Quality and Agricultural Products.

After the initial analyses performed in the lab, the fruits were split and stored in three different conditions: 1) normal atmosphere (NA) with 1°C and 95% RH, 2) controlled atmosphere conditions with 1°C, 95% RH, 3% and 5% CO_2 (CA 5% CO_2), and 3) controlled atmosphere conditions with 1°C, 95% RH, 1.5% O_2, and 10% CO_2 (CA 10% CO_2).

Organic plums samples were analyzed in six different moments, for 'Stanley' variety stored in NA conditions, five moments for 'Bluefree' variety stored in NA. For both varieties stored in CA with 5% CO_2 and CA with 10% CO_2, seven moments of analyses were performed.

Figure 1. Initial moment of analysis - Stanley variety

Figure 2. Initial moment of analysis - Bluefree variety

Chemicals

Methanol used to determine total phenolic content was bought from Honeywell (Riedel-de Haën, Seelze, Germany).

Folin & Ciocalteu's reagent were purchased from Sigma-Aldrich Chemie GmbH (Riedstrasse, Steinheim).

Sodium carbonate anhydrous was bought from Lach-Ner s.r.o (Neratovice, Czech Republic).

For antioxidant activity determination, DPPH (1.1-diphenyl-2-picrylhydrazyl) and Trolox (6 - hydroxy - 2, 5, 7, 8 - tetramethylchroman - 2 - carboxylic acid) from Acros Organics, Fisher Scientific (Geel, Belgium).

Gallic acid was purchased from Carl Roth and Sodium hydroxide 0.1N was from Cristal R Chim S.R.L. (Bucharest, Romania) and anhydrous sodium carbonate was purchased from Lach-Ner, s.r.o. (Neratovice, Czech Republic).

Ultrapure water used it was made with a Milli-Q equipment (Millipore, Bedford, MA).

Quality indicators

Quality indicators were represented by total titratable acidity (TTA), pH, firmness, total soluble solids (TSS) and dry matter content (DM), methods being described forward.

TTA and pH analysis were performed using the automatic titrometer TitroLine, equipped with pH electrode. The analysis consists in weighting approximately 5 g of fresh homogenized sample mixed with 25 mL of bidistilled water, measuring the initially pH values and then titration with 0.1N NaOH until the final pH is 8.1 according with AOAC Official Method 942.15. For TTA, results were expressed in g malic acid /100g of fresh fruit similar with Petre et al. (2021).

Firmness results were obtained and expressed in kg/cm² using a digital penetrometer (53205 TR Italy) equipped with 8 mm piston.

The analysis of total soluble solids (TSS) were performed using Kruss DR301-95 digital refractometer (Cătuneanu et al., 2017).

Dry matter results were obtain using UN110 Memmert oven and drying approximately 1 g of sample at 105°C (Stan et al., 2020) until constant weight.

Phenolic content and antioxidant activity

For total polyphenol content (TPC) quantitative determination was used the Folin-Ciocâlteu method protocol. Extraction consist in trituration of 1 g fresh sample with 10 mL of

70% methanol and incubated overnight at dark and room temperature (approx. 22°C) in centrifuge tubes of 15 mL. Next day, the extraction continue with homogenization at 500 rpm for 1 h and, then centrifugation at 7000 rpm for 5 min at 4°C. The supernatant was recovered in 50 mL centrifuge tubes and the residue re-extracted two more times until the final volume of the extract of 30 mL. First step of determination of total polyphenolic content is by mixing 0.5 mL of extract with 2.5 mL of Folin-Ciocâlteu reagent and incubated for 2 minutes at room temperature (aprox. 22°C). Second step is represented by adding 2 mL of 7.5% sodium carbonate solution (Na_2CO_3) and incubate the mix at 50°C for 15 minutes. The third and final step is based on the absorbance read at Specord 210 Plus UV-VIS spectrophotometer (Analytik Jena, Jena, Germany) at the 760 nm wavelength. Results are expressed in mg GAE/100 g fresh weight.

Antioxidant activity determination was used the DPPH (2,2-diphenyl- 1-picrylhydrazyl) method, similar as Bujor et al. (2016) with variations presented forward. Mixing 0.2 mL of extract with 2 mL of 0.2 mM solution of DPPH in methanol and incubated in dark for 30 minutes, with homogenising. The absorbance of the samples was measured at 515 nm wavelength. Results were expressed as mg Trolox/100 g FW. Methanol was the blank reference used.

Statistical analysis

Statistical analysis of obtained data was standard deviation, represent the average of three replicates from the same sample with independent preparation.

RESULTS AND DISCUSSIONS

Quality indicators

Both varieties of plums registered quality indicators variation in all storage conditions.

Experiment were performed during 91 days of storage, but physiological disorders appear after 56 days of storage of in NA conditions (for ‘Bluefree’ variety), and 70 days of storage (for ‘Stanley’ variety). In both CA conditions the fruits were kept for 91 days.

pH values of 'Stanley' variety stored in NA decrease from 3.47±0.02 (initially) to 3.32±0.02 after 42 days of storage, but after 70 days of storage, at final moment of analyses increase to 3.66±0.01. In both CA conditions, pH values remain constant until last two moments, when increase to 3.72-3.74 (Table 1).

The initially TTA values of ‘Stanley’ variety were 1.11±0.04 g malic acid/100 g FW (Table 1), after 70 days of storage in NA, the TTA values decrease to 0.93 ±0.01 g malic acid/100 g FW, which means the acidity of plums decreases. 'Bluefree' variety stored in NA conditions show similar variation of TTA after 56 days of storage. For ‘Stanley’ variety samples stored in both CA, TTA values maintain constant until 56 days of storage, at the next two moments of analyses, TTA values decreases.

For ‘Bluefree’ variety, stored in both CA, TTA values maintain constant until 42 days of storage, at the next moment of analyses TTA values slightly increase. TSS values showed constant increases to all samples, in all storage conditions, fruits dehydrating considerable after 56 days of storage for 'Stanley' variety, and after 42 days of storage for 'Bluefree' variety, which increase the concentration of total soluble solids. During the storage period, the value of TSS for the fruits stored in NA with 1°C, 95% RH increase much more compared to the fruits stored in CA 1°C, 95% RH, 3% O_2 and 5% CO_2 and CA 1°C, 95% RH, 1.5% O_2, and 10% CO_2. Dry matter values maintain constant until 28 days of storage for 'Stanley' variety stored in NA and CA with 1°C, 95% RH, 3% O_2 and 5% CO_2 until 70 days of storage, after that dry matter content increase in all conditions. For ‘Bluefree’ variety, dry matter content increase after 42 days in NA and CA with 1°C, 95% RH, 3% O_2 and 5% CO_2, for samples stored in CA with 1°C, 95% RH, 1.5% O_2, and 10% CO_2.

The firmness values of ‘Stanley’ variety, recorded an important decrease in NA and CA with 1°C, 95% RH, 1.5% O_2, and 10% CO_2 after 42 days of storage, samples stored in CA with 1°C, 95% RH, 3% O_2 and 5% suffers massive decrease after 70 days of storage. Firmness values of 'Bluefree' variety showed a constant decrease from 4.73±0.41 kg/cm² to 1.07±0.17 kg/cm² after 28 days of storage. In both CA conditions, firmness values suffered a slight decrease, but maintain constant during storage period (Table 2).

Table 1. Variation of pH, total titratable acidity (TTA), total soluble solids (TSS), and dry matter (DM) content during storage of 'Stanley' variety

Variety	Storage conditions	Analysis moment (days)	pH	TTA (g malic acid/ 100g FW)	Total soluble solids (%)	Dry matter (%)	Firmness (kg/cm²)
Stanley	NA with 1°C, 95% RH	0	3.47 ±0.02	1.11±0.04	12.68±0.91	22.75±0.46	3.77±0.29
		14	3.42±0.08	1.00±0.02	12.85±0.71	23.19±0.72	3.35±0.39
		28	3.46±0.03	1.06±0.01	12.82±0.60	25.36±0.51	2.51±0.73
		42	3.32±0.02	1.00±0.006	13.19±0.68	23.46±0.72	1.65±0.46
		56	3.38±0.04	1.05±0.01	13.38±0.63	23.28±0.27	1.62±0.71
		70	3.66±0.01	0.93±0.01	13.47±13.02	23.32±0.25	1.01±0.54
		91	After 70 days analyses, no healthy fruits remained				
	CA 5% CO_2, 3% O_2, 1°C, 95% RH	0	3.41±0.02	0.97±0.01	12.60±0.50	24.7±0.80	3.40±0.60
		14	3.44±0.03	1.07±0.01	13.79±0.73	23.64±0.83	3.74±0.52
		28	3.37±0.05	0.96±0.02	14.35±0.72	25.08±0.81	3.82±0.38
		42	3.36±0.03	1.03±0.04	13.24±0.43	23.12±1.32	3.20±0.52
		56	3.73±0.01	0.92±0.01	13.27±0.62	24.14±0.19	2.96±0.64
		70	3.72±0.01	0.93±0.02	13.12±0.55	25.46±2.11	2.74±0.74
	CA 10% CO_2, 3% O_2, 1°C, 95% RH	0	3.43±0.03	1.06±0.03	13.51±1.02	25.21±1.09	3.24±0.50
		14	3.41±0.02	1.00±0.03	12.74±0.57	23.71±0.67	3.15±0.73
		28	3.37±0.02	1.03±0.03	13.01±0.74	21.68±0.20	3.02±0.33
		42	3.40±0.07	0.96±0.01	13.34±0.46	22.59±0.52	2.55±0.34
		56	3.74±0.04	0.95±0.01	13.49±0.65	27.88±1.42	2.43±0.74
		70	3.74±0.04	0.81±0.01	12.76±0.64	24.12±1.37	2.09±0.47

Table 2. Variation of pH, total titratable acidity (TAA), total soluble solids (TSS), a nd dry matter (DM) content during storage of 'Bluefree' variety

Variety	Storage conditions	Analysis moment (weeks)	pH	TAA (g malic acid/ 100 g FW)	Total soluble solids (%)	Dry matter (%)	Firmness (kg/cm²)
Bluefree	NA with 1°C, 95% RH	0	3.51±0.03	0.97±0.07	12.62±0.98	22.07±0.45	4.63±0.41
		14	3.42±0.03	1.03±0.01	11.82±0.88	24.44±1.42	4.28±0.60
		28	3.43±0.01	0.93±0.01	14.68±0.68	21.26±0.15	1.07±0.17
		42	3.42±0.11	1.01±0.01	13.98±0.82	23.37±0.27	1.08±0.46
		56	3.29±0.00	0.91±0.02	13.59±0.54	24.17±1.04	0.30±0.08
		70	After 56 days analyses, no healthy fruits remained				
		91					
	CA 5% CO_2, 3% O_2, 1°C, 95% RH	14	3.36±0.05	1.02±0.02	12.69±0.52	22.86±49	4.58±0.88
		28	3.42±0.06	1.09±0.01	13.37±0.98	23.55±0.68	3.50±0.56
		42	3.41±0.07	0.99±0.01	13.32±0.51	22.64±0.43	3.90±0.46
		56	3.36±0.02	1.04±0.04	13.53±0.79	26.11±0.51	3.50±0.39
		70	3.34±0.04	1.03±0.02	13.45±0.34	27.23±0.63	3.38±0.26
		91	3.63±0.03	1.06±0.01	12.96±0.54	27.65±0.87	3.35±0.34
	CA 10% CO_2, 3% O_2, 1°C, 95% RH	14	3.37±0.06	0.96±0.02	13.76±0.56	24.25±0.94	3.69±0.66
		28	3.38±0.04	1.11±0.02	12.57±0.98	23.94±1.43	4.14±0.79
		42	3.38±0.07	0.90±0.03	12.94±0.36	22.54±0.15	4.05±0.48
		56	3.32±0.02	1.08±0.01	13.05±0.44	24.15±0.21	3.50±0.65
		70	3.34±0.03	1.04±0.02	12.96±0.23	25.44±0.31	3.34±0.28
		91	3.20±0.01	1.08±0.02	12.38±0.12	26.41±0.54	3.18±0.45

Phenolic content and antioxidant activity

Total phenolic content (TPC) were determined from whole fruit and showed similar behavior for both plum varieties in all three storage conditions. For organic 'Stanley' variety, initially TPC values was 88.88±3.51 mg GAE/100 g FW, results similar with those obtain by Miletic et al. (2012). After 14 days of storage TPC values increase in NA with 1°C, 95% RH up to 112.68±6.14 mg GAE/100 g FW and in CA with 1°C, 95% RH, 1.5% O_2 and 10% CO_2, up to 100.23 mg GAE/100 g FW. In CA with 1°C, 95% RH, 3% O_2 and 5% CO_2, TPC values maintain constant after 14 days of storage

(Figure 3). Highest TPC value, 119.70±5.88 mg GAE/100 g FW were observed at fruits stored in CA with 1°C, 95% RH, 3% O_2 and 5% CO_2, after 91 days of storage, but the most constant values during storage were obtained from samples stored in CA with 1°C, 95% RH, 1.5% O_2 and 10% CO_2.

Initially total phenolic content for organic 'Bluefree' variety was 103.83±5.37 mg GAE/100 g FW and increase in all conditions during storage, up to 120.58±4.02 mg GAE/100 g FW in NA with 1°C, 95% RH, to 152.98±3.22 mg GAE/100 g FW in CA with 1°C, 95% RH, 3% O_2 and 5% CO_2 and up to 144.87±3.36 mg GAE/100 g FW in CA with 1°C, 95% RH, 1.5% O_2 and 10% CO_2, after 14 days of storage (Figure 4). The results showed most constant values during storage period of TPC were obtained from fruits stored in CA with 1°C, 95% RH, 3% O_2 and 5% CO_2. TPC values start to decrease after 70 days analyses for 'Bluefree' variety stored in both CA conditions, in comparation with previous moments of analyses.

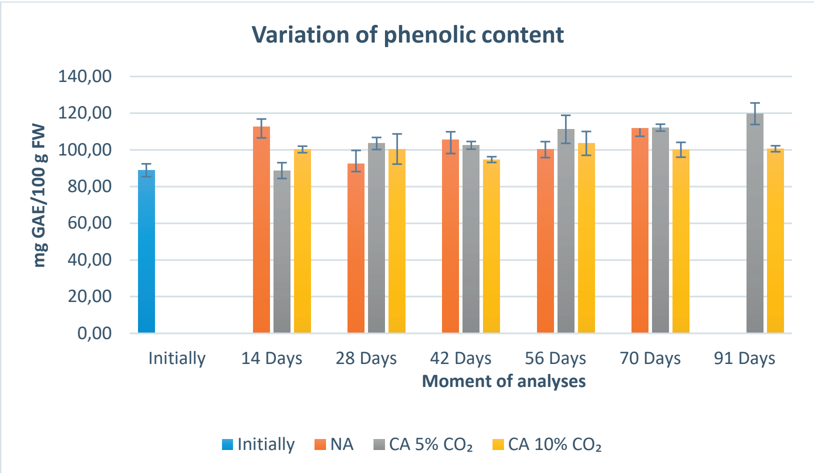

Figure 3. Total phenolic content variations for organic ‘Stanley’plums, registered during storage period

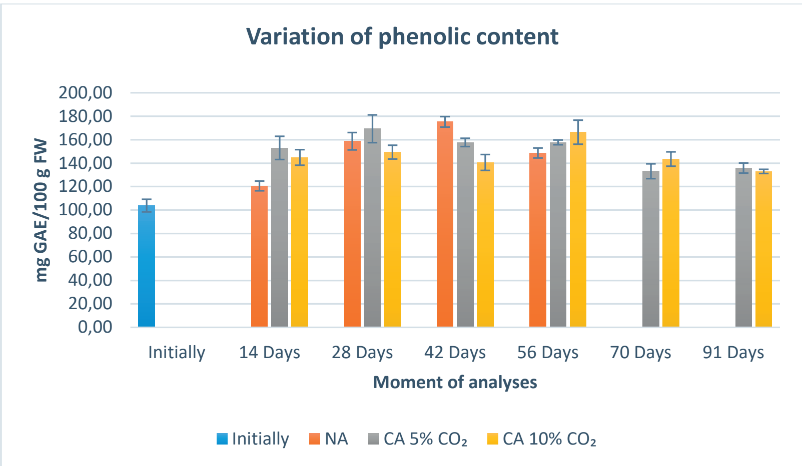

Figure 4. Total phenolic content variations for organic ‘Bluefree’variety, registered during storage period

Antioxidant activity of organic 'Stanley' variety increased from 805.89±74.10 mg Trolox/100 g FW (initially moment) up to 1038.42±94.00 mg Trolox/100 g FW after 14 days of storage in NA with 1°C, 95% RH, up to 902.62±11.62 in CA with 1°C, 95% RH, 3% O_2 and 5% CO_2 and up to 897.28±73.01 mg Trolox/100 g FW (Figure 5). 'Bluefree' variety showed a higher antioxidant activity, with values of 971.97±8.19 mg Trolox/100 g FW at the initial moment of analyses. After 14 days of storage antioxidant activity increased up to 1059.78±136.23 mg Trolox/100 g FW in NA with 1° C, 95% RH, up to 1398.66±90.02 mg Trolox/100 g FW in CA with 1°C, 95% RH, 3% O_2 and 5% CO_2 and up to 1339.49±70.25 mg Trolox/100 g FW (Figure 6).

Both organic 'Stanley' and 'Bluefree' plums showed increases of antioxidant activity during storage period (until 91 days of storage for 'Stanley' variety and until 56 days of storage for 'Bluefree' variety), variation trend of total phenolic content and antioxidant activity being similar during storage period in all three conditions.

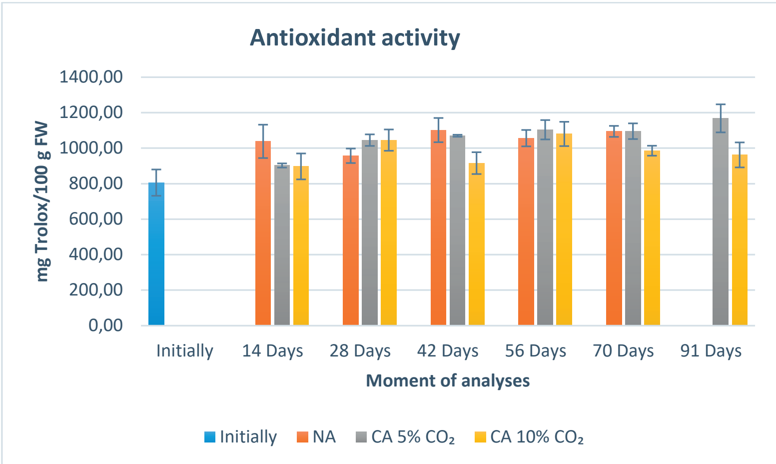

Figure 5. Antioxidant activity variations of organic 'Stanley' variety, registered during storage period

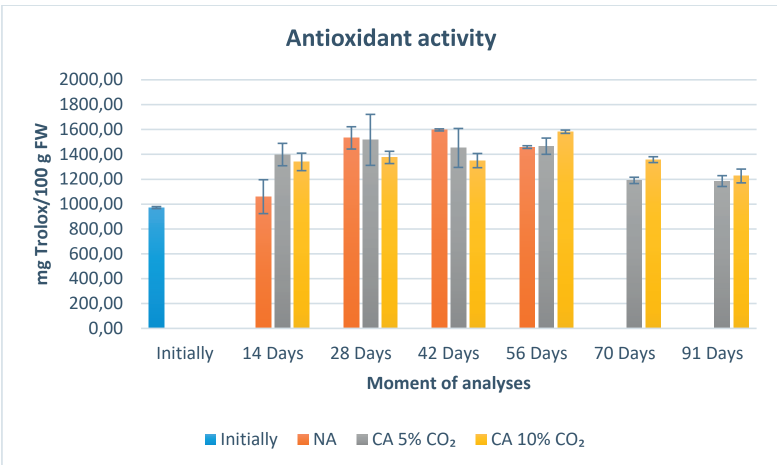

Figure 6. Antioxidant activity variations of organic 'Bluefree' variety, registered during storage period

CONCLUSIONS

In this paper the behavior of the two tested varieties of plums (‘Stanley’ and ‘Bluefree’) stored in three different conditions - normal atmosphere storage (NA), CA 5% CO_2 and CA 10% CO_2 was tested.

Compared to the normal atmosphere storage, rooms with controlled atmosphere extended the storage time of the fruits by 21 days for the ‘Stanley’ variety and 35 days for the ‘Bluefree’ variety, which may indicate that:

- quality indicators were maintained constant, except for the last two moments of analysis when both varieties presented lower values;
- antioxidant activity and total polyphenol content of ‘Stanley’ variety showed similar values, except samples from 91 days were fruits showed a slight increase compared to 70 days storage duration.
- antioxidant activity and total polyphenol content of ‘Bluefree’ variety recorded a decrease after 56 days in both storage conditions (CA 5% CO_2 and CA 10% CO_2) when compared with normal atmosphere storage (NA);

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EVALUATION OF FOUR SEA BUCKTHORN BIOTYPES FROM THE SPONTANEOUS FLORA OF ARGEŞ COUNTY, ROMANIA

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Abstract

Hippophae rhamnoides L. subsp. carpatica Rousi is one of the nine subspecies of the genus Hippophae, which belongs to the family Elaeagnaceae and its native area is East - Central and South - East Europe (Austria, Germany, Hungary, Romania, Serbia). In Romania, sea buckthorn grows spontaneously in the Subcarpathian area of Moldova and Muntenia but also may be found down to Black Sea coast, where sometimes forming groves or even dense bushes. This study aims to identify new biotips for horticulture studies, in breeding programs, as well to ensure the preservation of germoplasm resources of interest to spontaneous species of Hippophae rhamnoides out of the natural enviroment from the center of Romania. For this purpose, four biotypes named 'Leordeni 3', 'Leordeni 4', 'Leordeni 5' and 'Leordeni 6', were selected during 2020-2021 in Leordeni area from Argeş County. All these native biotypes were subjected to study in order to evaluate the fruits quality traits and the results were compared with 'Piteşti 1' cultivar. These researches identified the selection 'Leordeni 4' with the highest values of Vitamin C: 142,56 (mg/100 g FW) and also in the total of polyphenols content: 18,97 (mg gallic acid equivalent/ 100 g FW) meanwhile 'Leordeni 6' had good results at fruit weigh 0.59 (g) and soluble solid 12.62 (°Brix).

***Key words**: sea buckthorn fruits, biochemical constituents, biometrical measurements, wild flora.*

INTRODUCTION

Hippophae rhamnoides L. subsp. *carpatica* Rousi is one of the nine subspecies of the genus Hippophae , which belongs to the family Elaeagnaceae and its native area is East - Central and South - East Europe (Austria, Germany, Hungary, Romania, Serbia) (Yongshan et al., 2003; Ilhan G et al., 2021).

In Romania, sea buckthorn grows spontaneously in the Subcarpathian area of Moldova and Muntenia ranging from 0 to 1200 m. In the plate area, the sea buckthorn is found in riverbeds (Bistrita Valley, Siret Valley, Buzau Basin, Danube Delta), also in the hilly and submontane area exploring the sunny slopes. Sea buckthorn, being so widespread in wild flora, all cultivated Romanian varieties has the origin from wild flora (Ancu et al., 2017).

The plant has a great environmental plasticity and is extremely resistant to adverse ambient conditions, thus, it can be used for afforestation and wasteland management, which has led to its large-scale planting. It is resistant to urban conditions. Furthermore, as a xerophyte species, it tolerates drought, cold (up to −40°C), heat (up to 40°C), soil salinity, and air pollution. Sea buckthorn is a fruit species with a high nitrogen fixation capacity, thus improving soil quality; moreover, because of its extensive root system, it exhibits soil-binding properties (Ilhan G et al., 2021).

This study aims to identify new biotips for horticulture studies, in breeding programs, as well to ensure the preservation of germoplasm resources of interest to spontaneous species of Hippophae rhamnoides out of the natural enviroment from the center of Romania. Therefore, in this study, we attempted to determine the basic agro-morphological and biochemical traits of 4 seed-propagated sea buckthorn genotypes that grow naturally in the flora of Leordeni area from Argeş County, located in the center of Romania and the results were compared with 'Piteşti 1'cultivar.

MATERIALS AND METHODS

Research Institute for Fruit Growing Pitesti-Maracineni conducted numerous studies of exploring the wild flora in different areas of the country to identify new biotips for horticulture studies, to be used in breeding programs, as well to ensure the preservation of germoplasm resources of interest to spontaneous species out of the natural enviroment from Romania.

For this purpose, four biotypes named ’Leordeni 3’, ’Leordeni 4’, ’Leordeni 5’ and ’Leordeni 6’, were selected during 2020-2021 in Leordeni area from Argeş County. All these native biotypes were subjected to study in order to evaluate the fruits quality traits and the results were compared with ’Piteşti 1’ cultivar.

The samples were harvested at the optimal stage of maturity, between the second and the last decade of September.

The average weight of a fruit was determined by the weighing of a sample of 20 fruits with an electronic balance with an accuracy of 0.01 g, for each genotype and expressed in g/fruit.

The fruit length and diameters were measured using a calliper of a sample of 20 fruits for each genotype and expressed in mm.

The fruit size index was determined by measuring the length, large and small diameter (the longitudinal and polar diameter) for all 20 fruits of a sample for each genotype. The index size was calculated by formula: (height + large diameter + small diameter)/3 (Botu, 1997).

The fruit firmness was determined with a non-destructible penetrometer on a number of 20 fruits per genotype and expressed in units HPE.

Chemical analyzes and laboratory determinations were performed in three repetitions, this consisted in determining the soluble dry matter, organic acids, total sugar, vitamin C and total polyphenols.

Soluble solids content was determined in fruits juice by using a digital refractometer (Kern) and the results were reported as °Bx at 20°C (AOAC, 1999).

Total dry matter content was determined after extraction of water content for 8 hours at 105°C (AOAC, 1999) and expressed as g/100 g DW.

The organic acids (%) were measured by titration of the sampled fruits juice with a solution of 0.1 N NaOH, the results were expressed in g malic acid/100 g FW; ascorbic acid by the iodometric method and expressed in mg/100 g FW and total sugars (%) by the Fehling-Soxhlet method, 1965 (AOAC, 1968).

The acidity was determined by titration using of the sampled fruits juice (Ermakov et al., 1987); the results were expressed in g malic acid/100 g FW.

The pH of fruit juice was measured by squeezing the sea buckthorn fruits, using a multimeter C- 561, calibrated with 7 and respectively 4 pH solutions (AOAC, 1999).

The determination of total polyphenols was performed spectrophotocolorimetrically, by the Folin-Ciocâlteu method (Singleton et al, 1999) and was expressed as mg GAE/g fresh fruit.

The results were statistically calculated by Duncan´s Test at a significance level = 0.05.

RESULTS AND DISCUSSIONS

PHYSICAL CHARACTERISTICS OF THE FRUIT

Average weight of the fruit (g)

Regarding the average weight / fruit, the average of the 2020-2021 study years showed a significant difference between the genotypes studied as follows: 'Pitești 1' (0.57 g/fruit), 'Leordeni 3' (0.56 g/fruit) and 'Leordeni 6' (0.59 g/fruit) were statistically different from the genotypes 'Leordeni 4' (0.43 g/fruit) and 'Leordeni 5' (0.44 g/fruit) (Table 1).

Table 1. The evolution of average fruit weight (g)

Genotypes	Weight (g)		
	2020	2021	2020-021
Pitesti 1	$0.57{\pm}0.08^{a}$	$0.57{\pm}0.06^{a}$	$0.57{\pm}0.07^{a}$
Leordeni 3	$0.60{\pm}0.08^{a}$	$0.52{\pm}0.04^{b}$	$0.56{\pm}0.07^{a}$
Leordeni 4	$0.43{\pm}0.04^{b}$	$0.42{\pm}0.02^{d}$	$0.43{\pm}0.03^{b}$
Leordeni 5	$0.41{\pm}0.06^{b}$	$0.46{\pm}0.03^{c}$	$0.44{\pm}0.05^{b}$
Leordeni 6	$0.60{\pm}0.03^{a}$	$0.57{\pm}0.05^{a}$	$0.59{\pm}0.04^{a}$
Coeficient pearson	p=0.000	p=0.000	p=0.002

For 2020 and for 2021 the genotypes 'Leordeni 6' (0.60 g/fruit)/(0.57 g/fruit) and 'Pitești 1' (0.57 g/fruit)/(0.57 g/fruit) recorded the highest values. The results are in accordance with data from the literature Topala et al. (2020) obtained an average value of a fruit of 0.53 ± 0.17 g.

Size index of the fruit

In the case of the size index, the 'Pitești 1' genotype (10.31) had the highest average compared to the other genotypes studied in the two years, the lowest average values being registered by the 'Leordeni 4' selections (8.62), respectively 'Leordeni 5' (8.79) (Table 2).

Table 2. The evolution of size index

Genotypes	Size index		
	2020	2021	2020- 2021
Pitesti 1	10.59±0.45[a]	10.02±0.47[a]	10.31±0.54[a]
Leordeni 3	10.12±0.44[b]	9.09±0.51[b]	9.60±0.70[c]
Leordeni 4	9.01±0.34[c]	8.24±0.17[c]	8.62±0.47[d]
Leordeni 5	8.82±0.50[c]	8.77±0.30[b]	8.79±0.40[d]
Leordeni 6	10.14±0.31[b]	9.88±0.27[a]	10.01±0.31[b]
Coeficient pearson	p=0.000	p=0.000	p=0.000

For the year 2020 the genotype 'Pitești 1' (10.59) was followed by 'Leordeni 6' (10.14) and 'Leordeni 3' (10.12); the genotypes 'Leordeni 4' (9.01) and 'Leordeni 5' (8.82) remain the smallest.

The year 2021 had the same leader: 'Pitești 1' (10.02) followed by 'Leordeni 6' (9.88); the genotypes 'Leordeni 3' (9.09) 'Leordeni 5' (8.77) and 'Leordeni 4' (8.24) had lower averages.(Ancu et al., 2017) had similar results with values ranging between 7.07-9.80.

Table 3. The evolution of firmness

Genotypes	Firmness		
	2020	2021	2020- 2021
Pitesti 1	39.73±4.03[a]	36.93±6.63[a]	38.33±5.53[a]
Leordeni 3	27.49±7.60[b]	31.05±6.52[b]	29.27±7.13[b]
Leordeni 4	34.41±7.22[a]	29.55±3.29[b]	31.98±6.00[b]
Leordeni 5	27.84±8.90[b]	37.61±8.70[a]	32.73±9.92[b]
Leordeni 6	37.70±6.81[a]	29.43±5.10[b]	33.57±7.23[b]
Coeficient pearson	p=0.000	p=0.007	p=0.203

Firmness of the fruit (N)

Analyzing the average values of the two years of study in the case of Firmness we can see according to the data in Table 3 that the genotype 'Pitesti 1' (38.33 N) had the highest value compared to the other selections.

BIOCHEMICAL CHARACTERISTICS OF THE FRUIT

Soluble dry matter content (°Bx)

From the data recorded in Table 4 it is observed that the genotypes significantly influenced the soluble dry matter content in the two years of study obtaining five statistical classes with values between 11.10°Bx in 'Leordeni 5' and 12.86°Bx in 'Leordeni 3'.

In 2020 the genotypes 'Leordeni 3' (12.43) and 'Leordeni 6' (12.26) had the highest values and in 2021 they were exceeded: 'Leordeni 3' (13.28) and 'Leordeni 6' (12.98). Similar results were found at (Ancu et al., 2017) which has an average variation between 5.28 and 9.76.

Table 4. The evolution of soluble solid °Bx

Genotypes	Soluble solid °Bx		
	2020	2021	2020- 2021
Pitesti 1	11.46±0.97[bc]	12.50±0.73[b]	11.98±0.99[b]
Leordeni 3	12.43±0.48[a]	13.28±0.44[a]	12.86±0.63[a]
Leordeni 4	11.97±0.67[ab]	11.20±0.42[c]	11.59±0.67[c]
Leordeni 5	10.88±0.59[c]	11.32±0.39[c]	11.10±0.54[d]
Leordeni 6	12.26±0.55[a]	12.98±0.27[a]	12.62±0.56[a]
Coeficient pearson	p=0.000	p=0.000	p=0.000

pH of the fruits

For pH, mean values varied between the two years of study; according to them, the highest value was registered by the genotype 'Leordeni 5' (2.95) and the lowest being at 'Pitești 1' (2.60) (Table 5). Similar results were found at (Ancu et al., 2017) which has an average variation between 2.33 and 2.99.

Table 5. The evolution of pH

Genotypes	pH		
	2020	2021	2020- 2021
Pitesti 1	2.68±0.07[c]	2.52±0.11[c]	2.60±0.12[c]
Leordeni 3	2.86±0.11[b]	2.79±0.09[a]	2.83±0.10[b]
Leordeni 4	2.89±0.12[b]	2.70±0.04[b]	2.80±0.13[b]
Leordeni 5	3.11±0.12[a]	2.78±0.04[a]	2.95±0.19[a]
Leordeni 6	2.93±0.09[b]	2.73±0.09[ab]	2.83±0.13[b]
Coeficient pearson	p=0.000	p=0.000	p=0.000

Table 6 shows the evolution of the average values of the total organic acid content, Vitamin C content, total sugar content and total polyphenol content of the sea buckthorn fruits studied.

Total dry matter content (%)

Total dry matter content was significantly influenced by genotype. The mean values of the total dry matter content are grouped into four different series (Table 6). There is a significant decrease in the total dry matter content of the 'Leordeni 5' genotype (18.49%) compared to the other genotypes studied. The highest total dry matter content was recorded in the 'Leordeni 6' genotype (21.61%). The results are in accordance with the data in the literature (Olteanu et al., 2007) showing a variation of the total dry matter content of sea buckthorn fruits from 15 g % to 19.43 g %.

Total titratable acidity expressed as malic acid (%)

It is found that the average total content of organic acids decreased significantly starting from the genotype 'Pitesti 1' (3.32%) followed by the genotype 'Leordeni 6' and 'Leordeni 5' (3.01% and 2.88%). The lowest average content of organic acids expressed as malic acid in fruit was obtained in the 'Leordeni 2' genotype (2.60%). Variations in the organic acid content of fruits have been reported in the literature from 0.26% to 2.89% (Kawecki et al., 2004). The improvement of fruit quality is determined by the content of sugar and organic acids (Raffo et al., 2004).

Total sugar content (%)

Total sugar content was significantly influenced by genotype. The average values of the total sugar content of sea buckthorn fruits are grouped into three different series. The genotype with the highest average total sugar content was 'Pitești 1' (2.37%) followed by 'Leordeni 3' and 'Leordeni 6' (2.33% and 2.26% respectively). The lowest total sugar content was obtained in the genotype 'Leordeni 4' (2.07%). Kawecki et al., 2004, obtained a total sugar content in sea buckthorn fruits between 1.99% and 2.78 %.

Table 6. The evolution of the average values of the biochemical values

Genotypes	Soluble solid content (%)	Titratable Acidity (%)	Total Sugar %	Sugar/Acidity ratio	Vitamina C (mg/100g FW)	Total Phenolic content (mg GAE/g FW)
Pitesti 1	20.02±0.20[c]	3.32±0.09[a]	2.37±0.12[a]	0.72±0.05[b]	91.53±1.36[c]	14.71±0.14[c]
Leordeni 3	20.57±0.10[b]	2.60±0.09[d]	2.33±0.09[a]	0.90±0.01[a]	93.28±0.92[cd]	16.233±0.09[b]
Leordeni 4	19.92±0.06[c]	2.81±0.06[c]	2.07±0.06[c]	0.73±0.01[b]	142.56±0.45[a]	18.973±0.09[a]
Leordeni 5	18.49±0.14[d]	2.88±0.13[bc]	2.14±0.07[bc]	0.74±0.02[b]	113.96±5.67[b]	10.897±0.36[d]
Leordeni 6	21.61±0.08[a]	3.01±0.08[b]	2.26±0.09[ab]	0.75±0.01[b]	96.80±1.01c	16.043±0.09[b]
Coeficient pearson	p=0.000	p=0.000	p=0.008	p=0.000	p=0.000	p=0.000

Sugar/organic acid ratio

According to (Tang et al., 2001) increasing the sugar/organic acid ratio is an important factor in improving the flavor of sea buckthorn fruits. Sea buckthorn fruits are known to have a low sugar content and a high acid (Rongsen, 2006). Ohkawa et al., 2009, obtained a ratio of sugar/acid content between 0.34 and 1.14. In the studied genotypes, the highest ratio of sugar/acid content was obtained for the 'Leordeni 3' genotype (0.90).

Vitamin C content (mg/100 g FW)

Vitamin C content was significantly influenced by genotype. There are four different homogeneous series of Vitamin C content in the sea buckthorn genotypes studied (Table 6). The genotype with the highest Vitamin C content was 'Leordeni 4' (142.56 mg/100 g FW) followed by 'Leordeni 5' (113.96 mg/100 g FW) and 'Leordeni 6' (96.80 mg/100 g FW). The 'Pitesti 1' genotype had the lowest Vitamin C content (91.53 mg/100 g FW).

Total polyphenol content (mg GAE/g FW)

In the sea buckthorn genotypes, the content of total polyphenols varied between 10.90 mg GAE/g FW (genotype 'Leordeni 5') and 18.97 mg GAE / g FW (genotype 'Leordeni 4'). Similar results were found at (Criste et al.,

2020) with average values ranging between 10.12 and 18.66 mg GAE/g FW.

The evaluation of Table 7, highlights the correlations obtained between some biometric indicators of sea buckthorn fruits. Thus we can observe that there is a positive correlation, distinctly significant between the average weight of the fruit and the size index (r = 0.758), between the average weight of the fruit and the soluble dry matter content (r = 0.444). Also, the average weight of the fruits correlates negatively significantly with the pH of the fruit juice (r = - 0.231) and positively significantly with the textural firmness (r = 0.245). It is observed that the size index correlates positively, distinctly significantly with the soluble dry matter content (r = 0.274) and with the textural firmness (r = 0.327). Also, the size index has a significant negative correlation with the pH of fruit juice. There is also a significant negative correlation between the soluble dry matter content and the pH of the fruit (r = 0.215).

Table 7. Correlations

		Average fruit weight (g)	Size index (mm)	Soluble solid °Bx	pH	Firmness (N)
Greutate medie fruct (g)	Pearson Correlation	1				
Indice de marime (mm)	Pearson Correlation	0.758(**)	1			
Substanță uscată solubilă % Brix	Pearson Correlation	0.444(**)	0.274(**)	1		
pH	Pearson Correlation	-0.231(*)	-0.237(*)	-0.215(*)	1	
Fermitate (N)	Pearson Correlation	0.245(*)	0.327(**)	-0.008	-0.176	1

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

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

CONCLUSIONS

This study encompass four sea buckthorn genotypes 'Leordeni 3', 'Leordeni 4', 'Leordeni 5', and 'Leordeni 6', that grow naturally in the flora of Leordeni area from Argeş County, located in the center of Romania, and 'Piteşti 1'cultivar from Research Institute for Fruit Growing Pitesti-Maracineni. The results displayed important nutritional and bioactive compounds in the sea buckthorn berries.

Over the studied period the 'Leordeni 4' genotype had recorded the highest values of Vitamin C and total phenolic content and 'Leordeni 6' had good results at fruit weigh, size index, soluble solid °Bx and firmness versus the 'Piteşti 1' cultivar and studied genotypes, for that we recommend this genotypes for spread in culture and for further breeding programs targeting different breeding purposes.

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SENSORY EVALUATION AND CUSTOMERS' PERCEPTION OF SOME PAWPAW (*Asimina triloba* Dunal) PRODUCTS

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Abstract

Pawpaw (Asimina triloba Dunal) is a native North American fruit species that belongs to the Annonaceae family. In Romania, it was introduced for the first time in 1926, by Suciu family from Alba County, but for many decades remained unknown. Extended studies on the plant and new varieties are made at the Experimental Field, within the Faculty of Horticulture in Bucharest, starting with 2000. The purpose of this paper is to present the sensory evaluation of some pawpaw processed products: pawpaw ice cream; pawpaw yoghurt; pawpaw with sweet cottage cheese; pawpaw chocolate bar. Customers' perception consisted on the evaluation of general appearance, colour, texture, taste and flavour, noticed with grades from 1 to 7. The results showed that most of analysed products have been positively appreciated by consumers and their preferences varied with gender, age and origin.

Key words*: customers' preferences, food products, northern banana, raw materials.*

INTRODUCTION

Asimina triloba (L.) Dunal, or pawpaw, is the only temperate plant species that belongs to the *Annonaceae* family (Padmanabhan & Paliyath, 2016). Is a native North American fruit species including nine species of *Asimina*. Grows on the eastern part of the continent, from Florida to South Canada.

In the south-eastern part of Florida and Georgia State, there are eight other members of *Asimina* genus: *Asimina incarna* (flag paw-paw), *Asimina longifolia*, *Asimina obovata*, *Asimina parviflora* (dwarf paw-paw), *Asimina pygmaea*, *Asimina reticulata*, *Asimina tetramera* (oposum paw-paw), *Asimina* × *nashii* (Callaway, 1993; Stănică et al., 2008).

In Romania, the first Asimina plants arrived in Transylvania from North America at the beginning of the 20th Century. They were locally cultivated and remained unknown in the rest of country (Dănăilă et al., 2004).

Only after 2000, at the Faculty of Horticulture in Bucharest, was a scientific evaluation of this interesting species started with the goal of studying the propagation techniques, orchard management and its behaviour under Romanian conditions (Stănică, 2002; Stănică et al., 2004; Stănică et al., 2008).

The color of the fruit changes from creamy white through bright yellow to shades of orange. (Levine et al., 2015).

The flavor of ripe pawpaw fruit resembles a combination of banana (*Musa×paradisiaca*), mango (*Mangifera indica*), and pineapple (*Ananas comosus*); however, flavor varies among varieties, with some fruit displaying more complex flavor profiles (Padmanabhan & Paliyath, 2016).

Although pawpaw is sometimes confused with papaya (*Carica papaya*), but it is an entirely different species (Levine et al., 2015). Papaya is a tropical plant grown in tropical regions, but pawpaw can grow well in tropical regions as well as in humid microthermal climates. (Padmanabhan & Paliyath, 2016).

Regarding nutritional value, pawpaw is a nutritionally rich fruit with high levels of antioxidant compounds. Brannan et al. (2015), reported that pawpaw fruit contains a large amount of procyanidins, which have antioxidant effects, and Kobayashi et al. (2008), demonstrated that pawpaw fruit exhibits antioxidant activity. The pawpaw antioxidant

content is similar to values for strawberry and orange, and is almost ten times higher than values for banana and apple (Nam & Jang, 2018; Pellegrini et al., 2003).
Minerals and vitamins are comparable to banana, apple and orange. Pawpaw fruit are high in vitamins such as vitamin C, niacin, protein and minerals, amino acids and they can be considered an excellent source of potassium, calcium, phosphorus, iron and magnesium, all very important micronutrients that are often lacking in the diets of children and seniors, along with unique taste, make it an interesting alternative to the most commonly consumed fruits (Galli et al., 2007; Templeton et al., 2003).

MATERIALS AND METHODS

The objective of this study is to present sensory evaluation and customers' perception of some pawpaw (*Asimina triloba* Dunal) products.
All the products were prepared at the Research Center for Studies of Food Quality and Agricultural Products, in the Integrated Fruit Growing Laboratory.
The tested sensory characteristics included overall aspect, color, texture, taste and flavor.
For sensory evaluation, consumers were asked to rate the products on a 7-point Hedonic scale: 1=very unpleasant/dislike it very much; 2=unpleasant/dislike it; 3=a little unpleasant; 4=indifferent; 5=a little pleasant; 6=pleasant; 7=very pleasant.
Colour is important sensor character on which the consumer preferences dependent.
Color affects our perception of food and drink in different ways. The most obvious of these refers to the color of the product itself, which will change even the taste and the perceived aroma (Spence et al., 2010; Spence, 2016; Spence, 2018).
The products were evaluated by consumers represented by 70% women and 30% men with ages between 10 and 72 years old (Figure 1).
Pawpaw fruit can be consummated as fresh fruits or as processed products. The products prepared and tested were:

Figure 1. Products testing

Pawpaw ice cream
All the ingredients were mixed: yoghurt, pulp of pawpaw, honey, and sea buckthorn, for the second option, and put it in the special ice cream machine (Figure 2).

Figure 2. Pawpaw ice cream with honey/
Pawpaw ice cream with sea buckthorn

DAIRY PRODUCTS WITH PAWPAW

Yoghurt with chokeberry or sea buckthorn
Mixed yoghurt with pulp of pawpaw, juice of chokeberry or sea buckthorn and little honey (Figure 3).

Yoghurt with biscuits
Yoghurt with pulp of pawpaw and crushed biscuits (Figure 3).

Sweet cottage cheese and pawpaw
Mixed sweet cottage (0.2 fat) with pulp of pawpaw and putted on the crackers (Figure 3).

Figure 3. Dairy products with pawpaw: Yoghurt with pawpaw and chokeberry/sea buckthorn; Sweet cottage cheese and pawpaw; Yoghurt with pawpaw and biscuits

Cakes with pawpaw
Mixed all the ingredients: pulp of pawpaw, powder of jujube, juice of sea buckthorn or chokeberry and put them in equal quantities in wafer sheets and a chocolate glaze over (Figure 4).

Figure 4. Pawpaw cake with jujube powder and sea buckthorn/chokeberry

Chocolate bar with alcoholized pawpaw
Mixed all the ingredients: dark chocolate and pulp of alcoholized pawpaw and put them in special form for bars (Figure 5).

Chocolate bar with pawpaw
Mixed all the ingredients: dark chocolate and pulp of fresh pawpaw and put them in special form for bars (Figure 5).

Figure 5. Chocolate bar with alcoholized pawpaw / Chocolate bar with pawpaw

RESULTS AND DISCUSSIONS

The data collected from the questionnaires completed were processed into graphs.
The pawpaw ice cream with honey was the most appreciate (Figure 6). The flavor, being the most intense, made the difference between the two. The graph of ice cream pawpaw sensory characteristics can be found in Figure 7.
The mixture of sweet cottage cheese and pawpaw such was a success, maybe the used salty crackers too. The combination of sweet and salty was very appreciate. The graph dairy products can be found in Figure 8.
Among the three yoghurts presented, the most appreciated was the yoghurt with pulp of pawpaw and chokeberry. The most appreciated parameter was the texture, for the sweet cottage cheese with pawpaw, and the lower the texture of yogurt with pawpaw and biscuits (Figure 9).

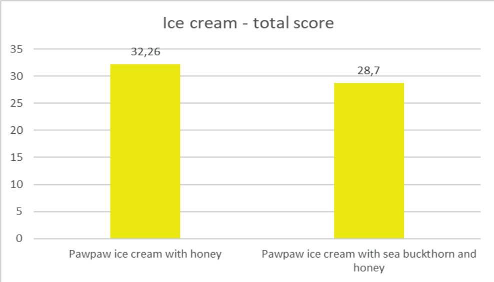

Figure 6. Pawpaw ice cream - total score

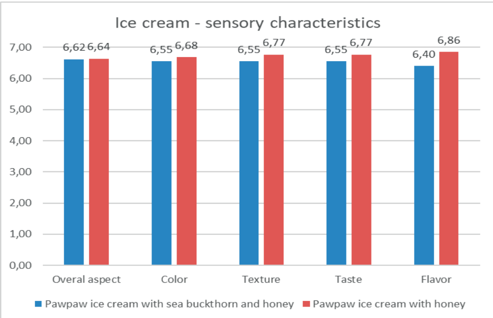

Figure 7. Pawpaw ice cream sensory characteristics

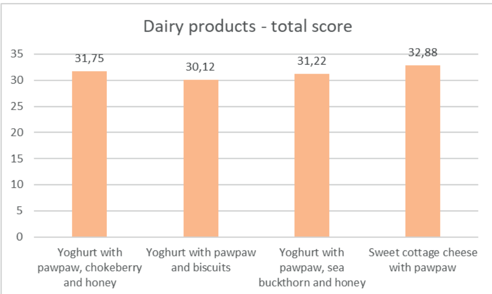

Figure 8. Dairy products - total score

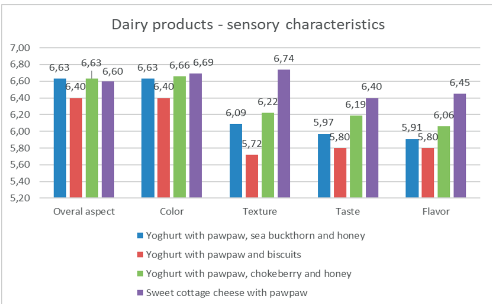

Figure 9. Dairy products sensory characteristics

Both of cake was very appreciate (Figure 10). The consumers appreciate much more the combination of pawpaw with chokeberry than with sea buckthorn (Figure 10).

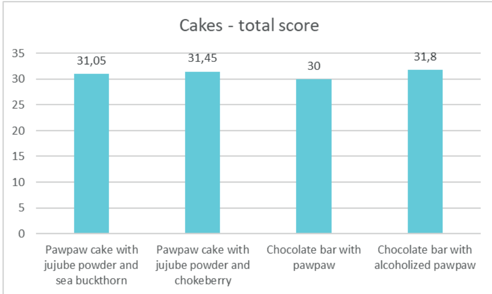

Figure 10. Pawpaw cakes and bars

The taste and flavor of chocolate bar with simple pawpaw was much more appreciated than chocolate bar with alcoholized pawpaw, maybe because of the alcohol, but the texture and overall aspect was very interesting for consumers for this bar (Figure 11).

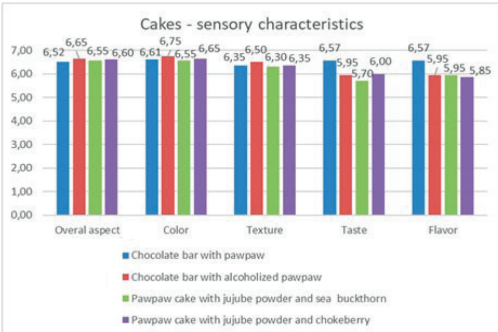

Figure 11. Pawpaw cakes and bars sensory characteristics

CONCLUSIONS

In conclusion, the results showed that most of the products in combination with the pawpaw offered to consumers for testing have been positively appreciated and their preferences varied with gender, age and origin.

The most appreciated products are the sweet cottage cheese and pawpaw and the pawpaw ice cream with honey.

All suggestions and recommendations for future products are taken into account.

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Ziziphus jujuba Mill. IN ROMANIA - SENSORY EVALUATION OF SOME FRESH FRUITS AND JUJUBE PROCESSED PRODUCTS

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Abstract

Jujube (Ziziphus jujuba Mill.) is the most important species of Rhamnaceae family and is one of the oldest cultivated fruit trees in the world. In Romania it was introduced for the first time in Dobrogea region, close to the Black Sea, some 2000 years ago. The first cultivated varieties, more than 20 genotypes, were introduced after 1997 at the Didactic Experimental Field, within the Faculty of Horticulture in Bucharest, from Shanxi Province, China. The purpose of this paper is to present the sensory evaluation of some fresh jujube fruits cultivars and customers' perception on some innovative products. The results were obtained during the international workshop "Prospective of Chinese jujube (Ziziphus jujuba Mill.) cultivation in Romania" organized in 2nd of October 2020, at the USAMV Bucharest. Among the most common jujube cultivated in Romania, only the ones that arrived at the ripening stage: Jun Zao, Hu Ping Zao, Hu Ping AA and Early Hu Ping Zao, were tasted. The fruit size and shape, fruit colour, firmness and juiciness, taste and flavour were noticed with grades from 1 to 5, five being the highest value. The jujube tested products were: dehydrated jujube (simple, with walnuts or almonds), jujube liqueur (with honey, with rose petals, with plums and honey, with plums and rose petals), jujube tea, jujube compote and jujube tart. The consumers' panel for sensory evaluation was represented by farmers, researchers, professors, specialists and ordinary people. The results showed that, fresh fruits and processed jujube products, was being positively appreciated by most of the participants and varied with the age, gender and origin.

***Key words**: food products, sensory evaluation, Ziziphus jujuba Mill.*

INTRODUCTION

Jujube is an edible and delicious fruit, which has various health benefits. It is extensively cultivating in tropical and subtropical regions, especially in East Asia (China, India), North Africa, and Middle Eastern countries. However, most of the species of jujube (*Ziziphus jujuba* Mill.) are native to China, where it has been cultivated from more than 4.000 years. There are over 400 cultivars available in China (Al-Saeedi et al., 2016; Li, et al., 2007; Liu et al., 2016; Raswan et al., 2020).
In Romania, jujube populations were found in the Dobrogea region (Ciocârlan, 2000), between the Danube and the Black Sea in the neighbourhood of antique sites as Greek, Roman, and Byzantine ruins at Ostrov, Jurilovca, and Mahmudia. Probably, those old civilizations had an important role in the introduction of this Asian plant to the area (Stănică, 2000; Stănică, 2009). The plant, nearly unknown, is named Dobrogea olive by the locals and the fruits are rarely used for eating. Only, Ostrov type, a real *Ziziphus jujube* tree, has interesting fruit for fresh and dry consumption (Ciocârlan, 2000; Stănică, 2009). Even it was introduced in the Dobrogea region, some 2.000 years ago by the Greek and Roman colonists, jujube plants and fruits are nearly unknown, as it happens in other countries from the Mediterranean basin (Stănică, 2009).
The first cultivated varieties were introduced at the Faculty of Horticulture in Bucharest from Shanxi Province, China within a common research project in 1997 (Stănică, 2019; Stan G. et al., 2021).
The peel and pulp of the fruits are considered the main part, which contains most of the bioactive compounds. The scientific evidence has shown that jujube fruits contain a high amount of various bioactive compounds, including

ascorbic acid, phenolic acids, amino acids, saponins, cerebrosides, polysaccharides, flavonoids and mineral constituents (Xu et al., 2019; Feng et al., 2019a; Ji et al., 2017; Raswan et al., 2020).

Therefore, jujube fruit is consumed as traditional and functional food worldwide. It can be consumed as freshly squeezed jujube pulp or it can be used for the preparation of some food products, such as beverages, jams, jelly as well as pickles, liqueur and compotes. The dried pulp can also be used as active ingredient in the food industries, such as dried products (Chinese dates, the ingredient of tea, slices snack, bread, cakes, etc.). Moreover, it can be added to other foods/products to improve the nutritional value and quality of the final products (e.g., goat milk yogurt, red jujube yogurt, etc.) (Feng et al., 2019b; Krška et al., 2009; Raswan, 2020; Wojdyło et al., 2016).

In today's society, the consumption preferences are varied and deeply changed comparative with the past; consumers are no longer categorized only about income, lifestyle and demographic variables (Popa et al., 2017; Farruggia et al, 2016). Now, consumer preference is influenced also by intrinsic quality attributes discovered before (colour, taste, flavour, and texture) (Sulistyawati et al., 2020). This perception is used to assess other more quality attributes of the product, such as health (nutritional value) and to determine the overall quality of a food product (Asioli et al., 2017; Sulistyawati et al, 2020). Besides product sensory and health properties, a key role in consumers' food choice is played by product familiarity and health consciousness, including for fresh fruit (Pollard et al., 2002) and dried fruits (Asioli et al., 2019). Moreover, healthiness of the product is another reason in consumers' food choice (Grunert, 2013; Milošević et al., 2012; Januszewska et al, 2011) and is often associated with flavour, taste, colour, texture and natural content characteristics (free of additives and with natural ingredients) (Chambers et al., 2018; Puska & Luomala, 2016; Stan et al., 2020).

Shin et al. (1992) investigated various processing methods for *Ziziphus jujuba* fruits. Based on sensory evaluation and chemical analysis it was found that dried fruits, nectar, jam, fruit extracts and a powdered tea were the most promising products.

The aim of this study is to evaluate different products of *Ziziphus jujuba* Mill. accepted by consumers and how they characterize the final product.

MATERIALS AND METHODS

The products with jujube (**fresh fruit**: Jun Zao, Hu Ping Zao, Hu Ping AA and Early Hu Ping Zao; **dehydrated**: simple, with walnuts or almonds; **tart**; **compote** and **liqueur**: with honey, with rose petals, with plums and honey, with plums and rose petals) were evaluated by consumers, represented by 60% women and 40% men, with ages between 22 and 53 years old.

From of all the jujube varieties grown in our country, only the ripe ones (at that moment), were tested.

The fruits were harvested, sorted and washed (Figure 1).

Figure 1. Fresh jujube

Dehydrated jujube

Drying was removing moisture from jujube fruit. The fresh and fully ripened jujube fruits were selected.

Any fruits that showed mould decay, or bruises were sorted and discard out. Such defects could affect all foods being dried. Then they were dried at 45°C in dehydrator.

The length of time needed to dry fruits would depend on the size of the pieces being dried, humidity and the amount of air circulation in the dehydrator.

At a drying temperature of 45°C, 40 to 48 h were planned for jujube fruits.

Figure 2. Dehydrated jujube (simple, with walnuts or almonds)

Jujube tart

For jujube tart were used the same ingredients as for traditional apple tart, such as: flour, eggs, milk, butter, salt, baking powder and jujube slices. All the ingredients were put in the oven and seasoned with powdered sugar.

Jujube compote

Fruits were cleaned and the unwanted parts of the fruit were discarded. The natural sugar in jujube should be calculated. When the fruits had low natural sugar more sugar must be added to the solution. The glass was filled with fruits, 1 spoon of sugar, water and for flavour were added cloves. All the ingredients were mixed together and then it was sterilized in water bath at 85°C for 15 min.

The jujube tart is presented in Figure 3 and the jujube compote is presented in Figure 4.

Figure 3. Jujube tart

Figure 4. Jujube compote

Jujube liqueur

For liqueur was used fruits, honey, alcohol with 96% strength and water. The fruits (jujube and plums) were washed, putted in a 2000 ml jar, over which was added honey and alcohol. For flavour were added rose petals. After few days was added water, until 32 vol. %.

The jujube liqueur is presented in Figure 5.

Figure 5. Jujube liqueur

RESULTS AND DISCUSSIONS

All the products were prepared at the Research Center for Studies of Food Quality and Agricultural Products, in the Integrated Fruit Growing Laboratory. The products were evaluated by consumers with ages between 22 and 53 years old (Figure 6).

The fruit size and shape, fruit colour, firmness and juiciness, taste and flavour were noticed with grades from 1 to 5, five being the highest value. For the fresh fruit, **Jun Zao** was the most appreciate. **Hu Ping AA** for simple dehydrated jujube and **Hu Ping Zao** for dehydrated fruit with nuts.

All results can be found in the following figures.

Figure 6. Workshop “Prospective of Chinese jujube (*Ziziphus jujuba* Mill.) cultivation in Romania”

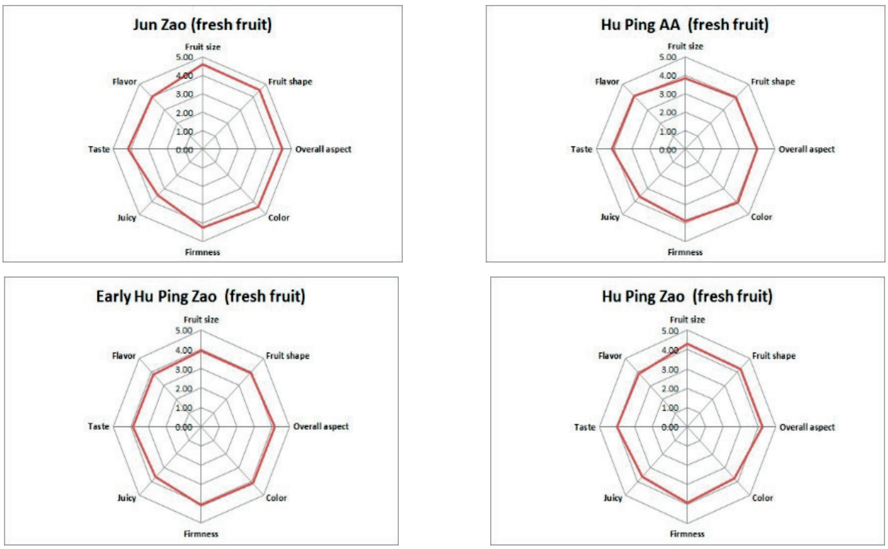


Figure 7. Consumer acceptability scores on a 5-point Hedonic scale for jujube fresh fruit

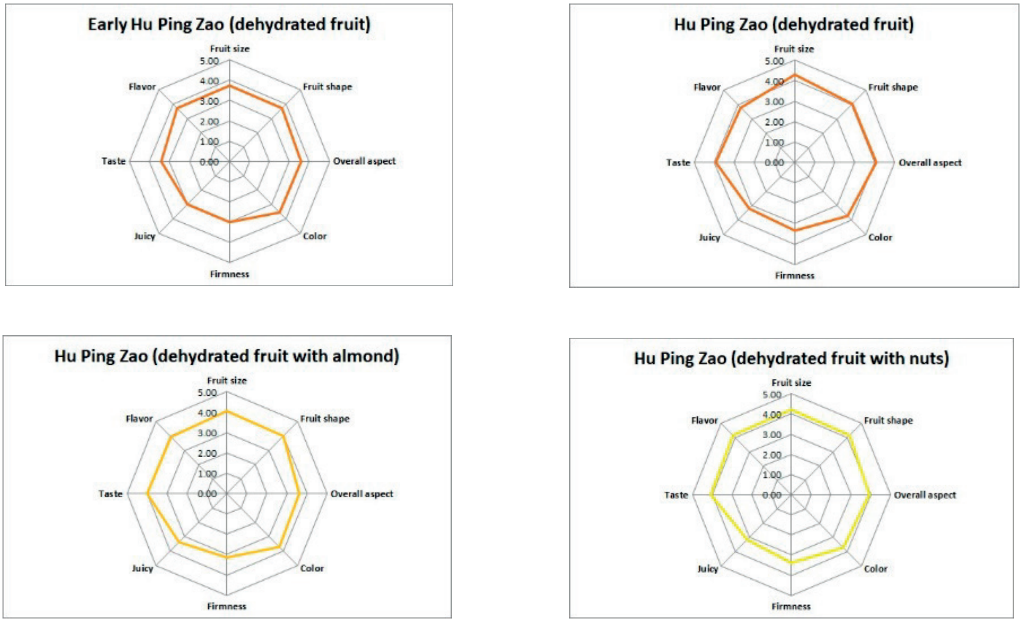


Figure 8. Consumer acceptability scores on a 5-point Hedonic scale for dehydrated jujube

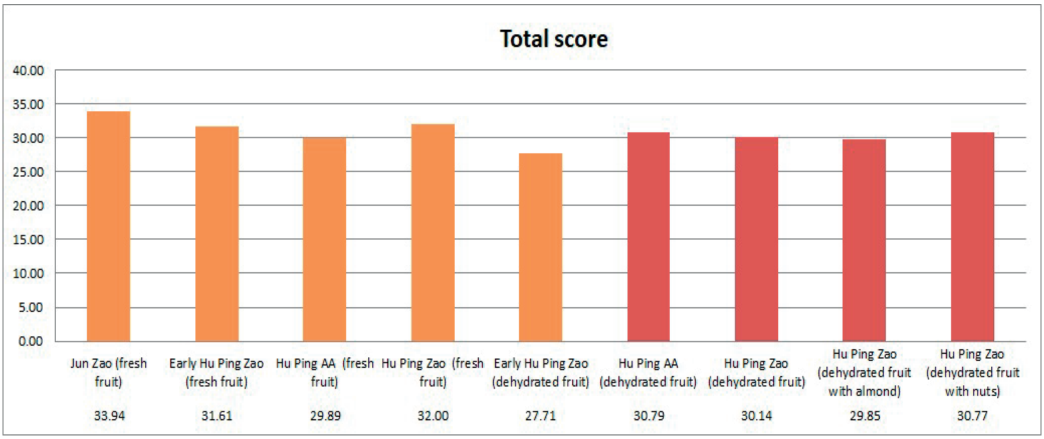
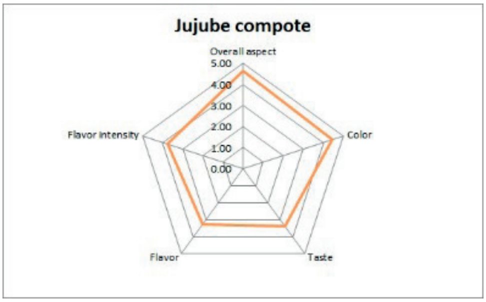


Figure 9. Total score of fresh and dehydrated fruits

The compote was very well appreciated for its overall aspect as for its color, only that its clove aroma was not very much appreciated. Jujube tart was appreciated very well for her overall aspect and for its taste and flavour. Jujube liqueur with plums and rose petals (for colour and flavor intensity) and jujube liquer with plums and honey (for taste and flavor) was the most appreciate from four variants. The results obtained in the present study suggest that sensorial analysis plays an active part in choosing the best variants.



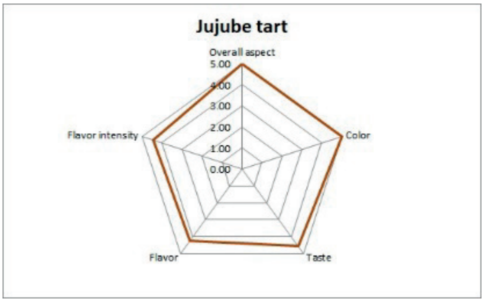
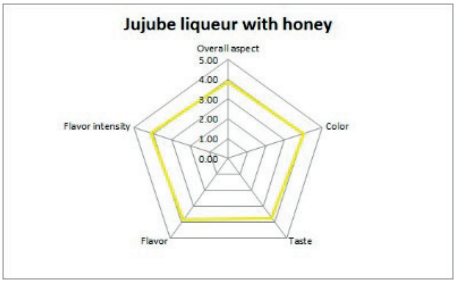
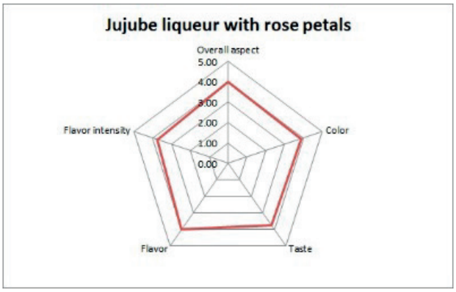
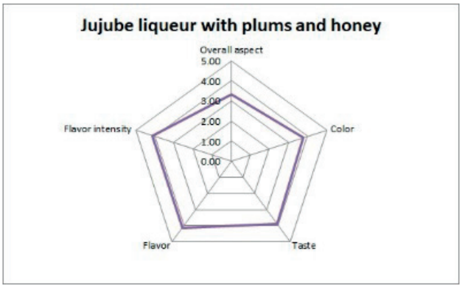


Figure 10. Consumer acceptability scores on a 5-point Hedonic scale for jujube compote

Figure 11. Consumer acceptability scores on a 5-point Hedonic scale for jujube tart







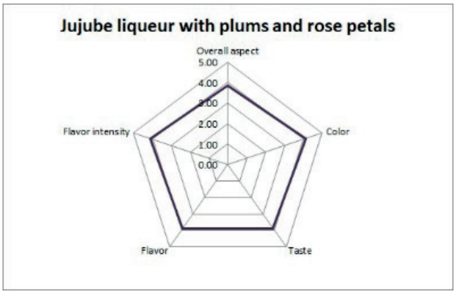


Figure 12. Consumer acceptability scores on a 5-point Hedonic scale for jujube liqueur

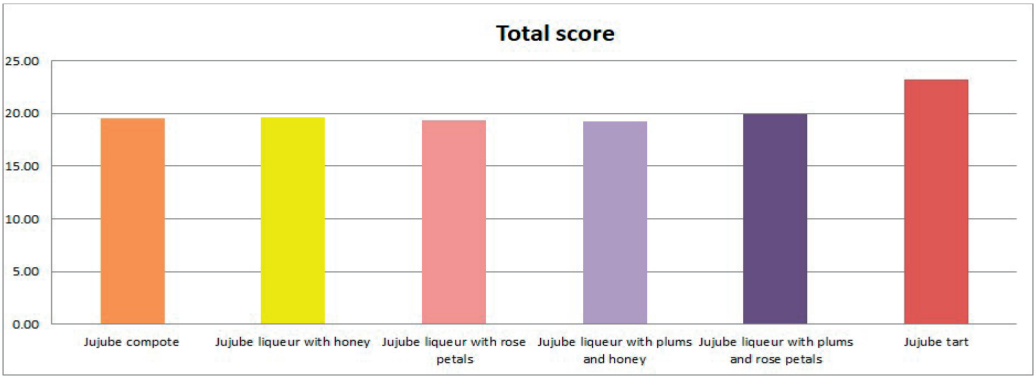


Figure 13. Total score of processed products of jujube fruits

CONCLUSIONS

The consumers' panel for sensory evaluation was represented by farmers, researchers, teachers, specialists and ordinary people.

The results showed that, the consumers perception varied with the age, gender and origin. Fresh jujube fruits and processed jujube products, were positively appreciated by most of the participants.

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ADVANCED RESEARCH ON THE DEHYDRATION OF THE BLACK CHOKEBERRIES (*Aronia melanocarpa* Linn.)

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Abstract

Five varieties of black chokeberries were analysed at fresh, intermediate (after the osmotic treatment) and dried stages, (after dehydration) comparing their soluble solids, total sugar, titratable acidity, vitamin C, phenols and anthocyanins content. The study aims to find various correlations between the osmotic treatment and the sensory & nutraceutical qualities of fruits and time & energy savings, as well. The hypertonic solutions were represented by a natural concentrated fruits juice in the ratio 1:2, 1:3 and 1:7 and by an inverted sugar syrup in the same ratios. Uncrushed berries were used, at the temperature of 50° C into syrup immersion and convective dehydration, subsequently.

Key words: *Aronia, bio-compounds, hypertonic solution, osmotic treatment, weight reduction.*

INTRODUCTION

Aronia melanocarpa Linn., known as black chokeberry belongs to the *Rosaceae* family which originates from the eastern parts of North America and East Canada (Kulling and Rawel, 2008). Aronia has been traditionally used in Native American medicine (Erichsen-Brown, 1989) by the Forest Potawatomi people for the treatment of colds and preparation of nutritious and lasting foodstuff, while berries and the bark as an astringent (Smith, 1933). The black chokeberries are used for juice, jam and wine production and are a source of natural pigments (Bridle and Timberlake, 1997). Chokeberries are rich in nutritious ingredients, including dietary fibres, organic acids, sugar, fat, proteins, minerals and vitamins (Juranovic et al., 2017). The chokeberry’s polyphenols content is higher than the content of other berries (e.g., blueberry, cranberry, etc.), which exhibits various physio-logical activities, such as antioxidant, anti-inflammatory, anti-diabetic, anti-cardiovascular diseases and so on (Banjari et al., 2017; Cebova et al., 2017; Turcan and Todiras, 2003; Zhu et al., 2020). One quarter to one-third of the fruits and vegetable production goes waste due to the lack of proper retailing and adequate storage capacity. Preserving food to extend its shelf-life, is a central preoccupation of the food industry and on this matter, the real challenge starts immediately after production. Nowadays, a renewed interest exists in the use of more traditional preservation methods and the ways they can be combined with newer technologies. Food preservation is the process of treating food to stop or slow down its spoilage caused or accelerated by the activity of micro-organisms (fungi, bacteria, etc.). The preservation retards cell oxidation and inhibits the natural ageing and discoloration that can occur during food preparation, such as the enzymatic browning reaction (Yadav and Singh, 2014) observed after cutting different kinds of fruits and vegetables.

Widely spread methods used for food-materials preservation are: drying (convective dehydration), freezing, freeze-drying, spray drying, vacuum-packing, canning, syrup preservation (osmotic dehydration), sugar crystallization, food irradiation, adding preservatives or inert gases (e.g., carbon dioxide) etc., while others are modern methods, such as the ultrasonic vacuum pre-treatment silver nano-particles treatment or pulsed electric field assisted osmotic dehydration. A successful application in the preservation of fruits is represented by osmotic dehydration (O.D.). This method is used from ancient times till nowadays when the new technologies transformed it into an innovative method. O.D. is a method of preservation that involves partial moisture loss from food-materials to improve their shelf-life.

It is done by immersing food materials either in whole or units into a hypertonic solution which allows diffusion of solutes from the food material into the solution concurrently with solid impreg-nation into the food material (Ciurzynska et al., 2016).

As Tiwari (2005) has mentioned, osmotic dehydration is the phenomenon of water removal from a lower concentration of solute to a higher concentration through a semi-permeable membrane, resulting in the equilibrium condition on both sides of the membrane.

In osmotic dehydration, the basic hypertonic solutions used are generally sugar syrup with fruits and sodium chloride or brine with vegetables (Bonatsou et al., 2016). In the osmotic process, the water flows out from fruits or vegetables tissues to the solution and along with water some components of fruits and vegetables (minerals, vitamins, fruit acids etc.), also, they move towards the solution, while the sugar/ salt migrates towards the fruits and vegetables (Fernandes et al., 2016).

The osmotic dehydration method is preferred to others due to their vitamins & minerals, organic acids, colour (pigments) retention properties (Ahmed et al., 2016; Osorio et. al., 2007). The O.D. treatment saves energy and prevents oxidative browning (Chandral and Kumari, 2015).

The osmotic dehydration can be an independent or pre-treatment process improving functional and nutritional properties and it can precede processes such as freezing, deep-frying, pasteurization, air-drying, vacuum drying, microwave drying, or freeze (Masztalerz et al. 2020).

Mass transfer and kinetic parameters play major roles in determining the extent of preservation, water loss, solid impregnation, as well as during O.D. as functions of immersion time, solution concentrations and temperatures antioxidant activity status of food-materials (Azeez et al., 2019).

MATERIALS AND METHODS

In this study, 5 varieties of *Aronia melanocarpa* have been used: *Galitianka, Hugin, Merlom, Nero* and *Viking*. The berries of the *Merlom* variety have been supplied by the Institute of Research-Development for Fruit Trees Cultivation, Pitesti - Maracineni, Arges County and they were organically cultivated (without chemical pesticides & fertilizers), following with strictness all rules and regulations of Organic Farming in the U.E. *Galitianka* and *Viking* chokeberry varieties have been provided by a private farm, located in Dorohoi, Botosani County, while *Hugin* and *Nero* varieties of *Aronia* sp. have been cultivated at the Institute of Research-Development for Processing and Marketing of Horticultural Products-"Horting", Bucharest.

*Aroni*a fruits were harvested in September 2020 and stored in a refrigerator at 5°C until processing. Each variety has been supposed to be a specific experiment in which the main variables were the compounds of the osmotic solute and the ratio. In the case of two varieties (*Nero* and *Hugin*) was intentionally extended the immersion time into the syrup, in order to record the possible changes in the chokeberries' tissue.

The experiment has followed 2 directions:

- to compare the processes appearing in the *Aronia* fruits in case of osmotic dehydration (O.D.) to the convective one;
- to establish the composition of hypertonic solution, ratio and O.D. time for obtaining the most valuable results, in the sense of sensory & nutraceutical qualities of *Aronia* berries and time & energy savings.

In this regard, the fruits were divided into 2 samples, for each variety:

- the berries are osmotically treated before the convective dehydration;
- the berries are convectively dehydrated, without osmotic dehydration.

Before the osmotic treatment, the chokeberries were sorted, washed and softly blotted for removing water drops. Below is a detailed of how the experiment has been prepared for each variety of *Aronia melanocarpa:*

1. *Merlom* (organic berries)

a) Immersion into concentrated natural fruit juice, ratio 1:2 (one-part berries to two-parts

hypertonic solution). Chokeberries' immersion time into the juice was 5 hours at 50°C (inside dryer) and 15 hours at room temperature (T).
b) Immersion into inverted sugar syrup, ratio 1:2 for 5 hours (inside dryer) at 50°C and 15 hours at room T.
c) Immersion into inverted sugar syrup, ratio 1:7 for 5 hours at 50°C and 15 hours at room T.
2. ***Galitianka***
a) Immersion into inverted sugar syrup, ratio 1:2 for 5 hours at 50°C and 15 hours at room T.
b) Immersion into inverted sugar syrup, ratio 1:3 for 5 hours at 50°C and 15 hours at room T.
3. ***Viking***
a) Immersion into inverted sugar syrup, ratio 1:2 for 5 hours at 50°C and 15 hours at room T.
b) Immersion into inverted sugar syrup, ratio 1:3 for 5 hours at 50°C and 15 hours at room T.
4. ***Hugin***: immersion into concentrated natural fruit juice, ratio 1:3.
The immersion time of the berries into the juice was 8 hours at 50°C and 15 hours at room T.
5. ***Nero***: immersion into concentrated natural fruit juice, ratio 1:7.
The immersion time of chokeberries into the juice was 8 hours at 50° and 15 hours at room T.
The compounds of the hypertonic solution for the osmotic dehydration were:
1. The natural concentrated fruit juice contains: concentrated sour cherry juice (61°BRIX) concentrated apple juice (68,1°BRIX) and organic inulin of agaves (Fig. 1). The concentration of the osmotic agents and the osmotic solution was monitored by an Abbe refractometer (Zeiss JENA, Germany). The osmotic solution was prepared in the concentration: of 60% concentrated sour cherry juice + 20% concentrated apple juice + 20% organic inulin of agaves.

Figure 1. Concentrated sour cherry & apple juice and organic inulin of agaves

2. The inverted sugar syrup contains: sugar, lemon juice and plain water. The syrup used for treating the organic chokeberries (those of *Merlom* variety) has contained only organic ingredients. The syrup was prepared by boiling 10 minutes 1 kg of sugar with 500 ml of plain water adding the juice of one lemon. The black chokeberries were immersed into the syrup when their temperature reached 50°C. The biochemical and physical analyses of *Aronia* berries at their initial stage (fresh fruits), intermediate phase (after osmotic dehydration) and final stage (dried fruits, after convective dehydration) were conducted at the Institute of Research - Development for the Processing and Marketing of Horticultural Products -"Horting".
Total soluble solids (TSS) were determined by refractometric method, according to the STAS 5956-71 for vegetable and fruit products. Titratable acidity (TA) was determined according to the STAS 5952-79 for vegetable and fruit products regarding the determination of titratable and volatile acidity. Total sugar (TS), expressed in inverted sugar (%) was determined by the Bertrand method (Heinze and Murneek, 1940). Vitamin C was determined by spectro-photometric method, using 2,6-dichlorophenol, indophenol after xylene extraction for obtaining the amount of ascorbic acid existing in vegetable and fruit products, according to the STAS SR ISO 6557-2. Total anthocyanin content (TAC) was determined using the differential pH method (Lee at al., 2009). Total phenol content (TCC) was determined using Folin-Ciocalteu method (Johansen, 1940). The research on *Aronia* berries' behaviour to different dehy-dration methods was conducted using only uncrushed (entire) fruits. Drying uncrushed fruit maintains a higher level of antioxidant bioactive components (especially anthocyanins), than drying the crushed fruits (Oszmianski and Lachowicz, 2016). To the values obtained at the above determinations, the confidence interval (95%) and the standard error were calculated.
The chokeberries were dipped for 5 hours, and 8 hours, respectively (only *Nero* and *Hugin* varieties), into the osmotic solution of the above concentration at the temperature of 50° C and then other 15 hours at room temperature for all varieties. From time to time, the fruits were softly agitated. The osmotic dehydration is faster in fruits when syrup is circulated (Hawkes and Flink, 1978). It is important to

use an optimum ratio of solution to sample for the economic considerations and not only. In general, as the solution to sample ratio increased, the osmosis rate increased up to a certain level and then its level decreased. Chaudhari et al., (1996) and Ispir & Togrul (2009) concluded that water loss and solid gain were dependent on temperature and osmotic solution concentration.

The reported temperature limit is 60° C (Petkovic et al., 2019). Le Maguer (1988) and Ponting (1973) have however reported enzymatic browning and flavour deterioration above 49° C. In the osmotic dehydration, a simultaneous flux of water and solutes from and into the material takes place. This method can be used as a pre-treatment before air drying to reduce from 30% to 70% of the water content of the food (Ruskova et al., 2016).

Osmotic dehydration preceding air-drying decreases colour changes and increases flavour retention in dried fruits (Lenart and Lewicki, 1988).

After the osmotic treatment, the berries were quickly rinsed and dried with an absorbent paper. Then, the fruits were weighted for calculating their weight reduction after the osmotic treatment and spread on the trays of the dryer (Figure 2).

Figure 2. *Aronia* berries on dryer's trays

The *Aronia*'s berries osmotically treated have been ground at the intermediate stage (after O.D.) using a Heinner grinder (http://www.heinner/ro/uploads/support/2f4b5-User-manual - HCG 200DGIX2.pdf). The black chokeberries from all varieties have been dried by a convective method in a professional dryer (B. Master model SR 18381, 2018, made in Italy, the Company Tauro Essicatori SRL) at the temperature of 50°C. B. Master dryer is made of stainless steel with 4 compartments, heating and ventilation unit with electric resistance, three-phase electric, automatic ventilation module and non-stick paper "Dry silk" (Figures 3 and 4).

The dryer is furnished with 40 trays which provide a drying surface area of 14 m².

The characteristics of B. Master dryer: maximum capacity -160 kg; average daily output; 40/60 kg; external dimensions - 192Wx140Hx82D cm; weight when empty - 190 kg; heating power levels available -3.4,5.1 and 7.5 kW; electric fan -400 mm; electric fan power -60 W; temperature control - electronic with LED display; moist air discharge control-slatted shutter; supply-220/ 400 V-50 Hz (https://www.tauro-dryers.com/en/b-master-line/b-master).

Figure 3. B Master professional dryer in order

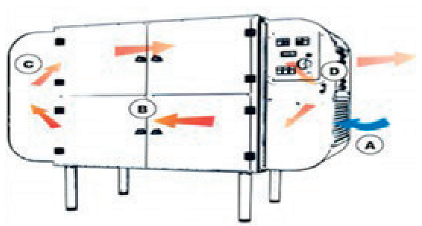
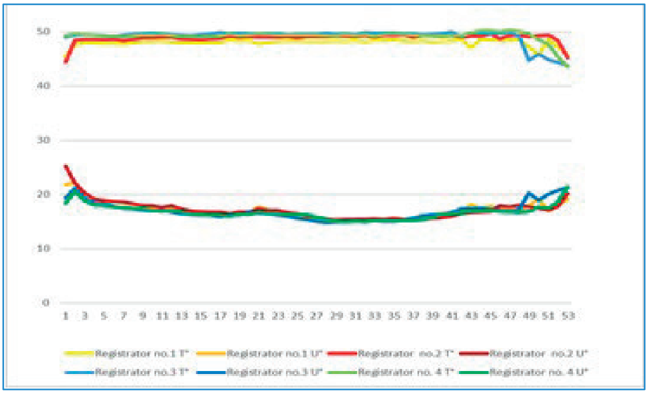


Figure 4. Air distribution inside B. Master dryer:
A - air entrance area; B - lower drying level
(2 compartments); C - upper drying level
(2 compartments); D - humidity exhausting area

RESULTS AND DISCUSSIONS

In the Figure 5 appears the variations of temperature (T) and humidity (U) levels, depending on the dehydration phase and recorders' place inside the dryer. In the first 3 hours of dehydration, although the dryer was set up at the working temperature of 50° C, the recorders have noticed a lower temperature and higher humidity, due to the changes happening inside berries' tissues and due to the changes of T & U between fruits and air dryer. At the end of the dehydration, when the dryer was set off, the T & U have turned to their initial values.

The curves of the chart show very slight differences in temperature & humidity inside dryer, as since the recorders have been placed near equidistantly on the trays. Airflow inside the dryer provides uniform temperature & humidity. The main indicators of fresh, osmotically treated (intermediate stage) and dried chokeberries (final stage) were analysed, as are represented in the Tables 1-3.



Recorders distribution inside dryer:
no.1- right side, bottom- upper level;
no.2 - left side, middle -upper level;
no.3 - right side, bottom - lower level;
no.4- left side, top - lower level.

Figure 5. Variations of temperature and humiditsy during dehydration

Of all fresh chokeberries (Table 1), the highest soluble solids content was represented by *Viking* (26.9°Brix), followed by *Merlom* (25.6°Brix) and the lowest content was obtained by *Nero* (12.2°Brix). The highest sugar amount was obtained by *Viking* (9.25%), followed by *Galitianka* (7.27%), whisle *Nero* obtained the lowest amount (4.09%). As regards the titratable acidity, the highest level was found at *Hugin* (1.04%), followed by *Merlom* (0.92%), while *Nero* obtained the lowest acidity (0.61%).

The best amount of vitamin C was found at *Hugin* (44.02 mg/100g), followed by *Nero* (39.02 mg/100g), while *Merlom* obtained the lowest amount of vitamin C (24.64 mg/100g). Concerning the anthocyanins, the highest content was represented by *Merlom* (454.41 mg cyd,3-glu/100g), followed by *Galitianka* (437.24 mg cyd,3-glu/100g) and the lowest content was obtained by *Nero* (220.87 mg cyd,3-glu/100g).

The highest content of phenol content was found at *Merlom* (1217.18 mg GAE/100g), followed by *Galitianka* (746.77 mg GAE/100g), while *Nero* has obtained the lowest content of total phenolics (342.38 mg GAE/100g).

Table 1. The main indicators of fresh Aronia berries

Variety	Total soluble solids (°BRIX)	Total sugar (inverted sugar %)	Titratable acidity (g malic acid/100 g)	Vitamin C (mg ascorbic acid/100 g)	Total anthocyanin content (mg cyd. 3-glu/100 g)	Total phenol content (mg GAE equiv./100 g)
Galitianka	23.00 - **A**	7.27 - **A**	0.66 - **A**	37.61 - **A**	437.24 - **A**	746.77 - **A**
Hugin	20.20 - **A**	6.06 - **A**	1.04 - **B**	44.02 - **A**	322.82 - **A**	636.88 - **A**
Merlom	25.60 - **A**	7.60 - **A**	0.92 - **A**	24.64 - **A**	454.41 - **A**	1217.18 - **A**
Nero	12.20 - **B**	4.09 - **B**	0.61 - **A**	39.02 - **A**	220.87 - **A**	342.38 - **A**
Viking	26.90 - **A**	9.25 - **B**	0.77 - **A**	36.95 - **A**	342.68 - **A**	675.65 - **A**
Average ($\bar{x}$)	**21.58**	**6.85**	**0.80**	**36.45**	**355.6**	**723.77**
Std. error	**2.61**	**0.86**	**0.08**	**3.93**	**37.32**	**141.30**

Legend: **A-** statistically no significant difference: **B** - statistically significant difference

After the osmotic treatment (Table 2), using as a hypertonic solution the inverted sugar syrup, the highest soluble solids value was found at *Viking* (34.1°Brix, ratio 1:3), followed by *Galitianka* (34.0° Brix, ratio 1:3). The highest sugar amount was obtained by *Galitianka* (12.38 %, ratio 1:3), followed by *Merlom* (11.83 %, ratio 1:7).

The best amount of vitamin C was gained in the berries of *Galitianka* (63.60 mg/100 g, ratio 1:3 and 62.21 mg/100 g, ratio 1:2), followed by *Viking* (53.71 mg/100 g, ratio 1:3 and 51.75 mg/100g, ratio 1:2).

The highest content of anthocyanins was represented by *Merlom* (514 mg cyd, 3-glu/100 g, ratio 1:7 and 508.98 mg cyd, 3-glu/100 g, ratio 1:2), followed by *Galitianka* (505.58 mg cyd, 3-glu/100 g, ratio 1:3 and 492.20 mg cyd, 3-glu/100 g, ratio 1:2).

The highest phenolic content was gained in the *Merlom* berries (1745.69 mg GAE/100 g, ratio 1:7 and 1700.65 mg GAE/100 g, ratio 1:2), followed by *Viking* berries (982.38 mg GAE/100 g, ratio 1:3 and 971.3 mg/100 g GAE, ratio 1:2).

In the case of fruit juice hypertonic solution, the highest soluble solids value was reached by *Merlom* (28.2° Brix, ratio 1:2), followed by *Nero* (26.2° Brix, ratio 1:7). The highest sugar amount was obtained by *Merlom* (9.21 %, ratio 1:2), followed by *Hugin* (8.29 %, ratio 1:3). Relating the vitamin C amount, the highest content was reached by *Hugin* (60.43 mg/100 g, ratio 1:3), followed by *Nero* (43.12 mg/100 g, ratio 1:7). The highest content of anthocyanins was got by *Merlom* (519.48 mg cyd, 3-glu/100 g, ratio 1:2), followed by *Hugin* (308.71 mg cyd, 3-glu/100 g, ratio 1:3). The highest polyphenolic content was obtained by *Merlom* (1686.18 mg GAE /100 g, ratio 1:2), followed by *Hugin* (772.98 mg GAE/100 g, ratio 1:3).

Table 2. The main indicators of Aronia berries osmotically treated (intermediate stage) in 2020

Variety	**O.D. treatment**		**Total soluble solids (ºBRIX)**	**Total sugar (inverted sugar %)**	**Vitamin C (mg ascorbic acid/100 g)**	**Total antho-cyanin content (mg cyd. 3-glu/100 g)**	**Total phenol content (mg GAE equiv./100 g)**
Galitianka	sugar syrup	ratio 1:2	33.20 - **A**	9.28 - **A**	62.21 - **B**	492.20 - **A**	865.30 - **A**
		ratio 1:3	34.00 - **B**	12.38 - **B**	63.60 - **B**	505.58 - **A**	895.50 - **A**
Hugin	fruit juice	ratio 1:3	25.30 - **B**	8.29 - **B**	60.43 - **B**	308.71 - **B**	772.98 - **A**
Merlom	fruit juice	ratio 1:2	28.20 - **A**	9.21 - **A**	39.15 - **B**	519.48 - **B**	1686.18 - **B**
	sugar syrup	ratio 1:2	31.00 - **A**	11.24 - **B**	38.73 - **B**	508.98 - **A**	1700.65 - **B**
		ratio 1:7	32.00 - **A**	11.83 - **B**	39.20 - **B**	514.26 - **B**	1745.69 - **B**
Nero	fruit juice	ratio 1:7	26.20 - **B**	7.24 - **B**	55.12 - **A**	215.70 - **B**	500.42 - **B**
Viking	sugar syrup	ratio 1:2	33.50 - **B**	9.73 - **A**	51.75 - **A**	403.27 - **A**	971.30 - **A**
		ratio 1:3	34.10 - **B**	10.22 - **A**	53.71 - **A**	407.58 - **A**	982.38 - **A**
Average ($\bar{x}$)			**30.72**	**9.94**	**50.21**	**430.64**	**1124.49**
Std. error			**1.14**	**0.56**	**3.39**	**35.90**	**154.09**

Legend: **A**- statistically no significant difference: **B** - statistically significant difference

After the osmotic dehydration, specific changes in the black chokeberry indicators appeared. Thus, near to all *Aronia melanocarpa* varieties, the values of the main indicators have increased, while others have slightly decreased (Table 3).

As the values appear in the Tables 2 and 3, the ratio of 1:7, followed by 1:3 has determined the highest levels of soluble solids, sugar, vitamin C, anthocyanins and phenols intake.

In the case of the berries maintained 8 h into the hypertonic solution (*Nero* and *Hugin* varieties), a higher rate of anthocyanins removal was noticed. Anthocyanins are very sensitive to temperature; light and immersion time and they have weak stability. A longer time of osmotic dehydration decreases the quantity and quality of fruit pigments (Landim et al, 2016). The process of osmotic dehydration leads to the stransfer of anthocyanin pigment and flavouring constituents to the osmotic solution. Due to their rich quantity of pigments and volatile consti-tuents, the osmotic solutions can be used as raw materials for developing natural additives (Osorio et al., 2007). In order to obtain accurate results, one variety of *Aronia* (*Nero*) was analysed in 2019 and 2020 (Table 4). The berries were organized both years into 2 experimental samples, following 2 directions of the research. The first sample was represented by the fruits osmotically treated and the second by the fruits without osmotic treatment. The berries of both samples were dehydrated convectively using B. Master professional dryer. Between the study of 2019 (Toma Singh et al., 2019) and that of 2020 at *Nero* variety existed few variables:

- <u>different place of cultivation:</u> in 2019 the *Aronia* berries of *Nero* variety were supplied by the Institute of Research - Development for Fruit Trees Cultivation, Pitesti - Maracineni, Arges County, while in 2020 the black chokeberries have been cultivated at the Institute of Research - Development for Processing and Marketing of Horticultural Products - "Horting", Bucharest.
- <u>fruit integrity</u>: in 2019 the fruits osmotically treated were crushed (pricking the berries - Figure 6 of the first sample and partially sectioning the berries of the second sample - Figure 7), while in 2020 for the osmotic dehydration uncrushed (entire) fruits have been used (Figure 8). In the case of the black chokeberries convectively dehydrated, without osmotic treatment, in 2019 and 2020 only uncrushed fruits were used.
- <u>osmotic dehydration time</u>: in 2019 the *Aronia* berries have been immersed for 4 hours into the concentrated natural juice at 50°C, while in 2020 the chokeberries have been immersed for 8 hours at 50°C and 15 hours at room temperature before their convective dehy-dration. The extension of fruit immersion time into the natural juice aimed to determine potential improvements as regard the weight reduction & shorter time of dehydration and higher values of sensory & nutraceutical quality of the fruits.

Figure 6. Pricking Chokeberries

Figure 7. Sectioning chokeberries

Figure 8. Uncrushed chokeberries in natural juice with inulin

Table 3. The modifications of main indicators of *Aronia* berries after osmotic dehydration in 2020

Variety	O.D.-Treatment		Total soluble solids (ºBRIX)	Total sugar (inverted sugar %)	Vitamin C (mg ascorbic acid/100 g)	Total anthocyanin content (mg cyd, 3-glu/100 g)	Total phenol content (mg GAE equiv. /100 g)
Galitianka	sugar	ratio 1:2	*↑ 44.35 % - **A**	↑ 27.65 % - **A**	↑ 65.40 % - **A**	↑12.57 % - **A**	↑15.87 % - **A**
	syrup	ratio 1:3	↑ 47.83 % - **A**	↑ 70.29 % - **A**	↑ 69.10 % - **A**	↑15.63 % - **A**	↑19.92 % - **A**
Hugin	fruit juice	ratio 1:3	↑ 25.24 % - **A**	↑ 36.80 % - **A**	↑ 37.28 % - **A**	*↓4.37% - **A**	↑ 21.37 % - **A**
Merlom	fruit juice	ratio 1:2	↑ 10.16 % - **A**	↑ 21.18 % - **A**	↑ 58.88 % - **A**	↑14.32% - **A**	↑ 38.52% - **A**
	sugar	ratio 1:2	↑ 21.09 % - **A**	↑ 47.89 % - **A**	↑ 57.18 % - **A**	↑12. 01 % - **A**	↑ 39.71% - **A**
	syrup	ratio 1:7	↑ 25.00 % - **A**	↑ 55.66 % - **A**	↑ 59.1 % - **A**	↑13.17% - **A**	↑ 43.41% - **B**
Nero	fruit juice	ratio 1:7	↑ 114.75 % - **B**	↑ 77.01 % - **B**	↑ 41.26 % - **A**	↓ 2.34 % - **B**	↑ 46.16% - **B**
Viking	sugar	ratio 1:2	↑ 24.54 % - **A**	↑ 5.19 % - **A**	↑ 40.05 % - **A**	↑ 17.68 % - **A**	↑ 45.40 % - **B**
	syrup	ratio 1:3	↑ 26.77 % - **A**	↑ 10.49 % - **A**	↑ 45.36 % - **A**	↑ 18.94 % - **A**	↑ 43.76 % - **B**
O.D. Fruits Average Gain ($\bar{x}$)			**↑ 37.75%**	**↑ 39.13**	**↑ 52.62 %**	**↑ 9.51 %**	**↑ 34.90 %**
Std. Error			**8.82%**	**10.84%**	**7.14%**	**2.66%**	**s 5.12%**

Legend: *↑ - increase; ↓ - decrease of initial values (fresh berries)
A - statistically no significant difference: **B** - statistically significant difference

Table 4. Comparison between the results obtained at *Nero* variety in 2019 and 2020

Year	STAGE	Total soluble solids (BRIX °)	Total sugar (inverted sugar %)	Vitamin C (mg ascorbic acid/100 g)	Total phenol content (mg GAE equiv./100 g)
2019	initial (fresh)	18.00	26.17	33.57	1000.10
	after O.D. 1:2 - pricked berries	29.04	41.47	38.80	1420.20
	after O.D. 1:3 - sectioned berries	28.01	37.77	36.94	1306.40
2020	initial (fresh)	12.20	4.09	39.02	342.38
	after O.D. 1:7 - entire berries	26.20	7.24	55.12	500.42

As the results obtained at *Nero*, an interesting aspect is connected to the fruit integrity. Better values were obtained at the uncrushed chokeberries. Between the crushed chokeberries, the pricked berries obtained higher values than those partially sectioned. It means that the mass and solutes kinetics are favourited by the maintenance of fruit integrity. The cell membrane allows a more efficient transfer from and into berry and osmotic solution in case the fruit integrity is not affected or less affected. Since there are more benefits of uncrushed berries, it conducts to time saving & lighter labour involvement. In the study of 2020, to all *Aronia melanocarpa* varieties, after 50 h of convective dehydration, the humidity level and weight reduction gain varied on the variety, type of osmotic solution, ratio and immersion time, as is shown in Tables 5 and 6 and Figures 10 and 11. In the Table 5, the highest weight reduction gain of dried chokeberries was obtained by the osmotically treated berries of all five cultivars. Among them, the berries immersed in the osmotic solution, the ratio of 1:7, followed by the ratio of 1:3 offered the best results, for all cultivars of *Aronia melanocarpa* found in this study. The berries immersion time at 50°C has shown that the weight reduction gain increased with extension time into the osmotic solution: the berries maintained 8 h into the hypertonic solution have obtained the best results. As the values show, it's proven that, as regards the humidity level and weight reduction gain of the dried berries, the greatest results were given by the immersion time in the osmotic solution (8 hours of berries immersion have offered a higher weight reduction gain than 5 hours of immersion). Considering the berries time immersion into the osmotic solution and the ratio, the humidity level and the weight reduction gain were mainly determined by the immersion time. Thus, the comparison between *Nero* and *Merlom* varieties shows that after 8 hours of berries immersion into fruit juice despite 5 hours of immersion in the same kind of solution, at the same ratio (1:7), has clearly pointed out a decrease of 12.52% in case of humidity and an increase of 12.71% in case of weight reduction gain. As regards, the type of hypertonic solution, *Galitianka* and *Viking* has been used only inverted sugar syrup, at Hugin and *Nero* only fruit juice, while at *Melrom* both types of hypertonic solution, inverted sugar syrup, respectively fruit juice. The *Merlom* chokeberries have been immersed for 5 hours at the ratio of 1:2. The difference consisted only in the type of hypertonic solution. Watching the results becomes easy to understand that the berries immersed in the inverted sugar syrup have got a level of humidity slightly reduced (with 0.19%) and a weight reduction gain slightly increased (with 1.59%). In the Table 6, the average humidity value of the dried chokeberries osmotically treated is near half of the average humidity value found in the dried chokeberries which were not osmotically treated_before the convective dehydration. The result is according to the information provided by other researchers. They have mentioned in their studies that the osmotic treatment applied to different fruits and vegetables substantially decreased the time of convective dehydration. This aspect has a direct and very important effect on energy saving and on the organoleptic and nutraceutical properties of fruits and vegetables. Khan (2012) considers that the osmotic dehydration process prior to drying reduces dehydration time, and retains many nutrients and organoleptic attributes, providing important characteristics to the finished products. So, the minimally processed fruits obtained through O.D. have recorded about 20-30% moisture reduction, while intermediate moisture fruits obtained from O.D. have recorded about 30-70% moisture reduction (Yadav & Singh, 2014). According to Sharma et al. (2003) and Falade et al. (2007) during osmotic dehydration always water loss is favoured over solid uptake leads to mass loss of the fruit. According to their experiments, all these parameters depend on the concentration of syrup and syrup to fruit ratio. Fruit ratio from 1 to 2, there was considerable change in water loss from 18.09% to 23.18%, mass loss from 9.26% to 20.06% and solid gain from 13.59 to 16.38%.

This process leads to the transfer of anthocyanin pigment and flavouring constituents to the osmotic solution. They also used osmotic solutions containing pigments and volatile constituents as raw materials for developing natural additives (Osorio et al., 2007).

In conclusion, the inverted sugar syrup appears more efficient for the dehydration process; it is less expensive and very easy to prepare; it has a better sweetening power than that of sugar or ordinary sugar syrup itself. On the other hand, it's important to specify that a concentrated fruit juice provides more benefits to our health (vitamins, minerals, amino acids, enzymes), improving substantially, also, the sensory characteristics (taste, aroma & colour). Comparing the behaviour of *A. melanocarpa* varieties to the convective dehydration process, Tables 5 and 6 and Figures 10 and 11 reveal very good results at *Nero* chokeberries, followed by *Hugin* and satisfactory results at *Viking*. The black chokeberries have been analysed at the dried stage (finally dehydrated) as concern the humidity level and the organoleptic characteristics (colour, features, taste/aroma). The main bio-chemical indicators of dehydrated chokeberries (sugar, vitamin C, anthocyanins & total phenolics) will be the subject of further research. After dehydration, the main modifications observed were shrinkage of cells, plasmolysis and folding of the cell wall (Mayor et al., 2011; Sette et. al., 2016).

After the convective dehydration, the Aronia berries pre-dehydrated through osmosis have obtained a nice & glossy aspect (Figure 9), due to the increase of carbohydrates and their shape & porosity look better, than the *Aronia* berries classically dehydrated, without osmotic treatment (Toma et al., 2019).

The osmotic dehydration has increased elongation and decreased roundness and compactness (Suelen et al., 2016).

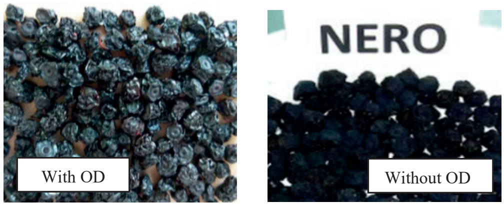


Figure 9. Dehydrated chokeberries:
Left side - after O.D.; Right side - without O.D.

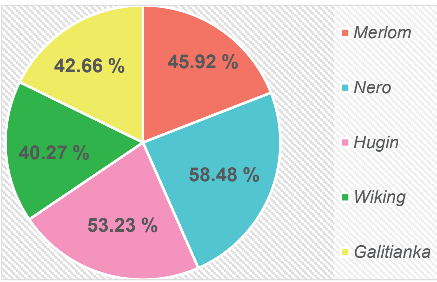


Figure 10. Average weight reduction gain* of dehydration to osmotically treated chokeberries
(*Of dried chokeberries without O.D.)

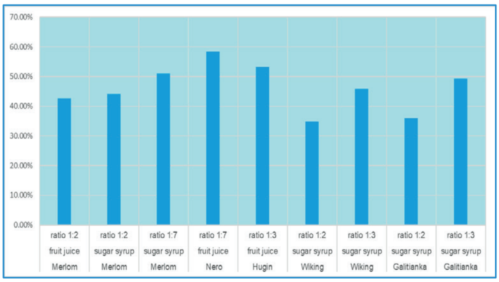


Figure 11. Weight reduction gain* after convective dried chokeberries - osmotically treated
(*Of dried chokeberries values without O.D.)

Table 5. The effects of the osmotic solution, ratio and immersion time on *A. melonocarpa* weight reduction gain

Variety	**O.D.-Treatment**		**Humidity (U° after 50 hours of convective dehydration at 50°C) %**
Galitianka	sugar syrup	ratio 1:2	6.49
		ratio 1:3	5.14
Galitianka	Non O.D.		10.14
Hugin	fruit juice	ratio 1:3	7.76
Hugin	Non O.D.		13.81
Merlom	fruit juice	ratio 1:2	6.83
	sugar syrup	ratio 1:2	6.64
		ratio 1:7	6.07
Merlom	Non O.D.		11.89
Nero	fruit juice	ratio 1:7	5.31
Nero	Non O.D.		12.79
Viking	sugar syrup	ratio 1:2	9.09
		ratio 1:3	7.54
Viking	Non O.D.		13.92
O.D. Berries Average (x̄)			**6.74**
Std. error			**0.54**
Non-O.D. Berries Average (x̄)			**12.51**
Std. error			**0.48**

Table 6. Levels of humidity after dehydration

Aronia melanocarpa **cultivars**	**Osmotic solution**	**Ratio**	**Immersion time at 50°C**	**Weigh reduction gain*** ***of dried berries without O.D.**
Merlom	fruit juice	ratio 1:2	5 h	42.56 %
Merlom	sugar syrup	ratio 1:2	5h	44.15 %
Merlom	sugar syrup	ratio 1:7	5 h	51.05 %
Nero	fruit juice	ratio 1:7	8 h	58.48 %
Hugin	fruit juice	ratio 1:3	8 h	53.23 %
Viking	sugar syrup	ratio 1:2	5 h	34.70 %
Viking	sugar syrup	ratio 1:3	5 h	45.83 %
Galitianka	sugar syrup	ratio 1:2	5 h	36.00 %
Galitianka	sugar syrup	ratio 1:3	5 h	49.31 %

The hypertonic solution used after the osmotic dehydration is very valuable. It is rich in fruit solutes which were released into the liquid, especially vitamins, minerals, acids, enzymes and pigments (anthocyanins), as mentioned by Ahmed et al. (2016) and Osario et al. (2007).
The Fig. 12 shows how the colour turns into a specific shade on the base of the ratio: a higher ratio determined a paler colour.

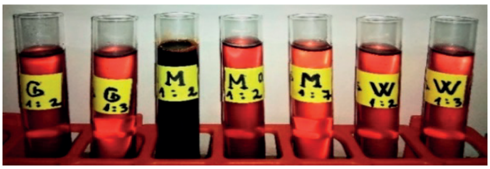


Figure 12. Stand with syrup/juice tubes after O.D.: From left to right: *Galitianka* - syrup ratio1:2; *Galitianka* syrup ratio 1:3; *Merlom* - juice ratio 1:2; *Merlom* - syrup ratio 1:2; *Merlom* -syrup ration 1:7; *Wiking* - syrup ratio 1:2; *Wiking* - syrup ratio 1.3.

The improved syrup/juice can be successfully used to prepare toppings, creams, jellies, toffies, cakes, etc. The osmotic syrup can be concentrated and reused at least 5 times without adversely affecting the fruit concentration (Bolin et al., 1983), flavour, colour, appearance and texture (Bongirwar and Sreenivasan, 1977). By partial osmosis dehydration, the product could be preserved for up to 1 year depending on conditions and packaging material and also gives the best results.

CONCLUSIONS

The *Aronia*'s berries osmotically treated before the convective dehydration obtained higher values to all bio-chemical indicators, comparing with the chokeberries dehydrated without osmotic treatment: average soluble solids gain: 37.75%; average sugar gain:

39.13%; average vitamin C gain: 52.62%; average anthocyanins gain: 9.51%; average phenols gain: 34.9%.

The increase of soluble solids conducts to a faster dehydration time and energy saving.

This aspect is very important because the vitamins (e.g., vitamin C) and other valuable bio-compounds remain in the berry's tissue and their bio - degradation due to the drying process is reduced to a minimum. The increase of sugar leads to a better taste and visual aspect, offering a longer shelf-life, as the sugar has a well-known role in food preservation.

The process of osmotic dehydration is more efficient in case the fruit integrity is not affected or the less it is. The average humidity of the dried chokeberries osmotically treated is near half of the average humidity value found in the dried fruits which were not osmotically treated before the convective dehydration. This is an important aspect for energy saving.

The highest weight reduction gain of dried chokeberries was obtained by the osmotically treated berries to all varieties.

The humidity level of the black chokeberries and their weight reduction gain was mainly determinate by the immersion time into hypertonic solution. The weight reduction gain has increased with fruits extension time into the osmotic solution.

The ratio of 1:7, followed by 1:3 has determined the highest levels of soluble solids gain, sugar, vitamin C, anthocyanins and phenols intake.

A longer time of osmotic dehydration decreases the anthocyanins' content due to their weak stability.

The inverted sugar syrup provided the highest gain of sugar and soluble solids to *Aronia*'s berries, while the concentrated fruit juice provided the best phenols' intake, vitamin C and anthocyanins to the fruits.

The hypertonic solution used after the osmotic dehydration is rich in fruit solutes released into the liquid (vitamins, minerals, enzymes, pigments, etc.)

Comparing the behaviour of *A. melanocarpa* varieties to the convective dehydration process, very good results have been obtained at *Nero* chokeberries, followed by *Hugin* and satisfactory results at *Viking*.

The differences found at the main bio-chemical indicators of *Aronia* varieties are due specially to soil and climate conditions, different technologies of cultivation and harvesting time.

The osmotic treatment is one of the best methods for fruits preservation because it improves their nutritional and sensory parameters. Moreover, since no preservatives were used, it does not affect the human body. This process is economical, gaining large savings of energy.

The osmotic dehydration can be used to reduce the post-harvest losses, to extend the shelf-life and to diversify the uses of the final products. This process could be used on small scale for the development of self-entrepreneurs and home scale industries. Consumption of such nutritional and valued products could be popularized through exhibitions and media.

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RESEARCH ON DISEASES AND PESTS DETECTED IN THE FRUIT TREE SPECIES IN BUCHAREST

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Abstract

The work is inspired by the concerns of biologist and botanist Traian Săvulescu (1889-1963), founder of the Romanian School of Phytopathology, who in 1928 founded the annual publication Phytosanitary condition in Romania, which presented the manifestation of various diseases in many host plant species, the first publication of this type in the world, followed by similar publications in the United States, Germany, and the Netherlands. Later, the entomologist Constantin Manolache (1906-1977), together with various collaborators, began to publish the situation of animal pests of plants grown in Romania. Both Traian Săvulescu and Constantin Manolache were teachers at the "Nicolae Bălcescu" Agronomic Institute in Bucharest. Fruit tree biodiversity is crucial for nutrition and ecosystem resilience around the world. Phytopathogenic and entomological biodiversity is of particular importance because diseases and pests of fruit trees cause significant loss of quality and quantity. Phytosanitary control plays an important role in reducing these losses. This paper presents the diseases and pests detected in fruit tree species in the experimental field of the Faculty of Horticulture-UASVM Bucharest, during the vegetation period of 2020 and 2021.

Key words*: fruit trees; biodiversity; diseases; pests.*

INTRODUCTION

The domestication of fruit trees took place several millennia after that of cereals and pulses (Janick, 2005; Zohary and Hopf, 2000).
Although domestication of fruit trees has received far less attention than that of annual crop plants, their significance as food sources in ancient times should not be underestimated. (Goldschmidt, 2003).
The domestication in the context of horticulture represent a “major positive change in the edibility of a wild, non-palatable fruit brought about by a rare genetic event that would disappear without human intervention” (Kislev et al., 2006).
Fruit trees can withstand the variability of rainfall better than annual crops due to the deep root systems and the perennial growth habit. Diversification into fruit tree-based systems generates high returns to the farmer and creates opportunities for value addition and avenues for employment creation at the village level (Dhyani et al., 2013).
In Romania, the cultivation of fruit trees has been practiced since immemorial time. The first information about the cultivation of fruit trees can be found in the language of our Daco-Roman ancestors. Historical sources specify that during the Roman rule, the economic base of Dacia was “cereal production, cattle breeding and fruit growing” (Baciu A.A., 2005).
Fruits are essential products for a rational diet, regardless of the age of the population. They are most often consumed as the plant produces them (without a culinary or industrial preparation). Following the processing of fruits (cooked foods, compotes, jams, jellies, etc.) the initial value decreases, the content of vitamins is reduced, etc. (Ropan, 2000).
The nutritional value of fruits is given by their chemical composition, in forms easily accessible to the human body, to which are added various olfactory, visual and gustatory stimulants (Sumedrea et al., 2011).
Diseases and pests of fruit trees cause significant loss of quality and quantity. Phytosanitary control plays an important role in reducing these losses. Identification of pathogens and pests in different areas of Romania is a permanent goal for phytopathological and entomological scientific activity in our country (Istrate et al., 2006;

Istrate et al., 2019; Paraschivu et al., 2010; 2021; Popa et al., 2013; Zală, 2021). Some pathogens can develop high epidemics in Romania depending on its virulence and the susceptibility of the host plant (Paraschivu Mirela et al., 2015). Correct diagnosis of pathogens and pest is the primary requirement in any integrated management practice.
Traian Săvulescu in 1928 founded the academic journal Phytosanitary Status in Romania, which presented the symptoms of various diseases in many host plant species, the first publication of this type in the world, followed by similar publications in the United States, Germany, and the Netherlands. Later, the entomologist Constantin Manolache (1949), together with various collaborators, began to publish the situation of animal pests of plants grown in Romania. Taking the example of the forerunners, we also want to present what are currently the diseases and pests, in this case, in the fruit tree species in Bucharest.
Biodiversity refers to the diversity in living organisms in an area (Altieri, 1999).
Biodiversity represents an important factor of ecosystem stability and productivity (Tilman et al., 2014).
The various phytopathogenic microorganisms along with harmful insects are the most important component of the biodiversity of an orchard, on which depends, to some extent, ensuring food security. Abiotic factors, especially temperature and rainfall, have a decisive effect on orchard biodiversity.
In the context of sustainable agriculture, the recommended doses have been observed in the preventive and curative application of fungicides and insecticides, in an integrated management system, thus respecting the criteria imposed by the European Union, by the 2009/189 Directive: establishing a framework for Community action to achieve the sustainable use of pesticides, so that plant protection products must not have harmful effects on human or animal health and must not have unacceptable effects on the environment.

MATERIALS AND METHODS

The research was carried out in the fruit tree species in the experimental field of the Faculty of Horticulture - USAMV of Bucharest.
The observations were made on three species of seed trees of the Rosaceae family: apple, pear, quince and on six species of stone trees: cherry, sour cherry, apricot, plum, nectarine, peach.
They were noted 10 varieties of apple, 7 varieties of pear, 3 varieties of quince, 11 varieties of plum, 6 varieties of cherry, 5 varieties of sour cherry, 2 varieties of apricot, 7 varieties of nectarine and peach.
Visual observation is the fastest method to identify diseases and pests based on symptoms shown by infected fruit tree species.
For the flying insects were used the glue traps (Figure 1).
Scouting for diseases and pests attack has a particular importance in fruit tree species to establishing the attack value during the vegetation season.

Figure 1. Glue traps

The attack value is represented by frequency (F%), intensity (I%) and attack degree (AD%).
Frequency is the percentage of leaves or fruits attacked out of 100 examined leaves or fruits.
The intensity, visually estimated, indicates the degree to which the leaf or fruit is attacked. The intensity was noted directly in percentage. The attack degree present severity of disease or pest in the crop and was calculated using the frequency (disease incidence) and intensity (severity). Attack degree was calculated using the formula: A.D. (%) = F (%) x I (%)/100.
The period analysed in this study was 2020-2021. For scouting optimization and for the observation of the climatic conditions necessary for the appearance and development of the disease, precipitation, wind speed and temperatures were taken into account (Paraschivu et al., 2020).

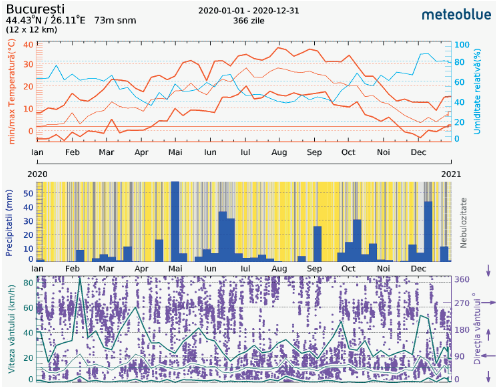

Figure 2. Climate parameters registered in 2020 in Bucharest (source:https://www.meteoblue.com/ro/vreme/historyclimate/weatherarchive/bucurești_românia_ year=2020)

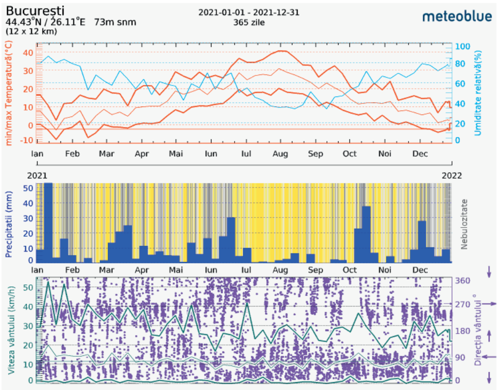

Figure 3. Climate parameters registered in 2021 in Bucharest (source:https://www.meteoblue.com/ro/vreme/historyclimate/weatherarchive/bucurești_românia_ year=2021)

The phytosanitary treatments applied in 2020 can be found in Table 1.

Table 1. Phytosanitary treatments applied in 2020

Date	Species	Product
31.01.	All species	Ovipron Top-1.5%
06.03.	Apple	Boedeaux mixture-0,5%+Confidor Energy-0.06%
18.03.	Nectarine, Peach	Syllit-0,2%+Dithane-0.2%+ Calypso-0,02%
	Apple, Pear, Quince	Calypso-0,02%+Luna experience-0.05%
	Cherry, Sour Cherry, Apricot, Plum, Walnut	Novadim progress-0.1%+ Dithane-0.2%
05.04.	Cherry, Sour Cherry, Apricot, Plum, Walnut, Nectarine, Peach	Calypso-0,02%+Luna experience-0.05%+ Syllit-0,2%+Dithane-0.2%
	Apple, Pear, Quince	Aliette-0,2%+ Microthiol-0,5%+Envidor-0.04%
03.05.	Cherry, Sour Cherry, Apricot, Plum, Walnut, Nectarine, Peach	Calypso-0,02%+ Syllit-0,2%+ Nissorun-0.03%+Topsin-0.07%
	Apple, Pear, Quince	Topsin-0.07%+ Nissorun-0.03%+Prev-Am-0.6%+Nurelle-0.08%
18.05.	Cherry, Sour Cherry, Apricot, Plum, Walnut, Nectarine, Peach	Luna experience-0.05%+ Syllit-0,2%+Envidor-0.04%+ Cropmax-0.3%
	Apple, Pear, Quince	Microthiol-0,5%+Flipper-0.2%
10.06.	Cherry, Sour Cherry, Apricot, Plum, Walnut, Nectarine, Peach	Calypso-0,02%+ Syllit-0,2%+ Luna experience-0.05%+ Zeamă Bordeleză-0,5%
	Apple, Pear, Quince	Nurelle-0.08%+ Nissorun-0.03%+ Microthiol-0,5%+Flint plus-0.15%
03.07.	Apple, Pear, Quince	Flipper-0.2%+ Luna care-0.3%+Furry-0.02%
	Cherry, Sour Cherry, Apricot, Plum, Walnut, Nectarine, Peach	Alcupral-0.5%+ Furry-0.02%
10.08. 21.08.	Apple, Pear, Quince	Alcupral-0.5%+Copfort-0.25%+Microfort-0.25%
13.11.	All species	Zeamă Bordeleză-2%

The phytosanitary treatments applied in 2021 can be found in Table 2.

Table 2. Phytosanitary treatments applied in 2021

Date	Species	Product
03.03.	Cherry, Apple, Sour Cherry	Ovipron Top-1,5%
26.03.		Zeamă Bordeleză-0,5%+ Microthiol-0,5%
	Nectarine, Peach	Zeamă Bordeleză-0,5%+Syllit-0,2%+Calypso-0,02%
	Apricot, Plum, Pear, Quince, Walnut	Zeamă Bordeleză-0,5%+ Calypso-0,02%+Teldor-0,08%
16.04.	Apple	Calypso-0,02%+Luna experience-0.05%
	Apricot	Calypso-0,02%+Luna experience-0.05%+Dithane-0,2%
23.04.	Nectarine, Peach, Apricot	Dithane-0,2%+Funguran-0.04%+Kerafol-0.3%
07.05.	Pear, Apple, Walnut	Luna experience-0.05%+Aliette-0,2%+Movento-0,75%+Cropmax-0.3%
	Nectarine, Peach, Apricot, Plum, Cherry, Sour Cherry	Movento-0,75%+Cropmax-0.3%+Alcupral-0.5%+Envidor-0.04%
12.05.	Pear, Quince, Apple, Walnut	Aliette-0,2%
15.06.	Cherry, Apple, Apricot, Plum, Sour Cherry, Nectarine, Peach	Super Fifty-0.5%+Prev-Am-0.6%+Microthiol-0.5%+Zeamă Bordeleză-0,5%+ Nissorun-0.03%
24.06.	Nectarine, Peach Apricot, Plum	Super Fifty-0.5%+ Zeamă Bordeleză-0,5%+Kerafol-0.3%+ Microthiol-0,5%+Garex-0.2%+Mimox-0.3%
	Pear, Apple, Quince, Walnut	Super Fifty-0.5%+Luna care-0.3%+ Envidor-0.04%+OmyraPro Calcium-0.5%

07.07.	Sour Cherry, Cherry Peach, Nectarine, Apricot, Plum	Super Fifty-0.5%+ Kerafol-0.3%+ Zeamă Bordeleză-0,5%+ Garex-0.2%+ Prev-Am-0.6%
	Pear, Apple, Quince, Walnut	Super Fifty-0.5%+ Kerafol-0.3%+ OmyraPro Calcium-0.5%+ Microthiol-0,5%+Decis-0.25%
27.10.	All species	Zeamă Bordeleză-2%

Samples of crop units (leaves and fruits) were taken randomly from every fruit tree variety from 4 points of the crown. In each point, 100 leaves were noted.

The apple (*Malus pumila* Mill.) varieties studied were: Enterprise, Braeburn, Florina, Red Elstar, Florina, Jonafree, Redcats, Goldcats, Suncats and Starcats.

The pear (*Pyrus comunis* L.) varieties studied were: Conference, Abatele Fetel, General Leclerc, Williams, Red Favoritka, Red Williams and Untoasă Bosc.

The quince (*Cydonia oblonga* Mill.) varieties studied were: Ekmek, Tinella and Bereczki.

The cherry (*Prunus avium* L.) varieties studied were: George, Ludovic, Bucium, Van, Regina and Vega.

The sour cherry (*Prunus cerasus* L.) varieties studied were: Pandy, Northstar, De Botoșani, Nana and Productiv de Debretin.

The plum (*Prunus domestica* L.) varieties studied were: Anna Späth, Stanley, Silvia, Centenar, Amers, President, Bluefree, Empress, Pescăruș, Grossa di Felisio and Ruth Gestetter.

The peach (*Prunus persica* L.) varieties studied were: Lucius, Gladys, Red Top, Sweet Dream, Royal Glory, Cardinal, and Springbelle.

The nectarine (*Prunus persica* var. *nucipersica* L.) varieties studied were: Honey Late, Nectaross, Stark Red Gold, Honey Royale, Big Top, Big Fire and Nectabelle.

The apricot (*Prunus armeniaca* L.) varieties studied were: Farbali and Farely.

RESULTS AND DISCUSSIONS

Taphrina deformans have been present in peach and nectarine since the end of March; in early April, *Podosphaera leucotricha* appeared on young apple shoots, *Monilinia laxa* made its presence felt on apricot stem; in May, *Monilinia laxa* appeared on the stem and fruits of the cherry, *Hyalopterus pruni* and *Grapholita funebrana* appeared on the plum, *Myzus cerasi* on the cherry, *Plum-pox* virus appeared on the leaves of some plum and apricot varieties, and on the apple *Dysaphis plantaginea*; In June, the few adults of the *Rhagoletis cerasi* were caught on the glue traps, *Erwinia amylovora* caused the burning of the pear leaves, *Xanthomonas arboricola* pv. *juglandis* on walnut stem and fruits, and on cherries *Monilinia laxa* on leaves and fruits; in July *Monilinia laxa* appeared on peach and apricot fruit, *Epitimerus pyri* on leafs and fruits of pear, *Eriophyes erinea* on leafs of walnut, *Wilsonomyces carpophilus* on peach and nectarine leaves, and *Quadraspidiotus perniciosus* on apple fruit; in August, *Monilinia fructigena* on apple fruits and *Monilinia linharthiana* on quince fruit.

The attack degree of powdery mildew on apple was highest in the Redcats variety - 2.2% in 2020 (Table 3). Varieties Enterprise, Braeburn, Florina, Red Elstar, Florina and Jonafree showed no symptoms of powdery mildew.

Some apple fruits of varieties Redcats and Goldcats showed characteristic symptoms of purplish red colour of *Quadraspidiotus perniciosus* attack. Also on varieties Redcats and Goldcats we found some leaves curled downwards and brown-reddish as a result of the attack by *Dysaphis plantaginea*.

Among the pear varieties researched only at the Conference, the fire blight was manifested.

Some leaves reddish to yellowish green blisters and deformed fruits caused by *Epitimerus pyri* were detected in the variety Abatele Fetel.

The attack degree of brown rot of quince fruit was highest in the Tinella variety-1.9% in 2021.

Regarding the peach leaf curl attack, the highest values - 12.0%, in 2021, were recorded for the Gladys variety, and the lowest - 4.0%, in 2020, for the Springbelle variety. In the Cardinal variety, we did not find any symptoms of leaf curl. Also in peach, in two of the researched varieties we reported the presence of brown fruit rot, with the highest attack, of 5.4% in 2021, in the Springbelle variety and symptoms typical of shot hole disease, in two other varieties, with the highest attack-4.6%, in 2021, for the Cardinal variety.

In nectarine of the seven varieties analysed, in three (Honey Late, Nectaross and Nectabelle) we found symptoms of leaf curl, with the highest degree of attack-10.8% in 2020 in the Nectabelle variety and two other varieties (Big Top and Big Fire) presented brown rot on some fruits. Stark

Red Gold and Honey Royale varieties showed no symptoms of disease.

Brown rot of apricot fruit showed the highest degree of attack - 4.5% in 2021 for the Farbali variety, and the lowest - 3.8% in 2020 for the Farely variety. Mild light green discoloration bordering the leaf veins (vein yellowing) or yellow to light green rings, typical of *Plum-pox potyvirus* infection, could be observed on some leaves of both varieties.

Table 3. Note the attack of diseases on the varieties that showed symptoms

Orchard	Varieties	Disease/scouting/year					
		F (%)		I (%)		A.D. (%)	
		2020	2021	2020	2021	2020	2021
		Powdery mildew					
Apple	Redcats	22.5	18.0	9.7	9.3	2.2	1.7
	Goldcats	17.75	14.5	8.6	7.9	1.5	1.1
	Suncats	14.25	11.5	9.3	8.5	1.3	1.0
	Starcats	9.5	7.25	7.6	7.3	0.7	0.5
	Brown rot of fruit						
	Florina	3.75	4.25	19.4	21.8	0.7	0.9
Pear	**Fire blight**						
	Conference	5.75	7.0	68.2	87.3	3.9	6.1
Quince	**Brown rot of fruit**						
	Ekmek	2.75	3.5	39.9	40.1	1.1	1.4
	Tinella	3.25	4.0	45.8	46.6	1.5	1.9
	Bereczki	2.25	3.25	34.3	36.2	0.8	1.2
Peach	**Leaf curl**						
	Lucius	11.25	12.0	82.3	82.5	9.3	9.9
	Gladys	12.75	14.0	85.1	85.6	10.9	12.0
	Red Top	12.5	13.5	84.4	85.0	10.6	11.5
	Sweet Dream	9.5	10.5	77.8	78.7	7.4	8.3
	Royal Glory	8.75	10.0	77.6	78.2	6.8	7.8
	Springbelle	5.25	6.0	77.2	77.9	4.0	4.7
	Brown rot of fruit						
	Sweet Dream	4.25	4.75	95.5	98.1	4.1	4.7
	Springbelle	4.75	5.5	95.8	98.9	4.6	5.4
	Shot hole disease						
	Cardinal	52.5	55.1	7.43	8.4	3.9	4.6
	Gladys	27.25	30.5	6.8	7.2	1.9	2.2
Nectarine	**Leaf curl**						
	Honey Late	8.75	9.0	78.8	79.2	6.9	7.1
	Nectaross	11.5	12.0	80.2	80.4	9.2	9.6
	Nectabelle	13.25	13.0	81.4	81.7	10.8	10.6
	Shot hole disease						
	Big Top	31.25	32.5	8.5	8.8	2.7	2.9
	Big Fire	28.75	29.5	7.6	8.1	2.2	2.4
Aprico t	**Brown rot of fruit**						
	Farbali	4.75	5.0	90.2	90.5	4.3	4.5
	Farely	4.25	4.5	88.8	89.1	3.8	4.0
Plum	**Brown rot of fruit**						
	Stanley	6.25	6.75	89.9	90.2	5.6	6.1
	Centenar	4.25	4.75	87.3	87.5	3.7	4.2
	President	6.75	7.0	90.2	91.1	6.1	6.4
Cherry	**Moniliosis of blossoms, buds, leaves and twigs**						
	George	10.5	11.5	16.2	17.1	1.7	2.0
	Ludovic	11.25	12.5	17.5	18.0	2.0	2.3
	Bucium	10.25	11.0	15.1	15.9	1.5	1.7
	Van	9.75	10.5	12.6	13.8	1.2	1.4
	Regina	8.5	10.0	9.4	11.2	0.8	1.1
	Vega	11.5	12.5	18.7	19.9	2.2	2.5
	Brown rot of fruit						
	All varieties	-	90	-	100	-	90
Sour Cherry	**Moniliosis of blossoms, buds, leaves and twigs**						
	Pandy	2.25	3.5	6.7	7.3	0.2	0.25
	Noerthstar	2.0	2.5	6.6	7.1	0.13	0.17
	De Botoșani	0.5	1.0	5.0	5.4	0.03	0.05
	Nana	6.5	7.0	9.3	9.5	0.6	0.66
	Productiv de Debretin	1.25	2.0	6.1	6.6	0.08	0.13

Of the 11 plum varieties, only 3 (Stanley, Centenary and President) had fruit with brown rot. Of these, the highest degree of attack was manifested by the President variety: 6.1% in 2020 and 6.4% in 2021. Several leaves with symptoms typical of *Plum-pox potyvirus* infection have been reported in the Centenary variety. Also at the Centenary variety we discovered *Hyalopterus pruni* colonies underside of the leaves. Some fruits of the Pescăruș variety showed entrance holes and exude gum caused by the attack of *Grapholita funebrana* larvae. The plum varieties Anna Späth, Silvia, Amers, Bluefree, Empress, Grossa di Felisio and Ruth Gestetter did not show any disease or pest attack.

The attack of the *Monilinia laxa* fungus caused the wilting of flowers, buds, leaves and a portion of the twigs in spring, both on cherries and sour cherries; higher attack values are marked on the cherry. A few colonies of *Myzus cerasi* could be seen at the top of the shoots of the George variety.

CONCLUSIONS

The sporadic presence of bacterial burn and walnut erinosis was also noted.

Diseases and pests were detected under conditions of natural infection under the influence of climatic conditions during the vegetation period of the two years of studies.

Despite the application of phytosanitary treatments, the appearance of the typical symptoms of the attack of pathogens and harmful insects is attributed to the sensitivity of the varieties in which they were encountered. In view of the results obtained, it may be advisable to use other plant protection products for the species of fruit tree species in which we have encountered diseases and pests, which could have a better action against the complex of diseases and pests.

In 2020 the degree of attack was higher for apple powdery mildew, rosy apple aphid and San José scale, pear and walnut leaf blister mite, the plum fruit moth, cherry fruit fly and cherry aphid.

In 2021 the degree of attack was higher for leaf curl of peach and nectarine, shot hole disease of apricot, plum, peach and nectarine, walnut blight, fire blight of pear, moniliosis or brown rot of stone fruits: cherry, sour cherry, plum, apricot, peach; brown rot of apple and quince. Practically, the almost continuous presence of rainfall during the ripening of cherries has led to a total compromise of production in all varieties. The attack of the detected insects was sporadic.

The highest frequency was recorded in the attack of *Taphrina deformans* fungus, and the highest intensity of a disease was caused by brown rot of stone fruit.

Among the microorganisms that made up the phytopathogenic biodiversity were a virus, two bacteria and six fungus and entomological biodiversity was represented by eight species of insects.

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TĂMÂIOASĂ ROMÂNEASCĂ AND BUSUIOACĂ DE BOHOTIN GRAPES - VALUABLE SOURCES FOR WINE PRODUCTION

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Abstract

Varieties of Tămâioasă Românească (TR) and Busuioacă de Bohotin (BB) grapes were harvested in 2022 from Pietroasa Viticulture and Winemaking Research and Development Station that belongs to the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV). These grapes were taken in studies in order to highlight the quality of the grapes of the respective area following the analyzes carried out. These varieties are processed into aromatic wines at the Pietroasa. Sugar determination was performed by HPLC-RID analyzes on the grapes of the two varieties studied. The values of essential analyses such as soluble dry matter content (24,036% for TR grapes and 23,120% for BB grapes) and acidity content (0,477% for TR grapes and 0,537% for BB grape) are appropriate results for next step of wine production. While the glucose content is similar in both varieties of grapes, the fructose content in TR grapes (10.913%) is higher in comparison with the BB grapes (10.57%). The balance between constitutive (grains 96.8% and 98.9%, respectively) and structural (mesocarp 69.7% and 71.5%, respectively) uvological units for both varieties (TR and BB) with maximum economic potential in this area was demonstrated.

Key words: *grapes, Tămâioasă Românească, Busuioacă de Bohotin*

INTRODUCTION

Grapes (*Vitis vinifera* L.) represent a major fruit crop worldwide. TR and BB grape varieties, which are considered one of the most aromatic wines of the world, are cultivated in Pietroasa vineyard Romania) for obtaining dry or sweet wine. The aromatic puzzle of a wine is particularly influenced by the interaction between grapes and fermentation agents (Romano et al., 2022). Grape varieties can be aromatic and semi-aromatic, TR and BB being aromatic varieties because they have the aromatic substances not only in the epicarp but are also present in the mezocarp. In addition to the high concentrations of terpenoid molecules, these constitute the key substances or signature to recognize the variety (Visan et al., 2018). The taste of wine is given by the *terroir*. The quality of the grapes depends on the soil, the climate, the positioning of the vineyard (Schusterova et al., 2021). Polyphenols act as antioxidants and have a positive role in human health. The phenolic composition of the wine also changes during the aging process of the wine, which is reflected in the color and degree of astringency of the wine, the final product. Phenolic compounds can also be used as markers to discriminate wine origin (Niculescu et al, 2017). Visan et al (2015) determined the aromatic compounds for the TR wines from two Romanian wine-growing areas: Stefanesti-Arges and Pietroasa vineyards.

Also, the wine-growing region influences the organoleptic characteristics of wine and the aromatic content. BB grape variety has been cultivated in Romanian vineyards such as Pietroasa Viticulture and Winemaking Research and Development Station, Dragasani, Murfatlar, Cotnari, Husi (Bohotin centre). BB and TR varieties have both a specific vinification process (Colibaba, 2015). In this paper two variety of grapes - BB and TR - were analized from the following parameters: the content in polyphenol compounds, soluble dry matter content, sugar content as well as the value of the uvological indices.

MATERIALS AND METHODS

-The grapes varieties Tamaioasa Romaneasca (TR) and Busuioaca by Bohotin (BB) (Figure 1) were harvested in 2022 from Pietroasa Viticulture and Winemaking Research and Development Station (Figure 2).

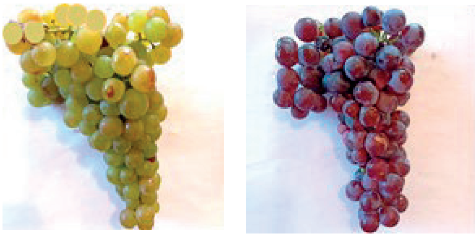

Figure 1. Grape varieties, TR variety on the left and BB variety on the right

Figure 2. Pietroasa vineyard

Physical-mechanical analysis of the TR and BB grape varieties

The technological potential of the studied grape varieties (TR and BB), grown in the Pietroasa vineyard, consisted in the determination of the uvological units (Matei et al., 2022). The percentages of each uvological unit of the grape and the berry and the yield in must and pomace of the grape were determined. With the help of these parameters, the uvological indices were calculated: structure index (SI), which is the ratio between berries (g) and clusters (g); the berry index (BI), which is the ratio between the number of berries and 100 g of grapes; the berry composition index (BCI), which is the ratio between the mesocarp (g) and the epicarp (g); the yield index (YI), which is the ratio between must (g) and grape marc (g).

Chemical analyses of the TR and BB grape varieties

Determination of soluble dry matter content - The dry soluble content (Brix value) was determined by refractometry using an Abbemat 550" refractometer (méthod Brix) and the results were expressed in g/100 g.

The sugar determination

Determination of sugars was performed by a HPLC method with refractive index detection (HPLC-RID), using a Shimadzu HPLC equipment with DGU-405 degassing unit; LC-40D pump; SIL-40autosampler/injector; CTO-40C column oven; RID-20A refractive index detector; Shimadzu LabSolutions software; Phenomenex Luna Omega SUGAR column, 105 x 4.6 m.

The folowing procedure was applied for samples preparation: 5 g of the sample was mixed with hot water in a 250 ml volumetric flask. In the solution cooled and then clarified solutions the Carez I and Carez II solutions were added, and then mixed and brought to boiling. The clarified solution was then filtered through a 0.45 mm filter and used for HPLC injection directly into the HPLC equipment. The elution was performed using acetonitrile/water mixture (80:20) as the mobile phase at a flow rate of 1.5 ml/min at a temperature of 25°C. The refractive index detector was maintained at 40°C, and the injection volume was 25µL.

The following external standards as 2% to 0.005% solutions were used for determinations: arabiosis, fructose, glucose, galactose, lactose, maltose, mannose, ribose, sucrosem xylose. The results were expressed as a percentage by mass/mass (g/100 g) of each sugar.

Acidity determination - Acidity determination was performed by titration with NaOH 0.1N in presence of phenolphthalein as indicator. The acidity value was expressed in NaOH 0.1N/ 100 g and in tartaric acid (g/100 g) respectively using the 0.075 conversion factor.

Total polyphenols determination - To determine the concentrations of total polyphenols, the Folin-Ciocalteu method was used (Stan A. et al., 2020). The method assumes that in an alkaline medium, polyphenols reduce the reagent resulting in a blue color with different intensities depending on the concentration of polyphenols. The analyzes were performed in triplicate, the final result being the average of the repetitions and expressed in mg GAE/100 g fresh sample.

RESULTS AND DISCUSSIONS

Physical-mechanical analysis of the grapes of the TR and BB varieties

The quality of the grapes determines the quality of the wine, in terms of constituents and their chemical composition. The composition and mechanical and chemical properties of grapes are studied by *uvology*, one of the viticultural sciences. The production of grapes (kg/vine or t/ha) is an important indicator for establishing the adaptability of the variety to the pedoclimatic conditions of the vineyard (Table 1). A very high production (12.5±1.4 t/ha) can be observed for the BB variety compared to the average obtained in Pietroasa for this variety (8.4 t/ha), demonstrating a high quantitative potential for this ecosystem (Ion et al., 2018).

The distribution of different chemical compounds in the constitutive parts of the grapes is particularly important, because they show changes during the ripening of the grapes as a result of the influence of external factors that have an effect on the quality of the grapes. Grapes are composed of clusters and berries. In table 2 the average weight of a grape for the varieties studied (TR and BB), as well as the percentage of each individual component (clusters, berries, skins, mesocarp, seeds) are presented.

Table 1. TR and BB production (kg/vine and t/ha)

Vine	Nº grapes		kg		t/ha	
	TR	BB	TR	BB	TR	BB
1	19	15	2.34	3,71	8.88	14.06
2	12	12	1.48	2,97	5.61	11.25
3	15	13	1.85	3,22	7.01	12.18
Media	**15.3±3.5**	**13.3±1.5**	**1.9±0.4**	**3.4±0.4**	**7.2±1.6**	**12.5±1.4**

Table 2. Average weight of a grape and the percentage of each individual component (clusters, berries, skins, mesocarp, seeds) (TR and BB)

Variety	average weight grapes (g)	% clusters	% berries	% mesocarp	% epicarp	% seeds
TB	123.4	3.0	96.8	69.7	25.8	4.5
BB	247.4	1.1	98.9	71.5	27.1	1.4

Regarding the average weight of a grape, it can be seen that BB grapes (247.4 g) are twice as developed as TR grapes (123.4 g), BB exceeding the average value (220 g), instead TR being below the average value from the literature (250 g) (Pomohaci et al., 2000). Regarding the percentage of components, the determined values are similar, except for the bunches (1.1%) and seeds (1.4%) in the BB variety, where the observed percentage values are below the known average value (2.2%, respectively 2.9%). On the other hand, for both varieties, a high value of the epicarp percentage was observed (25.8% for TR, respectively 27.1% for BB). The epicarp is composed of several layers of cells important in the wine-making process (epidermis and hypodermis), and pigments and flavors are accumulated in the hypodermis (Pomohaci et al., 2000).

Establishing the technological skills of the varieties can be done on the basis of uvological indices (Table 3).

Table 3 Values of the uvological indices in the varieties studied (TR and BB)

Variety	SI	BI	BCI	YI
	berries /bunches (g)	number of berries/100 g grapes	mesocarp/epicarp (g)	must/grape marc (g)
TB	29.9	73.7	2.7	3.1
BB	94.2	57.5	2.6	2.6

The structure index (SI) has high values for top quality wines, in the case of the BB variety, the value of this index is 94.2. Regarding the berry index (BI), its value varies between 45 and 100 (Pomihaci et al., 2000), high values suggest technological skills for branded wines, which can be seen in the TR variety (73.7). The berry composition index shows low values in the case of both varieties, which proves that superior quality wines can be obtained (2.7 TR, respectively 2.6 BB). The yield index must be correlated with the must volume and the sugar content. The values obtained (3.1 TR, respectively 2.6 BB) indicate varieties with a high potential for sugar accumulation in the Pietroasa area.

Chemical composition of the TR and BB grapes TR and BB

Acidity

- White grapes TR variety: 6.36 ml NaOH 0.1N/100 ml = 0.477% (as tartric acid)
- Red grapes BB variety: 7.16 ml NaOH 0.1N/100 ml = 0.537%

Dry soluble content (Brix value)

- White grapes TR variety: 24.036%
- Red grapes by BB variety: 23.120%

The sugar spectrum for the studied grape varieties is represented in the figures below (Figures 3 and 4), and the corresponding concentration of sugars in the Table 4.

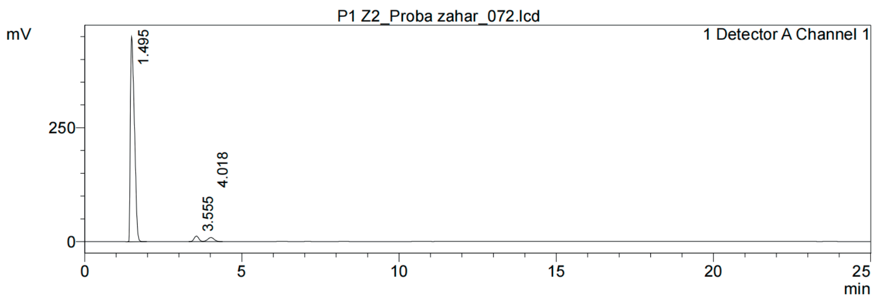

Figure 3. Chromatogram representing the sugar spectrum for the BB grape variety

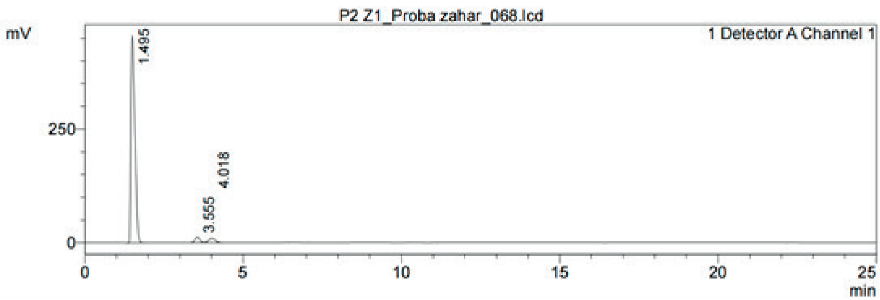

Figure 4. Chromatogram representing the sugar spectrum for the TR grape variety

Table 4. Sugars determination for grapes sample

Samples	Sugar analyzed	Concentration (%)
BB	Glucose	9.872
	Fructose	10.57
	Ribose	0
	Xylose	0
	Arabinose	0
	Manose	0
	Galactose	0
	Sucrose	0
	Maltose	**0**
	Lactose	0
	Glucose+Fructose	**20.442**
TR	Glucose	10.871
	Fructose	10.913
	Ribose	0
	Xylose	0
	Arabinose	0
	Manose	0
	Galactose	0
	Sucrose	0
	Maltose	0
	Lactose	0
	Glucose+Fructose	**21.784**

Busuioacă de Bohotin and Tămâioasă Românească grape varieties was higher in GAE compared to Merlot variety reported by Abe et al. (2007) who found 337 mg GAE 100 g^{-1} and also with Petite Syrah grapes reported by Abe et al. (2006) and (A. Franco-Bañuelos et al. (2017) (388 mg GAE 100 g^{-1}).

Table 5. Content in total polyphenols

Grapes variety	Total polyphenols	
	Average (mg GAE/100 g sample)	STDEV (mg/100 g sample)
BB 2022	453.77	±8.5
TR 2022	423.61	±8.19

CONCLUSIONS

- The technological suitability of the two analyzed grape varieties (TR and BB), in the Pietroasa area, based on the uvological indices analyzed, demonstrates the potential for obtaining wines with controlled designation of origin.
- The balance between constitutive (grains 96.8% and 98.9%, respectively) and structural (mesocarp 69.7% and 71.5%, respectively) uvological units with maximum economic potential in Pietroasa area was demonstrated.
- The sugar content from TR grapes was 21.784 % (10.871 % glucose + 10.913 % fructose) and for BB grapes 20.442 % **(**9.872 % glucose + 10.57 % fructose);
- The concentration for the rest of the sugars analysed was below the limit of quantification;
- Soluble dry matter content from TR grapes was 24,036% and for BB grapes 23,120%;

- The acidity content for BB grape (0,537% as tartric acid) was higher in comparison with TR grapes (0,477% as tartric acid);
- The content of total polyphenols for the BB grape variety was 453.77 ±8.5 (mg GAE/100 g sample), slightly higher than the TR grape variety showing 423.61 ±8.19 (mg GAE/ 100 g sample).
- These grapes varieties were used for the vinification process at Pietroasa winery.

ACKNOWLEDGEMENTS

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RESEARCH ON ADAPTATION MEASURES OF VITICULTURE TO CLIMATE CHANGE: OVERVIEW

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Abstract

The scientific literature of the last decades presents studies on the influence of global warming on vine, a sensitive plant, considered an indicator of climate change. Adaptation of grapevine to climate change is a major challenge for the vine-growing sector. More attention has been given lately to the methods of mitigating its effects, to maintaining the quality and to production sustainability. The latest research identifies many short-term measures (canopy management, application of sunscreen substances, soil management, pest and disease control, irrigation), medium-term (new training systems, minimal pruning, late pruning, shading nets) and long-term measures (relocation of vineyards, planting systems, land selection, scion/rootstock varieties, photovoltaic panels) to combat the negative effects of this phenomenon. This paper aims to present a synthesis of the studies conducted both in our country and worldwide, regarding measures to ensure the adaptation to new conditions.

***Key words**: viticulture, adaptation, climate change, strategies.*

INTRODUCTION

Climate is the main factor that determines the development of physiological processes, phenophases, the quality of grapes and wine, depending on the variety and local conditions. Numerous studies over time have established the optimal temperature values for photosynthesis and sugar accumulation in berries (25-30°C), as well as for the accumulation of anthocyanins (17-26°C). Lower values are also favorable for the accumulation of aromatic compounds (Pirie & Mullins, 1977; Mori *et al.,* 2007a; 2007b; Tarara *et al*., 2008; Spayd *et al*., 2002). Coombe's (1987) research in Australia has established that temperatures between 25 and 28°C ensure an optimal ratio between sugars and anthocyanins.

Exceeding these intervals, as a result of global warming, especially during the ripening of grapes, caused a number of negative reactions: advancing phenophases; excessive accumulation of sugars in berries, decrease of titratable acidity, insufficient accumulation of anthocyanins and aromatic compounds, obtaining unbalanced wines, lacking in typicality and with less possibilities of aging (Sadras *et al.*, 2012; Novello & de Palma, 2013; Bucur & Dejeu, 2014; Palliotti *et al.*, 2015; Martinez de Toda & Balda, 2015; van Leeuwen *et al*., 2019; Bucur *et al.*, 2019; Santos *et al.*, 2020; Reynold, 2021).

Climate change in recent decades requires the timely implementation of measures to adapt to changing conditions, depending on local conditions, in order to reduce the risks and preserve the typicality of wines. It is estimated that the development of strategies for adapting viticulture to climate change will be of paramount importance for the sustainability and competitiveness of production (Naulleau *et al*., 2021; Ollat & Tuzard, 2014; Barbeau *et al.*, 2015; Cataldo *et al.*, 2021).

These measures, which can be applied in existing plantations, have proven their effectiveness, can be applied mechanized, have immediate effects and have been considered short-term measures (Palliotti, 2013a).

A lot of data has been accumulated gradually, which will have effects not only in the short term but also in the medium and long term (Malheiro *et al.,* 2010; Neethling *et al.*, 2017; Santos *et al.*, 2020; Gutierrez-Gamboa *et al.*, 2021).

Given the effects of climate change (which will be amplified in the near future) on grapevine, this paper aims to review the latest scientific findings on strategies for adapting viticulture to climate warming and mitigating its effects. These have been grouped into short, medium and long term strategies.

SHORT-TERM ADAPTATION MEASURES

Canopy management

Studies in several European wine-growing countries have recommended the main options for adapting viticulture to climate change in the short term (Santos *et al.,* 2021) (Figure 1).

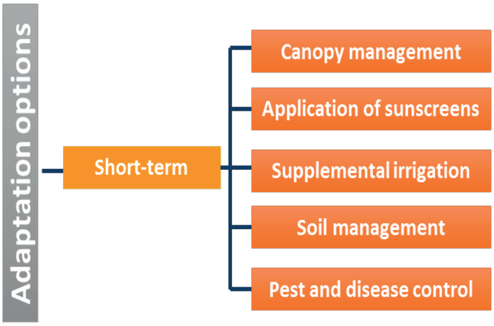

Figure 1. Short-term adaptations options to mitigate climate change impact on viticulture (Santos *et al.,* 2021)

The first measures to adapt to climate warming aimed at delaying the ripening of the grapes at moderate temperatures, avoids excessive heat during summer, maintaining a balance between sugars and acidity, with sufficient accumulations of coloring substances and aromatic compounds, which would ensure balanced, typical wines. As a priority measure to mitigate the effects of global warming on the grapevine, which can be applied during the growing season, shoots thinning, severe trimming of shoots, leaf removal, application of antiperspirants etc., are recommended.

Shoots thinning before flowering, keeping 14 shoots on the stem, followed by leaf removal applied in the area of the grapes, before the veraison lead to obtaining appropriate quality parameters for the Sangiovese variety, under conditions of climate change (Silvestroni *et al.,* 2016).

Severe trimming of shoots and increased competition from lateral shoots can contribute to delayed grape ripening (Martinez de Toda *et al.*, 2013). By delayed trimming of shoots, one week after the grape veraison, Filippetti *et al.,* (2011) obtained a significant reduction in the concentration of sugars in the must, without changing the pH, organic acids, the content of anthocyanins in the skins of the berries and the tannins in the seeds. With a severe trimming of shoots applied one week after the berries were tied, the harvest date of the Grenache grape was delayed by two weeks, reaching the same concentration in sugar and a higher one in anthocyanins, compared to the non-trimming control (Martínez de Toda *et al.,* 2013).

Leaf removal has proven to be an important measure of adaptation to climate change, by delaying grape ripening. In the past it was recommended to apply the leaf removal to the veraison, in the area of the grapes, aiming at their better maturation and reducing the attack of gray rot.

Today, under the conditions of global warming, the intended effect of this operation is different: reduction of the effect of excessive temperatures and solar radiation on grapes (Mereanu, 2010; Palliotti *et al.,* 2014; Bucur, 2021). The recommendation is to remove 30-35% of the leaves above the grape area, which are photosynthetically active, preventing excessive sugar accumulation, sunburn on the grapes.

Severe leaf removal from the area above the grapes before veraison has delayed the ripening of the grapes in the Rhin Riesling variety by about 2 weeks (Stoll *et al.,* 2009).

Leaf removal applied after grape veraison, at the age of 50-60 days (the most active photosynthetically) causes a slowdown in the accumulation of sugars. Research conducted by Palliotti *et al.* (2014) on the Sangiovese variety in central Italy, with the removal of 30-35% of the leaf area of the shoots (from the middle-upper part) when the sugar concentration of the must was 14-15°Brix reduced it by 1.2°Brix when the grapes were harvested, without affecting the titratable acidity, the anthocyanin content and the total polyphenols.

Large-scale removal of leaves is an important measure for delaying grape harvesting and obtaining low-alcohol wines with pleasant aromas in hot and dry years, without affecting the quality of white wines (Heßdörfer, 2020). By changing the ratio of leaf area to yield ratio, the development of phenophases is delayed, harvesting of grapes takes place after the heat waves and the quality of production improves (Martinez de Toda & Balda, 2013; Parker *et al.*, 2014; 2015).

Leaf area to fruit weight ratio, which decreases as a result of applying these green operations at values of 0.6-0.8 m^2/kg, contributes to the delay of the grape harvest and to the decrease of the sugar concentration of the berries. The reduction of this ratio to the Fetească regală variety to values lower than 0.6 m^2/kg (Figure 2) lead to a significant reduction of the sugar concentration, by over 20 g/L (Dejeu *et al.*, 2005).

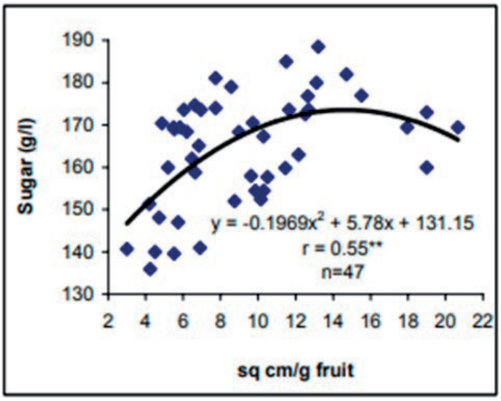

Figure 2. Correlation between cm^2 leaf area/g fruit and the sugar accumulations in the fruit (g/l), 2004

The research carried out at Valea Călugărească for the Cabernet Sauvignon variety confirmed that at low values of the leaf area to fruit weight ratio (6-8 cm^2/g grape, respectively 0.6-0.8 m^2/kg) the obtained sugar concentrations was 180-185 g/L, compared to 197-205 g/L (Figure 3), at optimal values of 1.0-1.7 m^2/kg (Belea, 2008).

Application of sunscreen substances

The use of substances with antitranspirant action causes a partial closure of the stomata, a reduction of photosynthesis and water loss through transpiration, preventing the berry shrivelling under conditions of water stress (Rosati, 2007).

Foliar treatments with ***kaolin***, a natural clay rock, white $Al_2Si_2O_5(OH_4)$ cover the leaves and berries with a film of nanoparticles, provide sun protection, reduce the effects of heat stress, reduce leaf temperature by 4-6°C, prevent phenomena photoinhibition, avoids leaf discoloration, sunburn, and improve the quantity and quality of yield (Frioni *et al.,* 2019).

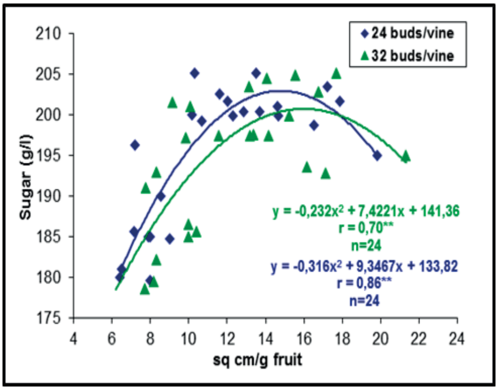

Figure 3. Correlations between the values of the ratio cm^2 leaf area/g fruit and the sugars accumulation in the fruit (g/l), depending on the buds load, average 2004-2006 (Belea, 2008)

It has been found that the use of antitranspirants reduces water loss through transpiration by preventing the berry shrivelling and sunburn under conditions of heat, water and radiation stress. These substances provide sun protection, forming a film of inert particles on the surface of leaves and berries that improves plant metabolism under such conditions (Santos *et al.,* 2020).

The use of kaolin under conditions of heat and water stress, combined with deficit irrigation, increased the anthocyanin content in Merlot berries (Song *et al.,* 2012).

The use of kaolin with pinolen in Cabernet Sauvignon under water stress in southern Italy has increased the efficiency of water use, without affecting the weight of grapes and berries, or the concentration of sugars; instead, the anthocyanin content increased significantly, the quality of the wine obtained being improved (Brillante *et al.,* 2016).

Leaf removal, combined with kaolin treatment, has increased the anthocyanin content of berries in the Muscat de Hamburg variety, without affecting the composition of volatile substances (Kok & Ball, 2018).

By foliar application, immediately after the grape veraison, of a repeated treatment with kaolin (5%), Dinis *et al.* (2016) under conditions of heat and water stress, the Touriga National variety cultivated in the Douro region

(Portugal) showed a 40% increase in the total phenol content, 24% increase in flavonoids and 32% in the anthocyanin content. It has also improved the antioxidant capacity of berries as a result of increasing the content of secondary metabolites.

Other authors have showed the protective effect of kaolin on the anthocyanin content of grapes (Kok & Bal, 2018).

The use of kaolin and ***zeolite*** in a concentration of 3%, in two rounds at the beginning of the grape ripening, in the Sangiovese variety, on leaves and grapes, determined a reduction of the berries' temperature, without affecting the gas exchange, grape yield and sugar accumulation. Moreover, the cooling effect of the berries contributed to the increase of the anthocyanin content in grapes and wine (Valentini *et al.,* 2021).

Chitosan, a natural biopolymer applied to leaves and grapes, also has an antitranspirant effect, thus avoiding the negative effects of water stress (Khalil & Badr Eldin, 2021).

Other sunscreens have also been used with good results, such as ***calcium carbonate*** ($CaCO_3$) and ***potassium silicate*** (K_2SiO_3) (Santos *et al.,* 2020).

A natural product obtained by distilling coniferous resins, ***based on pinolen*** (2%) applied after the grape veraison, leaves in a few hours after treatment, a thin, transparent film, which partially limits the gas exchange for a period of 40-50 days, causing a significant reduction or delay in the accumulation of sugars in the berries (Palliotti *et al*., 2013b). The treatment is recommended to be applied when the concentration of sugars in the must is about 14-15° Brix.

Foliar treatments with a natural ***product derived from inactivated yeast*** (*Saccharomyces cerevisiae*), in a dose of 1 kg/ha, applied in two stages, at the beginning of the grapes veraison and the second, after 12 days, determined an improvement in balanced wines of the Syrah variety grown in Hungary (Villangó *et al.,* 2015).

Foliar treatments with 3 elicitors ***(methyl jasmonate, commercial yeast extract and chitosan)*** applied to Tempranillo variety, to the grape veraison and one week later, increased the anthocyanin content of grapes and wine, in the case of the first two substances (Portu *et al.*, 2016).

Application before veraison of ***exogenous auxins*** (naphthyl acetic acid) at a concentration of 50 mg/L on grapes of the Shiraz variety grown in Australia (two treatments every 5 days) delayed the ripening of the grapes by 10 days, without affecting the accumulation of sugars, the anthocyanins and the organoleptic properties of wines (Böttcher *et al.,* 2011).

Irrigation

Irrigation is used to improve grape yield and quality, whenever rainfall is scarce, to ensure water requirements for vines, especially under conditions of drought exacerbated by climate change.

The measure is based on deficit irrigation (DI) strategies (e.g.: deficit regulated irrigation, sustainable deficit irrigation, partial drying of the root zone) to take advantage of the relationship between soil water, plant and yield/quality ratio. Considering these strategies, drip irrigation is generally implemented as the most efficient method of saving water (Santos *et al.,* 2020).

Deficit irrigation during grape ripening has led to a significant increase in the content of anthocyanins and tannins in the Merlot variety (Bucchetti *et al.,* 2011).

Deficit irrigation applied to the Sangiovese variety, with the use of a volume of water equivalent to 33% of the consumption by evapotranspiration achieved in the previous week, in the interval between the beginning of berries growth and grapes veraison led to an increase in yield, a reduction in soluble content from must, without affecting the concentration in anthocyanins and phenolic substances (Lanari *et al.,* 2014).

Late irrigation, applied to the veraison, has a greater effect if combined with the shoots topping because it causes a greater number of lateral shoots, which intensifies the competition for synthesized substances (Palliotti *et al.,* 2014; Santesteban *et al.,* 2017).

The combination of irrigation with the shoots topping causes a delay in the grape ripening, allowing full ripening at lower temperatures and a pronounced synthesis of aromas and phenolic substances.

Late application of irrigation, combined with the shoot topping, can be used to invigorate vegetative growth, leaving young lateral shoots, thus slowing down the accumulation of sugars in the berries.

The application of irrigation only during the veraison-harvesting period, under conditions of a warm, arid climate in Spain has led to a reduction in the accumulation of sugars in berries of Cabernet Sauvignon, without changing the composition of phenols and wine quality (Fernandez *et al.*, 2013).

For efficient water use, the use drip irrigation, deficit irrigation strategies is recommended. The application of deficit irrigation with the partial replacement of water consumed by evapotranspiration in different periods (even in the veraison - grapes harvest) under conditions of water stress has led to an optimization of vegetative, productive and qualitative performance of vines (Costa *et al.,* 2016; Keller *et al.,* 2016).

Numerous studies have been conducted, highlighting the deficit regulated irrigation, as well as that by partial drying of the root zone, which improve the efficiency of water use, in areas affected by drought (Romero *et al.,* 2022).

Deficit controlled irrigation together with Zn foliar fertilization of Alphonse Lavallée and Italy varieties have improved berries' color and their physical characteristics (pedicel peeling strength and resistance to berry cracking) (Sabir *et al.,* 2021).

Soil management

In the face of climate change, soil management needs to be reconsidered and less applied to keep water in the soil, especially on shallow and eroded soils on sloping land.

The application of the soil management must take into account the pedoclimatic conditions (the capacity of the soil to retain water, the volume of precipitation, excessive temperatures, increased evapotranspiration, etc.).

In general, under conditions of water stress, it is recommended to reduce the number of soil mobilizations (Walg, 2009; Dobrei *et al.*, 2015). Cataldo *et al.* (2021) appreciate that proper soil management for new conditions improves the quality of production.

It is recommended to apply those works that favor soil fertility, water loss, avoiding the mineralization of organic matter in the soil. Under such conditions, the system of minimum tillage practices, soil mulching and green cover are of particular importance.

Minimum tillage is a system of surface practices characterized by tillage without turning the furrow and partial incorporation of plant debris. The use of the „minimum tillage" system, together with mulching (partial or total) with straw, under conditions of thermo-hydric stress had a favorable effect on production and quality (Enache & Donici, 2014).

Soil mulching made with different materials causes a significant increase in the content of anthocyanins in grapes, soil improvement, improves the microclimate in the vine and prevents weed growth. In such cases, the vine suffers less from heat and water stress (Fraga & Santos, 2018), it prevents soil erosion, maintains soil moisture, prevents water loss through evaporation and the efficient water use improves the penetration of water into the soil. Both organic substances (grape marc, compost, vine pruning, tree bark, plant debris, animal manure, straw) and inorganic substances (plastic, geotextiles, etc.) can be used for mulching (Hosteler *et al.,* 2007).

Following the influence of soil maintenance by mulching in viticulture in an experiment conducted in 6 wine centers in Romania, Şerdinescu *et al.* (2013) found a decrease in the disruptive effect of the climate change. Compared to the maintenance of the soil as a black field (total or partial) mulching has ensured, under conditions of severe drought, reduced water loss through the soil by 14-20%, higher yields by 29-49% in the case of total mulching with straw and 6-22% for partial mulching with grape marc compost. There was also a significant increase in the total number of microorganisms in the case of soil mulching, more pronounced in total straw mulching, compared to maintenance as a black field (Brînduşe *et al.,* 2013; Şerdinescu & Brînduşe, 2014a; 2014b).

Green cover in viticulture (with spontaneous or cultivated species), especially during rainy periods, must take into account the minimum competition for water, favoring the infiltration of water into the soil, improving soil fertility,

preventing erosion and leaching in depth of nutrients etc. At the same time, green cover provides favorable conditions for predators and parasites, useful in biological control.
It has been found that the species *Festuca ovina, Festuca arundinacea, Lolium perenne, Hordeum vulgare, Trifolium incarnatum* and *Poa pratensis* and grasses in general are more adapted to global warming. Also, under conditions of excessive temperatures and dry periods, the soil temperature values were lower at green cover.
Abad *et al.,* (2020) found in Spain that the species *Trifolium fragiferum* have favorable influences on the soil and vines, when used for grassing in the direction of the rows (under-vine cover crop), preventing the development of weeds.

Pest and disease control

Climate change can help lower the pressure on diseases and pests, requiring fewer treatments. Disease and pest control must take into account the phenology of the vine that is greatly affected by global warming, changes in plant susceptibility to pathogens, and the behavior of vectors (Reineke & Thiéry, 2016).
Trichogramma, as parasitoid eggs of grapevine moth (*Lobesia botrana*), a wasp useful in biological control is favored by high temperatures and drought.
Scaphoideus titanus, a vector of flavescence dorée phytoplasma is rapidly expanding in the face of climate change.
The vineyards must be carefully controlled under the new conditions to detect the emergence of new diseases and pests.
Global warming influences the manifestation of diseases and pests in vineyards. Thus, it was found that the esca and flavescence dorée are more virulent under the new conditions (Maixner *et al.,* 2007). The main vector of phytoplasma, the causal agent of black wood disease (bois noir) *Hyalesthes obsoletus*, is also favored, and populations of predatory mites are greatly diminished compared to harmful ones (Corino *et al*., 2004).
According to research in northwestern Italy, Caffarra *et al.* (2012) appreciated that global warming is leading to an increase in the number of generations for grape moth (*Lobesia botrana*) and a sensitization of the vine to the attack of powdery mildew (*Erysiphe necator*).
Bois *et al.* (2017) found that gray mold (*Botrytis cinerea*) occurs less frequently in drier conditions.

MEDIUM-TERM ADAPTATION MEASURES

Training systems

Training system by increase trunk height delays grape ripening with 3-5 days, as a result of reducing maximum temperatures in the grape area (van Leeuwen & Destrac-Irvine, 2017). By switching from the low form of the vine to the semi-high form, Matei *et al.* (2009) found a lower accumulation of sugars in the berries of the Fetească regală variety in the period 2006-2008 by 7.2-19.2 g/L.
Carbonneau (2011) recommends lyre or modified lyre systems which could be adapted according to the actual weather situation for optimal berry development. Leaf removal is recommended, as another tool for keeping the traditional wine style.
The choice of pruning systems that do not allow the grapes to be exposed to direct sunlight (pergola, free cordon, Geneva double curtain) ensures conditions that contribute to maintaining the production-quality balance in the context of global warming (Novello & de Palma, 2013). These pruning systems provide diffused light to the grapes, especially in drought conditions.

Delaying of pruning

By delaying pruning when the apical shoots on the canes were 5 cm long, in the New Zealand variety of Merlot, Friend & Trought (2007) obtained a lower accumulation of sugar in the berries and a higher acidity content.
In the case of spur pruning system, applied after budding to the Sangiovese variety grown in central Italy, the content of soluble substances in the must was lower by 1.6°Brix, compared to the usual pruning during the rest period, and the titratable acidity - much higher by 1.8 g/L (Frioni *et al.,* 2016).
Buesa *et al.* (2021) found in the Bobal and Tempranillo varieties grown in eastern Spain, that the late pruning, before the budding of the basal budbreak, delayed the ripening of the

grapes, with a greater accumulation of anthocyanins in the berries. The delay of the ripening of the grapes by two weeks was obtained together with the late pruning, the minimum pruning and the severe shoot topping, as well as the leaf removal in the apical area of the shoots (Gutierez-Gamboa *et al.,* 2021).

Minimal pruning

Minimal pruning helps to increase the buds load and the number of shoots on the vine, resulting in an increased number of loose grapes, evenly distributed in the dense foliage wall, so that the skins of the berries become richer in phenols, harvesting can be done at small concentrations of sugars (Clingeleffer, 2007). Minimum pruning in the Rioja region has led to an increase in grape yield, a reduction in grape sugar content and an improvement in anthocyanin content in red wine varieties (Zheng *et al.,* 2017).

Use of shading nets

The use of shading nets is an effective means of mitigating the negative effects of excess radiation and temperature on grapes in warm climates. Nets that provide a shading of 35% installed on the veraison have led to delayed ripening, improved quality and better storage capacity for Sultana Seedless table grapes (Sen *et al.,* 2016).

In a study conducted in Italy, Valenti *et al.* (2012) found that the use of shading nets for Chardonnay and Pinot noir varieties led to a delay in grape ripening, preservation of acidity and improved sensory profile of wines.

The use of shading nets covering the area of grapes, 1 m wide, along the rows, from the veraison to the ripening of the grapes, which ensures a degree of shading of 20%, has led to an increase in the content of anthocyanins in the berries of the Cabernet Sauvignon variety (Martinez-Lüscher *et al.,* 2017).

It has been found that by using shading nets, the temperature of the grapes decreases by up to 7°C (Lobos *et al.,* 2015). Shaded hubs are more susceptible to prolonged summer drought when soil water reserves are low.

LONG-TERM ADAPTATION MEASURES

Relocation of vineyards

The long-term strategy provides for changes in the choice of land for the establishment of plantations (at higher latitudes, as well as at other altitudes, where the most favorable conditions are found). At the same time, land exposures with lower values of solar radiation and air temperature will be chosen.

According to the A1B scenario (2041-2070) Malheiro *et al.* (2010) finds a northward shift in favor of vine cultivation and a decrease in southwestern Europe (Figure 4).

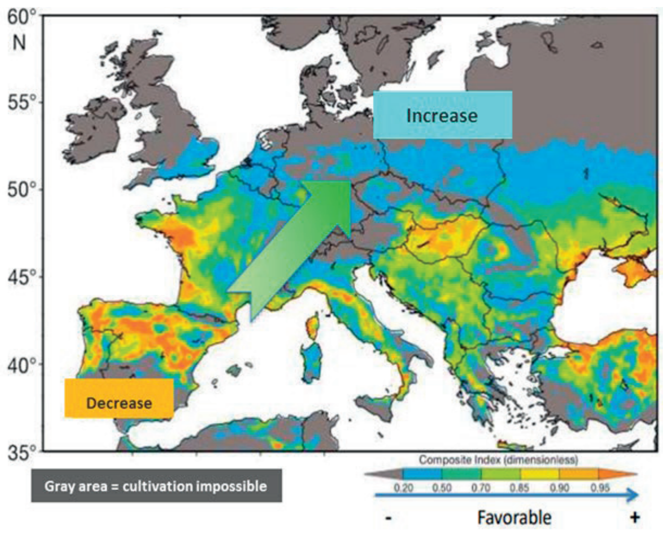

Figure 4. Evolution of lands favorable to vines at European level (Malheiro *et al.,*2010; Touzard *et al.,* 2021)

In Romania, will a decrease in climate favorability for vines in the southern part of the country and an increase in the northern parts of Moldova, center of Transylvania, the west of the country and the Black Sea coast.

This category also includes the orientation of the rows, which ensures better shading of the vines, which can ensure a proper maturation, avoiding sunburn and reducing the attack of gray rot (*Botrytis cinerea*) (Friedel *et al.,* 2013). In the future, the lands with northern, north-western and western exposures will be preferred, where the heliothermal resources reach lower values. Naulleau *et al.* (2021) emphasized the importance of east-west row orientation in reducing water stress.

If until three or four decades ago, the northern limit of cultivation of red wine and table grapes varieties with large berries was in the southern half of our country (Oşlobeanu *et al.,* 1991), as a result of the increase in thermal and solar radiation resources, the cultivation has expanded considerably throughout the country.

A good example is the success of the Syrah variety of Mediterranean origin, together with Merlot and Cabernet Sauvignon, in the Răteşti wine center - Beltiug area in Satu Mare county (Popescu, 2011).

Also, as a result of climate change, in the last decade, red wines have started to be produced in the Cotnari vineyard, and plantations for white wines were relocated to altitudes higher than 200 m (Irimia *et al.,* 2012; Quènol *et al.,* 2016).

It is also estimated that at European level, due to global warming, the northern limit of vine cultivation will move north to the latitude limit of 55° (Malheiro *et al.,* 2010). As early as 1992, Kenny and Harrison forecasted a displacement of the northward limit of the viticulture in Europe by 10-30 km per decade by 2020, with this rate doubling between 2020 and 2050.

The combined effect of heat, radiation and water stress can also be avoided by using land with different exposures to the southern ones (or close to them), which leads to a lower interception of solar radiation, and a decrease in water consumption through evapotranspiration.

The risks of sunburn are lower if the rows are oriented, when setting up plantations, in an east-west direction (Friedel *et al.,* 2013).

Choice of varieties and clones adapted to global warming

The diversity of vine varieties is an important resource for adapting to climate change (Destrac-Irvine *et al.,* 2016; 2020; Antolin *et al.,* 2021).

The choice of varieties when setting up plantations is a long-term measure and an important means of delaying grape ripening by up to two weeks. This choice can be made from existing varieties (for example, Cabernet Sauvignon can be planted instead of Merlot, which ripens up to two weeks later, under more favorable conditions). At the same time, new varieties can be introduced, better adapted to climate change.

Viticulture has many varieties, with different ripening periods, from early to very late, the latter undergoing the phenophase of ripening grapes at more favorable temperatures in early autumn. Following the adaptation to climate change of a large number of varieties (52) cultivated in the Bordeaux region, Destrac - Irvine *et al.* (2016) found differences in flowering of up to 20 days, and in the veraison of about 40 days.

Sadras *et al.* (2013) found differences between varieties in terms of the effects of rising air temperatures; there were highlighted substantial changes in titratable acidity and pH in Cabernet Franc and Chardonnay varieties, while in Shiraz varieties the changes were insignificant.

Many research projects have been carried out in different European countries on measures to adapt to climate warming, with special reference to the behavior of varieties under the new conditions (LACCAVE, Clim4Vitis and ADVICLIM, etc.) (Quénol *et al.,* 2014; Ollat & Touzard, 2014; Santos *et al.,* 2021; Touzard *et al.,* 2021).

Under the conditions of climate change, it is interesting to cultivate many varieties with late maturation, resistant to heat waves and drought (Cabernet Sauvignon, Syrah, Sangiovese, Carignan, Grenache, Mourvèdre, Marcelan, etc.).

A priority objective for the genetic improvement of vines is to obtain varieties adapted to the new global warming conditions, which ensure sustainable, high quality production (Duchêne *et al.,* 2010). Based on the results obtained in some wine centers in the western part of the country, Dobrei *et al.,* (2015) appreciated that the local viticultural germplasm, well adapted to the new conditions, can be a viable alternative to climate change and an important source of typicity and authenticity of wines.

It is also important to obtain new varieties with late maturation, with superior quality characteristics, as well as to reconsider the old local varieties adapted to the new conditions.

An objective of the clonal selection is to obtain clones with lower accumulation of sugars in the berries, with higher content of titratable acidity, without affecting the aromatic and phenolic profile (for red wine varieties).

Urucu (2014) performed a comparative study on the behavior of different Cabernet Sauvignon clones in the Bolovanu-Sâmbureşti wine center, with 4 clones of the Cabernet Sauvignon variety (Table 1), two of French origin (169 and 15) and two from Romania (7-Dg and 4-Iş). This study highlighted a delay in grape ripening and a lower sugar content of 3 clones (169/SO 4; 15/101.14 and 7-Dg grafted on rootstock SO 4), the difference being up to 20-30 g/L, as an average of the period 1999 - 2011.

Choosing clones with larger, loose grapes could counteract the rapid accumulation of sugars in the berries.

It is estimated that viticultural interventions to adapt to climate change are less expensive than those in winemaking, such as de-alcoholization of wines by physical means, such as reverse osmosis and ultrafiltration (Novello & de Palma, 2013; Palliotti *et al.,* 2015).

Table 1. The main characteristics of the grape composition in 4 clones of Cabernet Sauvignon at the time of technological maturity, average data from 2009 -2011 (Urucu, 2014)

Clone/Rootstock	Sugar (g/L)	Titratable acidity (g/L tartaric acid)	Total poliphenols (mg tartaric acid/ kg grapes)	Total anthocyanins (mg/kg grapes)
169/3309	238	6,8	4345	1328
169/SO 4	203	7,5	4931	1504
169/101.14	212	6,2	4588	1332
15/3309	235	5,7	4792	1583
15/101.14	201	7,6	4701	970
15/SO 4	212	6,6	4740	1346
7 Dg/SO 4	208	6,03	2142	900
4 Is/SO 4	213	6,0	2946	1050

Choice of rootstocks

The rootstocks used in the new plantations must not only ensure the resistance to phylloxera, but also to drought, at high temperatures, which will induce the late varieties of grape ripening, without affecting their quality.

The rootstock used for grafting can delay the grape entry in veraison and ripening of the grapes by about a week. There are rootstocks with good drought resistance: 140 Ruggeri; 110 Richter; 1103 Paulsen; 1444 Paulsen etc.

Ion (2006) found the significant influence of the rootstock partner on the accumulation of sugars in the Cabernet Sauvignon, Merlot, Fetească neagră varieties, cultivated in the Valea Călugărească viticultural center. Thus, in the case of the Fetească neagră grape variety grafted on the 140 Ruggeri rootstock, lower concentrations of sugars in the must (209 g/L) were accumulated, compared to the Oppenheim Selection 4 - 4 (222 g/L).

Use of mobile photovoltaic panels

The use of mobile photovoltaic panels above the rows of vines at a height that allows the work to be carried out is also a means of combating climate change. The orientation of the panels to meet the physiological needs of the vine can also ensure maximum shading when the solar radiation is excessive and contributes to reducing water consumption by 20%. At the same time, the panels ensure the production of renewable electricity and under clean conditions. It was found that under the conditions of using solar panels, less alcoholic wines with an improved aromatic profile are obtained (Pelsy & Merdinoglu, 2021).

CONCLUSIONS

The effects of global warming (rising temperatures, solar radiation, drought) on vines require the adoption of measures to mitigate and adapt viticulture to the new conditions.

Given that this phenomenon is expected to intensify in the future, adaptation measures are becoming increasingly important.

We reviewed over 100 papers on adaptation strategies to climate change in vineyards. These have been grouped into short, medium and long-term adaptation strategies. The short-term ones, which become a priority, refer to canopy management, application of sunscreen substances, soil management, pest and disease control.

Medium-term strategies include a series of measures related to changing cultural practices: new training systems, minimal pruning, late pruning, irrigation, use of shading nets.

Long-term strategies for adapting viticulture to climate warming refer to: site selection, relocation of vineyards, row orientation, exploitation of the diversity of vine varieties and the breeding of new varieties and rootstocks tolerant to heat and drought etc.

The application of these measures must take into account climate change evolution and local conditions.

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EVALUATION OF QUALITY OF 'SULTANINE' AND 'CORINTH' RAISINS OBTAINED FROM GRAPES GROWN IN A COOL CLIMATE

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Abstract

Two Vitis vinifera grape varieties 'Sultanine' and 'Corinth' were used to obtain raisins. The vines are grown in a cool climate (Cluj Napoca - in NV of Romania, an area not included in a viticultural area). During 2020, the grapes were harvested at 195 g/l - 'Sultanine' and 234 g/l for 'Corinth'. The berries were dehydrated using a household Gorenje 240W drier, at 40°C, for 24 hours. The soluble dry matter content for raisin was higher for raisins of the 'Corinth' variety (61.11%) and for the 'Sultanine' (59.89%). Regarding the moisture of the finished product - raisins, a high content was determined for the variety 'Sultanine' - 19%, followed by the variety 'Corinth' - 15.61%. The rehydration power of raisins had values of over 80% for both types of raisins. For the amateur culture, these varieties can be recommended, given that, in recent years, due to climate change, there have been no temperatures in this area during the rest period that could cause frost over the winter. The warm autumn favoured reaching full maturity for these varieties, even in the conditions in Cluj. Raisins can be easily obtained in the household, by using household dryers..

***Key words**: 'Corinth', dried berries, dried grapes, grape, raisin, 'Sultanine', quality.*

INTRODUCTION

Drying fruit is an ancient practice for the preservation of food that is still in use nowadays (Coimbra et al., 2011). Raisins are obtained by drying different cultivars/varieties of *Vitis vinifera* L. The 'Thompson Seedless' variety 'Sultana') covers approximately 95% of the market. Raisins have been included in the human diet since ancient times, for their energetic value but also for the presence of specific nutritional compounds (Restani et al., 2016). From the nutritional point of view, raisins are often considered negatively due to their high sugar content; as a consequence, their inclusion in the diet of children, obese and diabetic subjects is debated (Wang et al., 2016). The main raisin producers are Turkey, USA, China, and Iran, contributing to approximately 73% of the global raisin production for 2018/19 (USDA, 2019). The process of raisin production from grapes includes three main steps, pre-treatment, drying, and post-drying. The pre-treatment step is optional but is usually employed to remove the waxy layer formed on the skin of the grape during the ripening stage, providing a barrier to permeability and water diffusion (Pangavhane and Sawhney, 2002; Carranza-Concha et al., 2012; Wang et al., 2012).

Grapes can be dried in the sun, in an oven, or in a food dehydrator by using the right combination of warm temperatures, low humidity, and air current (Lokhande et al., 2016). Mechanical or conventional drying was developed to coincide with the commercialization of raisins and the need to meet the consumer's demands. Grapes are placed in dehydration tunnels for at least 24 h, using air circulation at a controlled temperature (Benlloch-Tinoco et al., 2015).

It is known that raisin is grown in a warm climate, to obtain the best quality production (Uysal and Karabat, 2017). Each year, the weather can be different and consequently has a big impact on the harvest outcome, hence creating great, good, and poor grapes. The 'micro-climates' within a larger climate type can often occur. Cool climate regions definitely get just as hot as warm climates in vegetation season, but the temperatures drop off so quickly towards harvest and that makes it difficult for grapes to ripen (Tarko et al., 2014).

Our work aimed to characterize the quality of raisins obtained from 'Sultanine' and 'Corinth' grape varieties grown in a cool climate. In this study, we investigated the grape quality before drying, such as sugar content and total acidity. The dehydration was made using a household drier. The quality of the raisin was tested such as moisture content, total soluble mater, level of tartaric and ascorbic acid, pH, and rehydration capacity.

MATERIALS AND METHODS

Grapes were harvested during the 2020 season, at commercial harvest, in Cluj-Napoca (46°46′0″N, 23°35′0″E) located in the center part of Transilvania (northwest of Romania). Cluj Napoca is not included in a viticultural area, but the climatic condition of the 2020 year, favored the ripening of grapes. Three kilograms of grapes from both varieties were collected. One kilogram was used for mechanical analysis and two kilograms were used for raisin. For the mechanical analysis, the components of the grapes are determined gravimetrically and numerically: the number of berries, skin, seeds, and pulp. Based on those data, some uvological indices were calculated, as follows:

Grape structure index = berries weight/rachis weight

Berry index = number of berries on 100 g of grapes

Berry composition index = pulp weight/(skin weight + seeds weight)

Also, the 100 berries' weight was calculated, following the formula:

100 berries weight (g) = (total weight of berries/number of weighed berries) x 100

After weighing the samples, the content of sugar and total acidity level was determined. The samples were analyzed in the Laboratory of Oenology of USAMV Cluj-Napoca, for each sample, three repeats were used. The determination of sugar in must samples was performed using the hand refractometer Zi du Bompas F-49120 (Alla France) with an accuracy of 0.02, according to Bora et al., 2014. To determine the total acidity of the must samples, titration was used in the presence of phenolphthalein. The principle of the method consists in determining the must acids with an alkaline solution of sodium hydroxide (NaOH), based on the constant that a milliliter of NaOH 0.1N can neutralize 0.0049 g sulfuric acid. The total acidity is expressed in g/L H_2SO_4, according to Bora et al., 2014.

The other two kilograms of each variety were used to obtain raisins. After washing the grape samples and draining the rest of the water, the bunches sat on the grills of the Gorenje FDK24DW, 240W, 35-70° electric dryer. The grapes were dried at 40°C for 24 hours. After dehydration, the raisins were stored at 4°C until analysis.

Determining the degree of moisture content of dehydrated products was made according to the formula (AOAC 20 013, 1997):

Moisture content (%) = ([initial weight - final weight]/initial weight)

Establishing the rehydration capacity of dehydrated products as determined by the method proposed by Rozsa, 2020, and using the following formulas.

The rehydration ratio Rr represents the ratio between the mass of the rehydrated sample (R) and the mass of the dehydrated sample (D), is calculated according to the relation:

RR = the mass of the rehydrated sample/the mass of the dehydrated sample

The water content of the rehydrated sample is as follows:

$WC = \{R-[D-(DxU)]/R\} \times 100$

in which:

WC = amount of water of the rehydrated sample, in %;

R = mass of the rehydrated sample, in g;

D = mass of the dehydrated sample taken as analysis, in g;

U = the amount of water contained in one gram of product dehydrated in g.

The rehydration capacity represents the percentage of water in the rehydrated material and is calculated according to the relation:

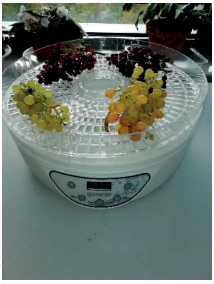
$Rc = R/[D-U/100-W)$

in which:

Rc = rehydration capacity, in %;

W = water content of the fresh product, in %

Products that absorb more than 80% of the water lost during dehydration in the rehydration process are considered to be of very good quality, and when the water absorbed is below 50%, the products are considered to be of poor quality (Rozsa, 2020).


a)


b)

Figure 1. a) The drying of the grapes to obtain raisin; b) left - the fresh berries before drying and right - the raisins

The soluble solids of the liquid phase of the samples (◦Brix) at 20°C were made using the refractometer Atago NAR-3T, Tokyo, Japan. Total acidity of the raisin was measured by titration with NaOH (0.1 N) and expressed in mg of the main acid (tartaric acid, TA) and ascorbic acid (AA) was determined by titration according to AOAC 985.33 (1997) and Carranza-Concha et al., 2012. The pH was measured with the InoLab PH 720 WTW pH-meter, with an accuracy of 0.001.
Statistical analyses were performed using the statistical software package SPSS (version 18.0; SPSS Inc., Chicago, IL, USA). The data were expressed as the mean of three replications for each sample analyzed. To determine the significant differences among values, an analysis of variance (ANOVA) was performed. The significance of difference was defined at 5%, 1%, and 0,01%.

RESULTS AND DISCUSSIONS

The resistance of grapes to external mechanical influences, to pressing, crushing, to the detachment of the pedicel, which largely determines the resistance to transport or storage, are the mechanical properties of grapes. Determining the mechanical properties of grapes is especially important for table and raisin grape varieties that are transported over long distances or kept fresh for a long time (OIV, 2016). The mechanical properties of grapes show high variability between different varieties.
The mechanical properties are dependent on the degree of ripeness of the grapes, on their position on the vine, on the position of the berries on the grapes, and, of course, on their sanitary condition. The size of the berry also plays an important role in determining the resistance to crushing or detachment of the pedicel. The mechanical composition of the grapes varies depending on the variety, the limits of variation, in this case, being large and characteristic of the specific characteristics of the varieties and their direction of use.

Table 1. Mechanical analysis per 1 kg of grapes

Vitis vinifera L. grape variety	No. of berries	% degraded berries	Pulp (g)	Skin + Seeds (g)	Rachis (g)
'Corinth'	931	36.29	641	272	86
'Sultanine'	417	10.63	769	166	65

In the studied varieties, the number of berries in a kg of grapes varied from 931 berries to the 'Corinth' variety, to 417 to the 'Sultanine' variety (Table 1). The health of the grapes particularly influences the data obtained from the mechanical analysis; therefore, it is necessary that in the study varieties for the knowledge of their qualitative potential. Under the conditions of 2020, during the vegetation period, large amounts of precipitation were registered, which led to the deterioration of the berries, differently, depending on the variety. In the 'Corinth' variety, the damaged grains were over 36%, while in the 'Sultanine', almost 11% of the berries in the bunches were damaged or attacked by *Botritys*.

Table 2. Uvological indexes

Vitis vinifera L. grape variety	Grape structure index	Berry index	Berry composition index
'Corinth'	10.67	101.97	2.35
'Sultanine'	14.38	44.59	4.63

The relationships between these uvological components are expressed by 3 indices. The structure index of the 'Sultanine' variety is 14.38, this being the higher of the two studied varieties. The 'Corinth' grape variety had the lowest value of the structure index, of 10.67, this is because, in this variety, the amount of rachis is higher.

The berry index, on the other hand, shows the lowest values for table varieties and the highest, sometimes exceeding 100, for wine varieties with very small berries. Regarding the berry index, from the data in Table 2, it is noticed that the 'Corinth' variety has the highest value of this index (101.97) which shows, among the analyzed varieties, for this variety, the berries are the smallest. Instead, the 'Sultanine' variety has the largest berries, with a berry index value of 44.59.

The composition index of the berry has high values, 10-15, for the varieties with large grain, in which the pulp massively dominates the skin, such as 'Afuz Ali', 'Muscat of Hamburg' or 'Cardinal'. Regarding the composition index of the berries, the varieties that have a thinner skin ('Sultanine') have a value of this index of 4.63. In contrast, for the varieties with thicker skin ('Corinth'), the values of this index were 2.35 (Table 2).

Regarding grape quality (Table 3) under the conditions of 2020, the best accumulation of sugar in the berries was in the Corinth variety, with 243 g/l. Instead, the lowest amount of sugar was in the 'Sultanine' variety, with 195 g/l, although, the Cluj area is not a viticultural area. The highest value of the mass parameter of 100 berries was determined for the 'Sultanine' variety, with 224 g. In exchange, the 'Corinth' variety had the highest value of the mass of 100 berries, with only 98 g.

Table 3. Evolution of Milk Production (kg/year)

Vitis vinifera L. grape variety	Grape weight (g)	100 berries weight (g)	Sugar content (g/l)	Total acidity level (g/l H_2SO_4)
'Sultanine'	434^{***}	224^{***}	195^{o}	3.67^{o}
'Corinth'	66^{ooo}	98^{ooo}	234^{*}	4.43^{*}
Mean	250	161	219	4.05
DL 5% =	12.90	3.79	12.25	0.32
DL 1% =	29.94	8.80	28.42	0.98
DL 0.1% =	94.88	27.87	90.00	2.23

In general, fresh fruit consists of water and dry matter. If the water is removed from the fruit, the dry matter remains. The remaining part is generally called total dry matter. This is because dry matter consists of two different elements.

Part of the total dry matter is composed of water-insoluble matter. These water-insoluble substances are called water-insoluble dry matter. Part of the total dry matter is composed of water-soluble substances (Rosza, 2019). In general, drying treatments caused a decrease in sugar content. After drying, the raisin content of the total dry matter was 59.89% Brix for 'Sultanine' and 61.11% Brix for 'Corinth'. Our results are lower than the values obtained by Isçi and Altındisli, 2015 for 'Sultanine' raisin, with 68.89-78.67% total soluble solids content in different drying methods.

It is known that tartaric acid is the most abundant organic acid in grape varieties (Chayjan et al., 2011). The summary of pH and tartaric acid results of the raisin sample is presented in Table 4. In the raisin samples, pH values ranged from 3.92 for 'Sultanine' to 3.31 for 'Corinth'. On the other hand, tartaric acid values ranged from 509.11 mg/100 g for 'Sultanine' to 422 mg/100 g. Ascorbic acid is the most potent enhancer of nonheme iron absorption. The enhancing effect occurs largely because of its ability to reduce ferric to ferrous iron. In the raisin samples, ascorbic acid values ranged from 9.82 mg/ 100 g for 'Sultanine' to 7.79 mg/100 g.

Both acids are affected by the drying treatments, causing losses in almost every case. This is because the skin of the grapes could change much in the drying process and could not protect the acid from the effects of oxygen. On the other hand, it is well known that ascorbic acid is seriously affected by high temperatures (Vikram et al., 2005).

For fruit, the value of 25% was maintained for a long time as the upper limit of the final humidity; today this limit has been reduced for many fruit species, depending on their chemical composition and, in particular, their sugar content (Rosza, 2019). Due to the considerable high sugar and moisture content, seedless grapes are very susceptible to microbial spoilage during storage (Carranza-Concha et al., 2012).

If the raisin after being dried and washed has more than 18% of moisture, it will be contaminated with molds, and if the moisture content is less than 11%, it will have an undesirable flavor and have a hard mouthfeel (Kowalska et al., 2017). The most appropriate moisture content for raisins should be between 14 to 16% (Bakhshipour et al., 2012). The moisture level of a raisin in our experiment was 19.41% for 'Sultanine' and 15.61% for 'Corinth'. Other authors obtain a moisture level between 9.70-16.27% for 'Sultanine' raisins, depending on the drying method.

An important indicator of the quality of dried material is its rehydration capability (Figiel, 2007). Rehydration is a very important quality property for dried products. It is a complex process intended to restore the properties of the fresh product by contacting dried products with a liquid (Winiczenko et al., 2018). The methods of removing water from food result in a variety of changes in the tissue which impairs the possibility of regaining volume, mass, and water content.

The best rehydration power was determined for raisins obtained from the 'Sultanine' variety - 88.55%. The value for this parameter obtained for raisins obtained from the Corinth variety was 80.70%. For the dried fruit rehydration, the drying method was the most important factor in the ability to absorb water.

The key to the quality of raisins is the drying process and their water content. If the raisins are too dry their nutritional value and flavor are diminished, while if they are too wet, they degrade very quickly and will not survive storage or transportation.

Table 4. Raisin quality

Vitis vinifera L. grape variety	SDM Brix %	Moisture %	Tartaric acid (mg/ 100 g)	Ascorbic acid (mg/ 100 g)	pH
'Sultanine'	59.89^{o}	19.41**	509.11**	9.82*	3.92*
'Corinth'	61.11*	15.61^{oo}	422.90^{oo}	7.79^{o}	3.31^{o}
Mean	60.50	17.20	466.00	8.80	3.61
DL 5% =	0.58	0.76	8.08	0.53	0.20
DL 1% =	1.35	1.76	18.66	1.23	0.46
DL 0.1 % =	4.29	5.61	59.39	3.91	1.45

SDM - soluble dry matter

The low water content ensures a better microbiological behavior and prevents unwanted chemical reactions (oxidation, discoloration, non-enzymatic browning, etc.) during the storage of dry products. However, enzymatic phenomena are not inhibited by this reduction in water content.

CONCLUSIONS

The research carried out on the two grape varieties for raisins 'Sultanine' and 'Corinth', aimed to better understand their behavior in the pedoclimatic conditions in Cluj-Napoca, the suitability of their cultivation in this area, and obtaining new information about their production and quality. Sugar content at harvest for both varieties was good, with 195 g/l sugar for Sultanine and 234 g/l sugar for Corinth.

Following dehydration, the soluble dry matter content was higher for raisins of the 'Corinth' variety, with values of 61.11%, followed by the 'Sultanine' variety, with a content of 59.89% soluble substance. Regarding the moisture of the finished product - raisins, a high content was determined for the variety 'Sultanine' - 19%, followed by the variety 'Corinth' - 15.61%. The rehydration power of raisins had values of over 80% for both types of raisins. For the amateur culture, in the Cluj area, these varieties can be recommended, given that, in recent years, due to climate change, there have been no temperatures in this area during the rest period that could cause frost over the winter. Also, the long autumns favored reaching full maturity for these varieties, even in the conditions in Cluj. Raisins can be very easily obtained in the household, by using household dryers.

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RESEARCH ON LAUNCH AND IMPLEMENTATION STRATEGIES FOR A NEW LINE OF WINES IN THE DOMENIILE PRINCE MATEI, DEALU MARE VINEYARD

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Abstract

A wine company portfolio, like any Fast-Moving Consumer Goods (FMCG), should be diversified, innovative and very well correlated with the market requirements. Cash absorption and cash generation can be criteria for classifying the products portfolio. This research aims to identify the most efficient methods able to determine the structure and market positioning for Domeniile Prince Matei producer's portfolio. Such methods will be applied to design a new line of wines, to reduce the time for its production and implementation, to achieve the proposed results in the shortest time. The first step was to analyse the Domeniile Prince Matei's brand architecture, determine the profit for the present wine lines by using the Boston Consulting Group matrix, which is based on the relative market share and growth rate. The matrix application provided a general overview of company's products competitiveness and demand, which led to a portfolio rearrangement, a repositioning of the existing wines and creation of a new line of wines to replace the ones identified as unprofitable. Even if the Boston Consulting Goup matrix is a smart reliable tool and helps a lot, in practice is better to take the final decision based on the results of more enterprise strategic analysis methods.

Key words: *Boston Consulting Group Matrix, brand architecture, wine.*

INTRODUCTION

As a product, wine belongs to the category Fast Moving Consumer Goods, even if it has a shelf life of more than three years, because it is bought for immediate consumption. Because consumer goods have a high turnover, their market is not only very large, but is also very competitive (Penn, 2014; Kolmar, 2021). For this reason, companies in this sector must focus their efforts to attract and persuade consumers to buy their products (Dobronăuțeanu, 2012; Neacșu, 2012; Ageieva & Agarkova, 2018).

In order for a wine to become known, either massive investments in the media is needed or ensuring that customers get a direct experience with that wine (Bărbulescu, 2018; Vergamini *et al.*, 2019).

Brand architecture is an important part of any business and depends much on the creation plan. The main factor that helps to generate a strong and sustainable brand is the brand architecture (Bezos *et al.*, 2021).

The brand architecture helps companies to organize and structure a framework that allows for the independence and equal opportunities of the brand, increased attention and very well-directed communication. In short, it is about the structure of a portfolio within a company.

Regardless of the size of the business, the company needs a strong visual identity, a brand that will position it in the market, define the economic sector in which that product or service operates, transmit the company's values, be memorable and attract the attention of its customers (Kotler *et al.*, 2005).

The growth of any business also leads to the growth of the product portfolio, which determines a segmentation of the type of customers to whom it is addressed. It also gives the possibility to communicate directly to the specific customers through the brand. This is practically the time when it is necessary to create a structure, a coherent brand/portfolio architecture that meets the needs of the manufacturing company, ensuring positioning and further development, but also the requirements of customers who access that type of product or service.

Brand architecture can be of several types: the most well-known **branded house** and **house of brands**, to which the extended branded house, the house of a single brand etc. can be added.

A branded house and a house of brands vividly describe the two extremes of alternative brand portfolio strategies. While a **house of brands** contains independent, unconnected brands, a branded house uses a single master brand to span a set of offerings (Aaker, 2004;2008).

In the case of a **branded house**, a producer practically offers a second wine and even a third wine, more affordable, all under the Grand wine/main brand, but with a similar visual identity.

Château Angélus (Figure 1) is one of the top producers in Saint Emilion, which owns 27 ha of vines, grown with Merlot (65%) and Cabernet Franc (35%). For the first wine, Château Angélus, the average price is 2000 euros (the older it gets, the higher its value), for Carillon d'Angélus the average price is about 500 euros, and for No. 3 d'Angélus 250 euros (Geoghegan, 2020).

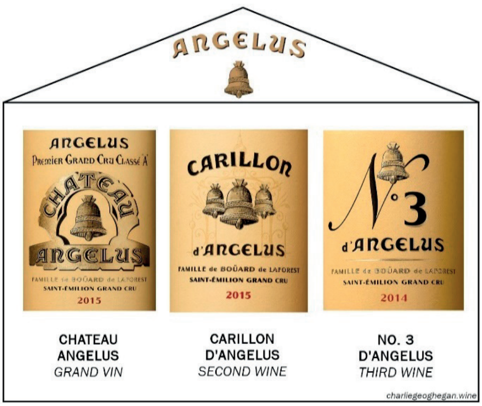

Figure 1. Example of Branded House architecture in Château Angélus, Bordeaux (Geoghegan, 2020)

Australian manufacturer ***Penfolds*** (Figure 2) also uses a similar visual identity for its entire product portfolio (7 lines, 44 products), whether a bottle costs $ 40 or $ 3000. In this way, wine lovers are offered the experience of wines that have the guarantee of the quality of the respective vineyard, but at affordable prices.

In the case of **house of brands,** the portfolio consists of distinct brands, independent of each other and independent of any major brand. Each brand has its own type of consumer, its communication being positioned entirely separately.

Figure 2. Example of Branded House arhitecture - Penfolds portfolio (https://www.penfolds.com/en/wines/lines/the-penfolds-collection)

The **house of brands** portfolio strategy is placed at the extreme opposite end of the spectrum to the branded house. Here, the portfolio consists of distinct brands, independent of one another and of any overarching master brand. An example of this is the producer ***Vignobles K*** (Figure 3) which owns seven properties located in four of the most famous regions of Bordeaux (Saint Emilion, Pomerol, Lalande de Pomerol and Castillion Cotes de Bordeaux). The wines in the producer's portfolio correspond to each property and are positioned differently, communicated independently having no common element (Geoghegan, 2020).

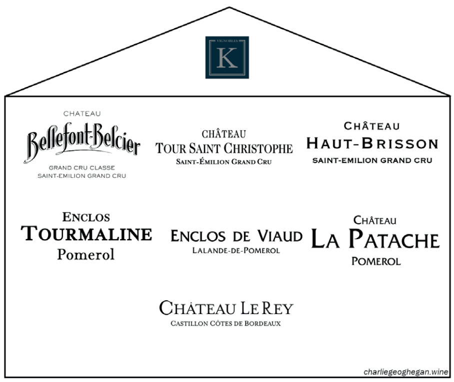

Figure 3. Example of House of Brands architecture in Vignobles K, Bordeaux (Geoghegan, 2020)

After achieving the portfolio architecture, by applying the **Boston Consulting Group Matrix**

(Chiu & Lin, 2020), the portfolio of products or services is ordered according to the relative market share and the market growth rate of each product, analysing in this way the profitability.
This method of analysing and determining a portfolio is an important tool to order the list of products and implement a new line of wines on the market.

MATERIALS AND METHODS

The study aimed to identify the most effective way to determine the structure and market positioning in the manufacturer's portfolio of **Domeniile Prince Matei** new line of wines, in order to reduce the time of realization and implementation, so as to obtain efficient results in the shortest possible time.
Winery location, cultivated areas and varieties. The research was carried out in the period 2019-2021, on the portfolio of products belonging to the wine company **Domeniile Prince Matei** (previously known as **Villa Zorilor Winery**, Zoresti).
Located in the viticultural region of Muntenia and Oltenia, at the confluence of the hill area with the plain, Villa Zorilor winery belongs, since 2019, to the holding company that also owns Viticola Sarica Niculițel S.A., Vintruvian Estate. That year, the new owners changed the name of the winery into **Domeniile Prince Matei S.R.L.**
Domeniile Prince Matei is located in the southern part of Romania, in Vernești commune, Zorești village, Buzău county (GPS: 45.172821; 26.704623) and has an area of 80 hectares with vines, located at altitudes between 70 and 202 m, on hills with eastern and south-eastern exposure. The age of the plantations is about 40 years, and the production obtained is between 4 and 8 tons/ha, destined for DOC wines.
The cultivated varieties are: Merlot, Cabernet Sauvignon, Fetească neagră, Fetească regală and Fetească albă.
Evolution of the product portfolio. The wine portfolio of **Domeniile Prince Matei**, at the end of 2020, consisted of the brands ***Prince Matei, Petit Matei, Red Paradox*** and ***Villa Zorilor***. Among these brands, compared to the portfolio of brands taken over in 2019, the only new wine label was ***Petit Matei.***
For the new wine, ***Petit Matei***, all the graphic/ visual elements of the other existing wine brands were kept untouched. After 2019, when the ownership changed, the new owner added also their logo to complete the visual identity of all the products.
At the end of 2020, the current shareholders decided to reposition **Domeniile Prince Matei** on the market by taking several measures: adding a new retail line of wines, restyling the old Prince Matei and Petit Matei labels, and removing the brands Red Paradox and Villa Zorilor wines from their product portfolio.
Realization a coherent brand architecture. The brand architecture must meet the needs of the manufacturing company, both in terms of positioning and further development (Hsu *et al.*, 2010; Osorio, 2021). Even though several types of brand arhitecture exist, the best known and used in this study are brand house and house of brands.
Branded house (Figure 4) is the portfolio architecture that has a master brand and several sub-brands, all derived from the main brand as far as their positioning is concerned, along with their visual identity and message.

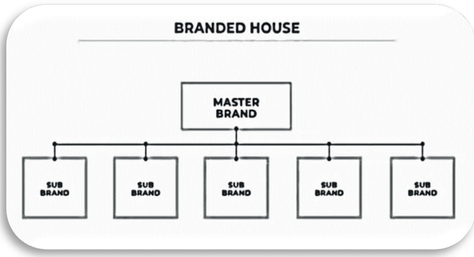

Figure 4. Portfolio architecture - Branded house (source: https://brandmasteracademy.com/brand-architecture/)

This portfolio strategy has clear advantages, by providing a simplified imaging solution for all products, and also reducing costs, due to the fact that no separate brand needs to be managed.
Customers accept new products or product lines much faster because they trust the main brand and if the main brand grows, automatically the sub-brands will receive recognition.

As a disadvantage, putting the main brand in the same basket with all other brands can lead to a loss of the main brand identity. Moreover, if one of the sub-brands does not live up to expectations or it proves to be a failure, the negative reaction will affect all brands in the basket, including the main one.

House of brands (Figure 5) is the portfolio architecture in which each sub-brand is treated as a unique brand with its own positioning, customer category, visual identity, communication style and tone of voice. The link between the main mark and the sub-marks is almost imperceptible or even non-existent.

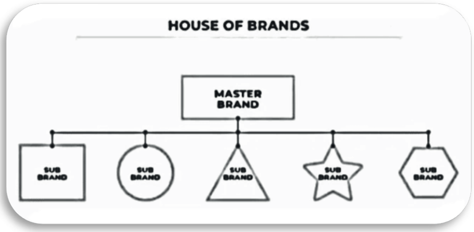

Figure 5. Portfolio architecture - House of brands (source: https://brandmasteracademy.com/brand-architecture/)

This type of product portfolio architecture offers more flexibility in terms of positioning, communication and customer type, because it does not have the constraints of a main company/brand philosophy that adapts its discourse according to the target audience.

If one of the sub-brands suffers an image loss or is considered of questionable quality, none of the other sub-brands or the main brand will be affected. In this case, resources can be allocated much better, because brands are very clearly differentiated in terms of positioning, and budgets will be clearly determined for each of them.

This portfolio architecture has its disadvantages as well, starting with the fact that managing brands that act independently, in accordance with their own identity, can be costly and cumbersome if the portfolio is large.

Because there is no main brand to support these sub-brands, they can only be based on their own reputation and, moreover, confusion can be created in the mind of the consumer regarding the identity of the manufacturing company.

When building its brand portfolio, every company must choose a strategy, according to the resources and architecture that suits best the products it wants to "place" on the market.

Applying the Boston Consulting Group matrix allows one to place the brands included in own portfolio, in a 2 x 2 square chart, based on the relative market share and the growth rate of each product / service.

The matrix is a table divided into four quadrants (Figure 6), each with its own unique symbol that represents a certain degree of profitability. Thus, the quadrants are the following: star(s), question mark(s), cash cow and pet or dogs or millstones (often represented by a dog).

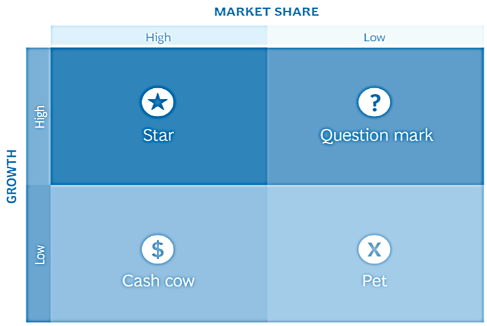

Figure 6. Boston Consulting Group Matrix (source: https://www.bcg.com/about/overview/our-history/growth-share-matrix)

By analysing and including each brand or service in one of these four categories, the decision makers in the company can later determine in which direction to focus their resources (human, research) and capital, to generate the highest profit, and where to reduces losses.

Each of the four quadrants is a combination of the relative market share and the growth rate of the market:

- ***stars*** have a relatively high market share and a high growth rate, which means high profitability with high financial need. Companies need to invest in these products because they have the potential to grow, to generate revenue, even higher than they produce at the time of evaluation;

- ***the question marks*** are products/services that have a high growth rate but a relatively small market share, meaning low profitability with high financial needs. In general, these are newly launched products, which have the potential to grow and generate profit, respectively, but in order to reach results, to become „stars", they need investment;

- cash cows have a relatively high market share, but also a low growth rate, that is high profitability with low financial needs. These are the products that generate mainly the profit of the companies and do not require investments because they no longer need having a large market share. The profit of these products should be reinvested in „stars” and „question marks” so as to increase the number of products in a company's portfolio that are revenue-generating, „cash cows”;

- dogs or millstones have a relatively small market share and a low growth rate, meaning low profitability with low financial needs. Because they do not generate any revenue and have no potential for growth, these products must either be discharged from the portfolio or repositioned.

According to Boston Consulting Group, there are *three scenarios* in which a company can be found: *the optimal route, the sequence of success* and *the sequence of disaster.*

RESULTS AND DISCUSSIONS

Establishing the new brand architecture at Domeniile Prince Matei. At the end of 2020 the Domeniile Prince Matei wine portfolio included the following brands: ***Prince Matei, Petit Matei, Red Paradox*** and ***Villa Zorilor.***

All wines are obtained from the Merlot variety, practically we are talking about a master brand, namely ***Prince Matei***, split in 4 labels (Figure 7), therefore having a structure of a ***Branded House***.

The branded house architecture relied only on red wines of 3 levels of quality: *first level wine* (***Prince Matei***), *second level wine* (***Petit Matei***) and *third level wines* (***Red Paradox*** and ***Villa Zorilor***).

In April 2021, a new image of the **Domeniile Prince Matei** portfolio was launched, with the following structure: ***Prince Matei, Petit Matei*** and ***Domeniile Prince Matei***, including not only red wines, but also white and rosé wines.

Visually, the new labels were designed to be modern and appealing to consumers. The old labels were redesigned to respect the style of the newly introduced labels Domeniile Prince Matei (Figure 8). All labels have common elements that clearly define the architecture of a Branded House.

Figure 7. *Domeniile Prince Matei* wine portfolio in December 2020
(source: Domeniile Prince Matei archive)

Figure 8. New labels in the portfolio *Domeniile Prince Matei*, April 2021
(source: Domeniile Prince Matei archive)

The current portfolio is still structured on three price levels, namely:

- ***Domeniile Prince Matei line*** - which includes three wines, white, rosé and red, is meant to be positioned as a middle price line; the affordable prices (about 30 RON) are intended for retail (off trade), being volume-based revenue generators;
- ***Petit Matei*** - *the second wine label in the portfolio*, is a wine designed for both on trade and off trade, with a price that indicates a superior quality (approximately 60 RON);
- the famous ***Prince Matei*** - *the first label wine*, the excellence of the portfolio is offered at a high-end price (approximately 130 RON), being positioned as a wine exclusively for on trade (bars, restaurants, coffee shops, clubs, hotels).

Considerations regarding the new portfolio of Domeniile Prince Matei. Analysis of the old and current brand architecture. The **Branded House** strategy was selected and applied by this producer's portfolio because it was used before and it was known in the market. The sub-lines of wines ***Petit Matei*** and ***Domeniile Prince Matei*** are visually identified with common elements with the master brand ***Prince Matei***, thus drawing from the notoriety of the most famous wine in the portfolio, which is already an established label (Figure 9).

This architecture has the advantage of using the image known for over 20 years of an iconic brand for quality Romanian wine, Prince Matei, which has been extended to other wines/lines, ***Petit Matei*** and ***Domeniile Prince Matei***.

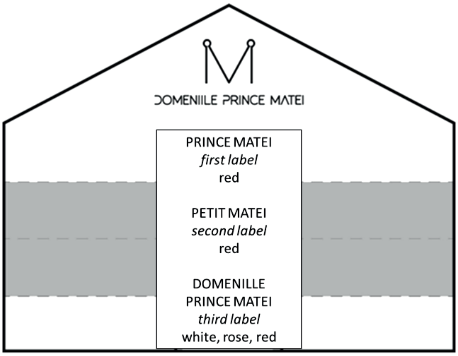

Figure 9. New portfolio of Domeniile Prince Matei - Branded House architecture (April 2021)

Introducing a new line such as *Domeniile* Prince Matei (shelf price 30/35 RON), to include for the first time in the portfolio white and rosé wines besides red, shows an opening to the consumer demands. This change proves that the new management sought a modern and non-conformist approach, but also shows a long-term development strategy, introducing a line that can generate income for the development of the company.

The visual identity for the labels of the new *Domeniile Prince Matei* line (Figure 10) borrows many elements from the *first wine label*, starting with the chromatic and continuing to the visual elements and finishing with the bottle type. In this way, the visual experience at the first contact a consumer has with these wines is similar to that of the famous Prince Matei label.

Figure 10. Domeniile Prince Matei Line Labels, April 2021

Thus, the image capital of the brand was used, without large additional investments to support the other brands. Each new wine benefits by associating its identity with the image of the master brand. The visual unity that is found in the producer's portfolio can be an advantage for the off-trade sales, when the consumer goes to pick a wine form the store shelves and is undecided.

Associating new labels to a master label which guarantees the quality of the vineyard and winery it is a marketing strategy often found in well-known wine-growing areas, in traditional countries such as France and Italy, but especially in the new world wine countries Australia or California. In Romania, the strategy is less used, therefore this can be a challenge.

Others experiences proved that it is a good strategy for a small portfolio, making it easier for the consumers to understand the offer. A single clear brand, carefully managed over time and extended to the full line of products, is a simpler proposition than a line of independent brands.

Branded house is an architecture that is best applied to small producers in the wine industry (no more than 100 ha) because it does not require a big communication effort.

As long as the *brand architecture is established* and correlated with the attributes of the manufacturing company (size, positioning, etc.) *the time to make a new brand will be shortened very much.* Implementation and communication will be much easier to achieve when there is a concept that expresses very clearly what message the manufacturer wants to convey.

Evaluation of the Domeniile Prince Matei portfolio using the Boston Consulting Group matrix to assess profitability. Following the establishment of the new brand architecture for Domeniile Prince Matei, the wine portfolio from the end of 2020 was analysed using the Boston Consulting Group Matrix (BCG) to assess its profitability. For each wine brand the relative market share and relative growth rate were evaluated and included in the BCG Matrix (Figure 11).

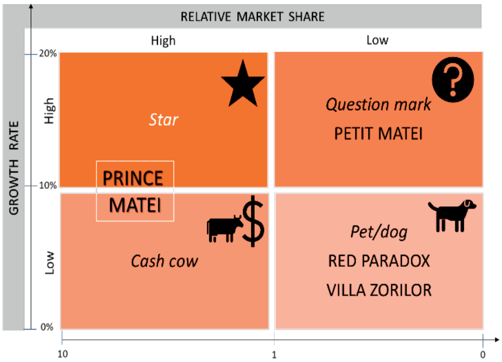

Figure 11. Portfolio analysis of Domeniile Prince Matei with the Boston Consulting Group Matrix before strategy change (December 2020)

Prince Matei brand, according to the BCG matrix, has a double role because, on one hand, *it is the star* of the producer's portfolio, contributing to the increase of the turnover, as well as to the improvement of its image, but on the other hand it is also considered a *cash cow*, meaning that it occupies a leading position in the market, but has nowhere to grow.

It represents the value of this producer, in the sense that it brings profit and provides liquidity to finance the other products in the portfolio. It is a wine that holds a steady position on the market, is well-known, but does not have a great possibility of development beyond the current state.

Petit Matei - launched in the past recent years, failed to impose itself on the mrket and can only be considered a *question mark / dilemma*.

Red Paradox and ***Villa Zorilor*** - although wines with affordable prices (30 RON in retail) were launched a long time ago and they do not perform anymore in the market, thus being only be considered *pets / dogs* in the BCG matrix.

In that context, the company was right to want to rearrange the portfolio, reposition some existing wines and create a new line of wines to replace the two unprofitable wines (Red Paradox and Villa Zorilor).

In the present structure of the manufacturer's portfolio, launched in April 2021 (Figure 12), we observe the new arrengement of the lines in the BCG Matrix, considering the relative market share and market growth rate.

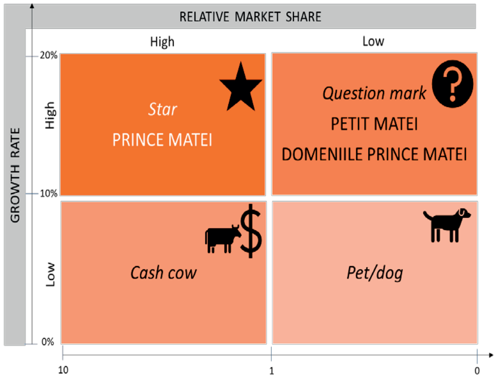

Figure 12. Portfolio analysis of *Domeniile Prince Matei* with the Boston Consulting Group Matrix after strategy change (April 2021)

Prince Matei brand remains *the star of the portfolio* with a larger market share now, dominating its market segment, but only with the need for permanent investment to keep up with the growth rate of competition and the preservation of its market share**.**

Petit Matei is the younger "brother" brand, which, being launched recently (in 2019), it did not have time to prove itself and thus remains in the square of the *question mark*. This means that it has a low market share, but a high market growth, on condition that the company invests in the brand to prove that it can occupy an important position in its market segment. The lately introduced **Domeniile Prince Matei** brand is also in the *question mark* square because it is a new and not-so-known line, that will need investment before it will establish itself a cash-generating wine brand, able to support the other wines in the present and future portfolio.

Results of the analysis of the portfolio of the manufacturer Domeniile Prince Matei using the Boston Consulting Group Matrix. After analysing the portfolio of the company, it was determined that, at the time of this study, the company does not generate profit and needs capital to support itself. The situation exists because there is only ***Prince Matei*** who is in the position *of star*, meaning that even if the brand generates some capital, it also needs permanent investment (visual identity promotion, communication, location on the shelf etc.), in order to maintain its position and eventually increase its market share.

The other two brands, ***Petit Matei*** and ***Domeniile Prince Matei*** are still in their infancy, in the position of *question marks*, so they need investment as well in order to make a profit.

The ideal situation for this type of portfolio would be for both ***Petit Matei*** and ***Domeniile Prince Matei*** to occupy a cash cow position, so as to generate capital for the development of new lines and maintaining the leading position in its segment for ***Prince Matei*** (Figure 13).

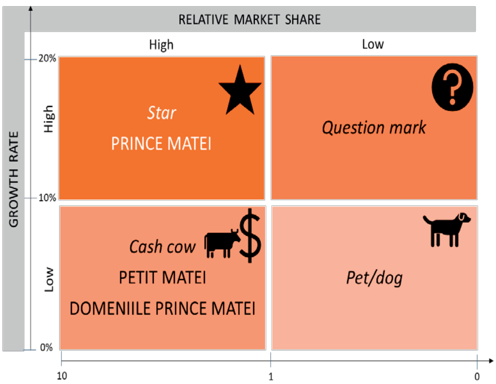

Figure 13. Ideal portfolio structure for Domeniile Prince Matei, shown by the Boston Consulting Group Matrix

However, what a production company wants and needs does not always happen in the real market, so that anytime the conditions can change and one or more of the brands in the portfolio can become a *pet/dog*. For this reason, the people in the marketing department, together with those in sales department, must constantly analyse the market, the competition, the external conditions etc. to know very well the market share for each brand, to realise what their goals are and, most importantly, to be aware of the resources they have.

CONCLUSIONS

After applying the Boston Consulting Group Matrix method for the portfolio analysis of the producer **Domeniile Prince Matei**, it was found that out of the four wine brands initially owned (***Prince Matei, Petit Matei, Red Paradox*** and ***Villa Zorilor***), only one generated capital, namely ***Prince Matei***, but not enough to support the entire company. Thus, at the end of 2020, it was established that a restructuring of the **Domeniile Prince Matei** portfolio was necessary, but with the preservation of the existent brand architecture, that is the one of a Brand House.

The *star* but also the capital generator - ***Prince Matei**, first label wine*, underwent a change of form, but not of core, maintaining its position as a grand wine, a star, thus improving its opportunities to increase its relative market share.

***Petit Matei**, the second label wine* in the portfolio, was brought much closer to the image of Prince Matei, but positioned in a younger market segment, remaining as a *question mark*, with the possibility of growth, but without generating significant capital.

The elimination of the non-profiable ***Red Paradox*** and ***Villa Zorilor** wine labels* and the creation of the new line for off-the-shelf sales, brings to the portfolio a new *question mark*, the ***Domeniile Prince Matei*** line (white, rosé and red).

In order to have a future successful portfolio the recommendation for this producer is that both ***Petit Matei*** and the ***Domeniile Prince Matei*** lines benefit from investments so that they perform better, increase their relative market share and end up producing enough capital to be considered *cash cows* (according to the Boston Consulting Group Matrix).

The company must also create the framework for further development of the star brand, ***Prince Matei***, which together with the other two wine lines would produce the necessary capital to support the business, but also for the development of new product lines.

In conclusion, the the Boston Consulting Group Matrix method is quite effective, but not enough to establish very clearly the success of a new line of wines. The manufacturer will have to use other marketing tools (market research, scientific research of the market sector, etc), to constantly measure the evolution of the market, to constantly adapt its portfolio to demand and to communicate permanently and coherently through its marketing specialists.

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COMPARATIVE BEHAVIOR OF VOLATILE AND AROMATIC COMPOUNDS OF TAMAIOASA ROMANEASCA AND MUSCAT OTTONEL GRAPE MARCS FERMENTED DURING TRADITIONAL STILL DISTILLATION

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Abstract

The demanding requirements of consumers in recent years and the desire of domestic producers to export as much as possible to European Union countries, make issues related to the quality, safety and authenticity of alcoholic beverages increasingly of concern for Romanian producers.
To investigate the behaviour of volatile compounds during a traditional alembic distillation, large numbers of important volatile compounds were identified and quantified by GC/MS analysis in different fractions (one of Head, three of Heart and one of Tail) of grape marc distillates made from two aromatic varieties Tămâioasă românească and Muscat Ottonel. Monoterpenes were confirmed to be responsible for a typical Muscat aroma, as well as for descriptors such as flowery, rose and spicy/menthol in distillates made from Muscat varieties. Due to the abundance of volatile terpene compounds, it has been considered beneficial to use the tail fraction as a raw material for re-distillation.

Key words*: grape marc, distillation fractions, traditional alembic distillation, volatile and aromatic compounds, GC/MS.*

INTRODUCTION

In Romania, the production of grape marc brandy, along with țuica plum distillate by traditional distillation with still, is a very important part of national identity.
Such traditional distilled beverages exist in many Mediterranean countries, such as Grappa in Italy, Tsipourro in Greece, Bagaceira in Portugal, Orujo in Spain, with specificity in terms of sensory properties, chemical composition and manufacturing techniques (Flouros et al., 2003)
Grape marc distillates are highly appreciated, especially after meals (Silva et al., 2000). The aroma of these distilled drinks comes primarily from the grape variety used but also from each successive stage of the manufacturing process: raw material processing, fermentation process, distillation method, and finally maturation (Mangas et al., 1996a).
Considering that grape pomace is the main by-product of the vinification process, a series of specific processes are involved in obtaining distilled pomace drinks that influence both their chemical composition and sensory attributes.
Thus, after pressing the grape pomace and obtaining the wine, the pomace obtained is stored and subjected to alcoholic fermentation. During this process, the most important volatile flavour compounds (alcohols, acids, esters and aldehydes) are synthesized together with the terpene compounds of the grapes used.
In the constitution of the varietal aroma, the terpenic compounds play a very important role being stored in the skin of the grape. Some of them end up in must and come through maceration-fermentation but some remain in pulp (Stoica et al., 2014; 2015)
After the completion of the fermentation of aromatic pomace, the ethanol produced and relatively large amounts of volatile substances as well as terpenes are recovered from the fermented pomace through the distillation process (Silva & Malacata, 1999).
The composition of distillates can be significantly affected by the distillation process (Cortes et al., 2009). Traditional alembic distillation is a discontinuous process that involves separating three major fractions that are distilled consecutively (Leaute, 1990). The first fraction is called the head fraction, the next is known as the middle fraction and the last is the tail fraction. Usually, the first fraction (head) and the last (tail) are eliminated because they contain compounds dangerous to human

health and worsen the sensory quality of the distillate (Stoica et al., 2015). However, they can be redistilled with the next batch of pomace to partially recover the flavours (Da Porto et al., 2010).
Stopping the distillation process in traditional distillation with still without computer control is done by measuring the concentration of alcohol during the process or by tasting. Therefore, the skills and experience of the distiller play an important role (Stoica & Giurgiulescu, 2016).
All these studies related to the raw material, the development of the maceration-fermentation processes but also the distillation represent the research work of several scientists over time (Di Stefano, 1986; Cortez et al., 2003; Geroyiannaki et al., 2007; Lukić et al., 2010; 2011; Stoica et al. 2019; 2020).
The aim of this study was to identify and quantitatively determine the most important volatile compounds in the different fractions of grape marc distillates. The distillation process was the traditional one with the copper still.
Two varieties of Tămâioasă românească and Muscat Ottonel aromatic grapes were used due to the abundance of aromatic compounds, but also due to the different aromatic profiles, highlighting the potential of this raw material - aromatic marc for obtaining natural distillates, of high quality.

MATERIALS AND METHODS

Grape marc samples.
For this study two varieties of aromatic white grapes Tămâioasă românească and Muscat Ottonel were used.
Grape pomace samples were obtained from the 2021 harvest, from a private vineyard plantation. Both varieties were used for the production of distillates, in Romania, especially in the Oltenia region (southwest of the country).
The samples were obtained according to the usual vinification procedures and characteristic of the variety used.
The grape marc from the Tămâioasă românească, an aromatic grape variety, was obtained following standard aromatic wine production practices (grape crushing, sulfitation) and was treated with 5 g/100 kg of pectolytic enzyme preparation. Knowing that terpenes are located in the deep layers of the skin, the mixture of berry skin and pulp of Tămâioasă românească was macerated for 48 hours at 20°C and then pressed. The pH of the marc resulted was 3.8.
The Muscat Ottonel variety was vinified in a similar way with the difference that the maceration period was shorter, 18 hours, as the terpenes are located in the upper layers of the skin. The must was also treated with 2.5 g/ 100 kg of pectolytic enzyme preparation, macerated for 18 hours at 20°C, then pressed. The pH of the resulted marc was 3.4.
Alcoholic fermentation of grape marcs
Grape pomace immediately after maceration was pressed and transferred to wooden containers with a capacity of 100 L, previously cleaned. The vessels were fille up to 70%. The alcoholic fermentation was spontaneous, with indigenous yeasts, at a temperature of 20°C ± 1°C.
Due to the fact that the surface of the pomace is susceptible to oxidation, it was sulphited with a 5% aqueous solution of sulphur dioxide and covered with plastic wrap. To ensure anaerobic fermentation conditions, the plastic foil was sealed by coating with a layer of sand.
The fermentation was carried out over a period of 21 days. Monitoring of the fermentation was performed daily and the fermentation lasted until the sugar concentration dropped to 4°Brix.
Distillation
The fermented grapes marc was distilled in a traditional copper still with shaker and without dephlegmator. Marc was placed up on a copper grate placed on the bottom of the boiler, under which 20 L of water was previously added to prevent the marc from burning.
Before distillation began, the alembic was sealed to prevent any vapor leakage. Heating the alembic was made by direct fire, with natural gas as a heating source. The distillation was induced by strong heating, which was continued for a short time after the condensate distillate began to leak, in order to prevent the discontinuation of the distillation process. During the distillation, the obtained flow rate was kept constant by the gradual increase of the heating temperature, due to a decrease in the ethanol/water ratio in the boiler and in the vapours. The water from the cooling tank was maintained between 20 and 22°C throughout the process.

The alcohol content of the distillates was monitored during the distillation process by an aerometer. The first fraction - head - consisted of the first 200 ml distilled. The next fraction of distillate - heart - was collected until the alcohol content of the operating distillate fell below 30% by volume. This was divided into three 100 ml single samples, marked as heart 1, heart 2 and heart 3. After collecting the heart (middle) fraction, 100 ml portions of the tail fraction were collected, until the alcohol content of the operating distillate decreased to 20% by volume, by the end of the distillation.

Analytical method

The determinations regarding the chemical composition of the distillates were made only on the middle fraction, which is destined to be consumed. The distilled fractions were stored in dark bottles, at 20°C for three months, and then analysed.

All 10 samples (five for each variety) were analysed using gas chromatography and following the method used by the Laboratory of the Department of Horticulture and Food science and the laboratory of National Institute for Cryogenics and Isotopic Technologies (I.C.S.I. Rm. Valcea).

The volatile compounds were extracted from the distillate fractions by liquid-liquid extraction following the method proposed by Lukić et al. (2010). This method consists in the use of a 12 mL volume of a fraction sample, which was diluted with 150 mL of deionised water, and 75 g of ammonium sulphate was added in order to improve the extraction efficiency. Then, a 250 µL aliquot of the internal standard solution (3-octanol, nonanoic acid, and methyl nonanoate in ethanol) was added to control the extraction. Volatile compounds were extracted with three 5 mL portions of dichloromethane. Dichloromethane extracts were combined, dried over anhydrous sodium sulphate, and concentrated to 0.5 mL. To control injection, 10 µL of a 3-heptanol ethanolic solution was added as another internal standard (Lukić et al., 2011).

Quantitative determination of volatile compounds was performed using a gas chromatography system, a VARIAN 450 gas chromatograph GC-FID detector (flame ionization detection) with a set of 275°C temperature for both the column TG-WAXMS 60 m, ID 0.32 mm, film, 0.25 mm, injector temperature 150°C, column temperature 35°C, 3 min stand, temperature increase rate of 20°C/ min., up to 70 to 150°C with 27°C/min., rest 2 minutes, increase to 200°C, rest 2 minutes, increase to 240°C with 20°C/min and rest for another 6 min. The carrier gas was helium (1.2 ml/min flow rate). Injection volume is 1 µL. The identification was made by comparing the retention times of standards from the calibration curve (Stoica et al., 2020).

A professional Cartier/Gay-Lussac 0-100 alcohol meter, metrologically approved and calibrated at 20°C, was used to determine the ethanol concentration (% vol).

Chemicals and standard physicochemical analysis

Standards of volatile aroma compounds were purchased from Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland). Dichloromethane (99.8%) and sodium sulphate (99%) were supplied by Kemika (Zagreb, Croatia). Pure deionised water was obtained from an Elix 3 purification system (Millipore, Bedford, MA, USA).

RESULTS AND DISCUSSIONS

Volume fraction of ethanol (%), methanol and concentration levels (mg/L anhydrous alcohol, a.a.) of volatile compounds in different fraction of fruit distillate obtained by traditional alembic distillation are presented in Tables 1 and 2.

Ethyl alcohol

Alcohols dominate the group of volatile compounds in grape distillates and have a significant effect on quality and sensory characteristics (Plutowska et al., 2010).

Most alcohols are produced by yeasts from amino acids through specific metabolic pathways, and only small amounts are made by the yeast by reducing the corresponding one aldehyde (Li X. et al., 2012).

In both distillates, the content of ethyl alcohol in the head fraction exceeds the value of 70%, while in the tail, it reaches a much lower value. Distilling further releases vapours that contain low-alcohol substances, but are rich in impurities, thus leading to lower quality products.

Methanol

A similar pattern of methanol distillation was observed both for Tămâioasă românească and Muscat Ottonel grape marc distillates (Tables 1 and 2).

Table 1. Volume fraction of ethanol (%vol) and value of volatile compounds (mg/L anhydrous alcohol) in different fraction of distillate from Tămâioasă românească obtained by traditional alembic distillation

Volatile aroma compound	Distillate fraction				
	Head	Heart 1	Heart 2	Heart 3	Tail
Methanol	3480.17	1123.01	823.72	266.91	22.76
Ethanol %vol	77.20	71.15	67.63	58.15	36.20
Higher alcohols					
1-propanol	502.99	404.67	303.21	239.41	111.51
Isoamyl alcohol	2540.46	2571.14	2250.01	1580.36	999.28
Isobutanol	740.25	670.92	558.563	350.14	201.49
1-hexanol	96.83	98.74	93.98	73.56	53.25
2 Phenylethanol	8.49	11.38	16.35	28.61	57.82
1-octanol	60.62	58.22	43.66	38.57	31.23
Benzyl alcohol	0.55	1.16	2.21	3.40	7.80
Esters					
Ethyl acetate	1334.22	1122.54	545.46	162.72	60.58
Isoamyl acetate	3.75	3.20	2.41	1.52	0.51
Isobutyl acetate	2.41	1.51	0.60	0.27	0.20
Monoterpenes					
α-terpineol	33.78.	50.45	66.15	87.15	95.35
Linalool	14.63	20.45	28.35	25.87	23.65
Nerol	6.70	10.98	12.75	15.95	19.31
Geraniol	9.89	9.52	14.32	19.42	28.54
Limonene	0.45	0.60	0.95	0.85	0.50
Aldehydes and ketones					
Acetaldehyde	553.28	458.13	390.35	370.60	439.12
Furfural	n.d.	2.15	4.68	6.65	9.15

Table 2. Volume fraction of ethanol (%vol) and value of volatile compounds (mg/L anhydrous alcohol, a.a.) in different fraction of distillate from Muscat Ottonel obtained by traditional alembic distillation

Volatile aroma compound	Distillate fraction				
	Head	Heart 1	Heart 2	Heart 3	Tail
Methanol	2126.17	1204.15	823.71	291.77	29.58
Ethanol (%vol)	72.10	70.50	65.65	55.29	34.80
Higher alcohols					
1-propanol	478.95	450.76	410.25	360.12	282.15
Isoamyl alcohol	1477.33	1505.80	1315.56	1025.78	985.63
1-hexanol	83.32	87.96	66.42	51.39	35.78
2 Phenylethanol	3.99	7.89	12.01	20.12	55.36
1-octanol	55.52	53.23	41.65	36.53	33.69
Benzyl alcohol	0.36	0.98	2.05	3.10	6.25
Esters					
Ethyl acetate	1789.80	1050.15	570.32	188.10	69.75
Isoamyl acetate	5.90	3.89	2.52	1.01	0.44
Isobutyl acetate	1.28	0.82	0.35	0.30	0.26
Monoterpenes					
α-terpineol	5.45	11.27	19.36	32.96	52.63
Linalool	33.86	50.25	69.10	67.40	62.51
Nerol	3.61	8.12	10.25	13.86	15.98
Geraniol	6.68	7.67	8.90	10.30	16.69
Limonene	0.17	0.19	0.38	0.47	0.24
Aldehydes and ketones					
Acetaldehyde	425.15	336.20	360.45	341.03	401.95
Furfural	n.d.	1.82	2.89	5.65	9.68

By distilling a mixture of completely soluble liquids having different boiling points and aiming at a gradual increase in the boiling temperature of the remaining liquid, the mixture can be divided into several fractions. Although methanol is of no importance to the flavour, its occurrence has been widely investigated. Methanol is a characteristic constituent of the head fraction. Initially, the concentration was fairly constant, followed by a significant decrease in the second half of the distillation. This result is in agreement with several previous research observations (Apostolopoulou et al., 2005; Geroyiannaki et al., 2007; Stoica et al., 2020). Other researchers (Silva & Malcata cited by Stoica, 2020) pointed out that methanol may form azeotropic mixtures with ethanol and water. This makes its distillation uneven. This also makes it difficult to remove them by traditional still distillation.
Compared to the Muscat Ottonel distillate, the Tămâioasă românească distillate registers much higher values of methanol, especially in the head fraction.

Higher alcohols
Higher alcohols, compared to methanol, are found in much smaller amounts in all fractions. From the data presented in Tables 1 and 2, it can be seen from a quantitative point of view isoamyl alcohol dominates, being followed by 1-propanol, with higher values in Tămâioasă românească. They are formed by the hydration of the corresponding acetals during the alcoholic fermentation of grape pomace. Benzyl alcohol, which is a rich aromatic alcohol, is present in both distillates, but is found in larger quantities only in the last fraction-tail. A different behaviour of aromatic alcohols was observed during distillation, as some decreases in their concentration during distillation was observed (Plutowska, 2010; Spaho et al, 2013 cited by Stoica et all., 2020).

Esters
Esters are qualitatively the most important class of aromatic compounds in distillates. Esters are formed, like higher alcohols, during the alcoholic fermentation of grape pomace. (Stewart, 2008). Esters are associated with a pleasant, fruity and flower aroma (Stoica et al, 2016).
The positive influence of esters on the aroma of distillates is given by their concentration in the beverage (Cortés et al., 2009; Stoica et al., 2020). Ethyl acetate is the main ester present in distilled alcoholic beverages. The importance of ethyl acetate in distillates, which even in small quantities can confer a pleasant aroma to these drinks, should be noted. However, in too large quantities, it can impart too strong of a flavour, that is not always appreciated by the consumer (Silva et al., 2000; Lukic et al., 2011; Spaho et al., 2013). According to the data in Tables 1 and 2, it can be seen that the values of the analysed esters are higher in the Muscat Ottonel distillate than in the Tămâioasă românească distillate. Also, they present the highest values in the head fraction, after which they start to progressively decrease. The second most abundant ester was isoamyl acetate, in both varieties distillates. Isoamyl ester has also been characterized by a steady decline in concentration with the progration of distillation (Tables 1 and 2). The decrease the most evident in the last fractions. Previous studies (Léauté, 1990; Hernández-Gómez et al.,2005; Lukic et al., 2011) showed that these esters belong to the group of compounds that distillate mainly in the head fraction and in the first fraction of the heart due to low boiling points.

Monoterpenes
According to the data presented in Tables 1 and 2, the behaviour of most monoterpenes during distillation was characterized by an increase in concentration. Final fractions (Heart 3 and Tail), with lower volume fraction of ethanol, which distilled at higher temperatures, contained higher total monoterpenes concentrations. Although due to the good solubility in ethanol and poor water solubility, higher concentrations were expected in the initial fractions, but their relatively high boiling the points were crucial in determining the distillation behaviour (Lukic et al., 2011).
The observed increase was expressed especially in the case of monoterpenes alcohols for which the concentration was almost constant, probably due to their higher polarity, hydrophilicity and boiling point relative to monoterpenes hydrocarbons. This hypothesis was corroborated, on one hand, by the fact that the last fractions of the heart and tail contained

notable amounts of acetates and polyols, which contain several polar parts in their molecular structure. One possible explanation for the increase in concentrations at the end of distillation is that the physical release of terpene compounds from grape seed cells and especially from skins was stimulated by the higher temperatures towards the end of the distillation process. This high content of terpenes compounds in the tail fraction leaves the possibility of its use for further distillation with a new batch of aromatic marc.

Another interesting aspect regarding the aromatic profile of the two distillates refers to the values of two monoterpenes, namely linalool and α-terpineol.

Both monoterpenes are found in distillates. While at the Tămâioasă românească distillate α-terpineol predominates, at Muscat Ottonel the major monoterpene is the linalool. This fact is reflected in the aromatic profile of distillates, which are similar as those of the grapes from which the beverage is prepared.

Other researchers (Ohta et al.1990; Lukic et al., 2011) have also shown that under slightly acidic conditions, such as pH 3.8 at 100° C, similar to the conditions in our study, geraniol and nerol were converted to linalool and α-terpineol in 30 min. It is possible that the conversion of nerol and geraniol into α-terpineol was the reason for which a much higher concentration accumulation rate was observed towards the end of distillation, despite the lower boiling points of the terpenes.

Aroma is one of the main characteristics that determine a brandy's organoleptic quality and style. This is the result of the contribution of hundreds of volatile compounds. They come from volatile chemical compounds resulting from grapes and vinification and distillation process (Stoica, 2008; Stoica et al, 2019).

A certain level of acetic aldehyde was found in all fractions of both aromatic distillates (Tables 1 and 2). The explanation is probably due to the complete solubility of acetaldehyde in both ethanol and water.

This result confirmed the findings of Lukic et al., 2011, but it was not in agreement with other researchers, who observed a significant decrease in concentration during distillation (Apostolopoulou et al, 2005; Leaute, 1990; Prado-Ramírez et al, 2005; Lukic et al, 2011). Slightly higher concentrations observed in the heads and the tails were probably the result of an increase in ethanol oxidation rate under higher temperature conditions, applied in our distillation phases.

The presence of furfural has been characterized by a steady increase in concentration, which has been in line with previous results (Soufleros et al., 2004; Colonna-Ceccaldi, 2008; Stoica et al, 2020). High boiling points and good water solubility have certainly supported such behaviour.

CONCLUSIONS

Based on the results obtained from the compositional and qualitative researches of the two distillates obtained from the aroma marc of Tămâioasă românească and Muscat Ottonel, it can be stated that both are quality drinks with a high degree of naturalness.

This study also showed the possibility of significantly affecting the concentrations of many classes of volatile aromatic compounds in grape marc distillates by choosing the appropriate points of fraction separations during the distillation by traditional alembic. An example would be the higher alcohols and esters that are distilled in the first head and heart fractions.

The use of traditional equipment (alembic) and the process of discontinuous distillation have proven to be quite inefficient in removing significant amounts of major toxic constituents such as methanol and acetaldehyde. Therefore, the removal of the first fraction of distillate, the head is fully justified.

The most important result of this research is the experimental evidence regarding the behaviour of varietal aroma compounds during traditional distillation.

This study showed that the concentration of terpene compounds increased during the distillation process, reaching a maximum in the tail fraction. This shows that this last fraction of distillate is a valuable raw material for re-distillation and enrichment with varietal aroma.

In conclusion, it can be argued that by using aromatic marc a natural and quality distilled beverage is obtained.

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MINIMUM QUALITY CHANGES AND WEIGHT LOSS OF TABLE GRAPES PROCESSED DURING STORAGE

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Abstract

To carry out this study, we started from the scientific argument that, after harvest, table grapes undergo a series of physico-chemical changes influenced mainly by the method of storage and its duration. During the cooling and the storage of the fruits, all the physiological processes continue to take place, but with reduced intensities compared to the previous stages of fruit growth and maturation, and the phytosanitary conditions of the grapes directly influence the shelf life of the fruits. The obtained results show that the samples from the variant with 5 % CO_2 registered lower weight losses during storage, and in terms of taste, more balanced. The practical outcomes of these results can be transferred to the grape vine - growing companies cultivating table varieties, but also to economic operators in the capitalization and retail chain who will be able to use the combination of different parameters (temperature, relative humidity and different fractions of CO_2, N_2, O_2) in order to increase the shelf life and ensure quality products for consumers.

Key words: *varieties, quality, postharvest, storage, table grapes.*

INTRODUCTION

Today, more than ever, food safety, product quality, guarantees of authenticity and originality in the range of healthy agri-food products offered by the consumer market are four of the main concerns of producers and consumers in the wine sector (O.I.V., 2020). For these reasons, traceability has become a useful tool, and also necessary to ensure the proper functioning and knowledge of the process of production, development and marketing of grapes, addressing the issue of food and nutrition security. Table grapes are part of the delicatessen fruit categort, they are the requested and consumed fruit-food, due to rich and varied chemical composition necessary for the health and vitality of the human body, and their consumption in fresh state has always been an object of need in human society. Regular and correct consumption in the fresh state also proved the therapeutic action, given by the concentration of phenolic compounds (over 500 compounds) accumulated in the skin, pulp and seeds of a series of microelements that promote metabolism, the presence of resveratrol - a powerful antioxidant, a true inhibitor of cancer cell growth (Mahanna et al., 2019; Tahereh et al., 2020; Benbouguerra et al., 2021), and last but not least, the presence of substances that promote the process of weight loss, with all of which also contain glucose (Haitao et al., 2021). Over the last decade, world production of table grapes (27 million tons) and their consumption in fresh state (3 million tons exported between countries) has increased significantly (O.I.V., 2019). Moreover, the consumer market both globally and nationally has grown significantly from year to year (FAO, 2020), drawing attention to the need to ensure competitive products in terms of sensory and visual characteristics.

However, a number of factors are involved in achieving a quality production, factors that are mainly related to the environment and climate change, but also to the genetics of varieties, applied technology, storage life, etc. (Crupi et al., 2012; Stroe & Bejan, 2014; Rolle et al., 2015; Stroe et al., 2016; Martins et al., 2021; Huwei et al., 2021; Ehtesham et al., 2021).

In this context, producers and researchers focus more on the quality of the product that can be described by specific and measurable indicators and characteristics of the fruit (size, shape, compactness, berry color, consistency, taste and aroma of the pulp, skin thickness and coverage) and coverage with plum odor, general, general appearance, discoloration and accelerated softening and degradarion of the berries, dehydration of the rachis, freshness, degree of ripeness or any other qualitative attribute specific to the fruit (Tyagi et al., 2020; Lingling et al., 2021) and which to be stored even after a storage period of more than 2-3 months. All these are in a close correlation, each influencing the response reaction of the vine and the product obtained. It is difficult to say which of the listed factors has a greater influence (Biniari et al., 2020), but the biological and technological barriers encountered on the flow of traceability in wine production must be removed.

The obvious defects of the quality of the grapes after storage can lead to the rejection of the product by the consumer, followed by the decrease of sales, the loss of markets and income, etc., and then it is no longer just a unique feature, recognizable is a dynamic concept, anchored in reality in which more and more emphasis is placed on product traceability and food safety.

Because of the growing interest and consumer demands, economic operators need to supply horticultural products of outstanding quality, respecting European quality stadards. For our country, however, the sale of fresh horticultural production has an uncontrolled, conjugal, disersed and very fluctuating aspect, which is aggressively completed by imports, through the attractiveness of the presentation (packaging), which are sold at a much higher price than similar domestic products. In our contry today, table grapes have a share of only 6.9% of Romania's wine production (O.I.V., 2020), and worldwide, table grapes represent 15% of total wine production.

This context causes a clear imbalance, which implicitly affects the shortening of the period of consumption in the fresh state. In addition to all of this, the storage of grapes in storage facilities is done at a very modest volume, although there is the potential for Romania to provide fresh table grapes on the market until February-March. Basically, we need a strategy that starts from the cultivation and capitalization of transportable varieties (promotion of Romanian creations), prone to storage, and also the collaboration between the research sector and producer assosciations through information and technology transfer, by upporting access to markets (supermarkets) in Romania of manufactures.

Fruit storage procedures are a topic of current research and are expanding more and more, testing and using different methodologies (Adamane Naouel et al., 2018) to extend this period. After harvest, table grapes undergo a series of physico-chemical changes influenced by the preservation method and its duration, because, during storage, physiological processes continue to take place, but with reduced intensities compared to previous periods of the fruit growth and maturation. The speed of biochemical reactions that take place in the fruit is influenced by microclimate conditions (Nicolosi et al., 2018).

Table grapes are non-climacteric fruits, perishable at room temperature, the perishability process evolving rapidly, especially if these were harvested after reaching the optimal time of harvest. Long-term storage causes problems, with weight loss, discoloration, dehydration of the rachis, loss of skin turgidity, all affecting fruit quality. Basically, in refrigeraton conditions with normal atmosphere, the losses are quite high: 10.1% after 3 months, 13.9% after 4 months, 18.1% after 5 months, 23.2% after 6 months (Rotaru et al., 2011; www.eurepgap.org). Based on these records, the present study aims to evaluate the weight loss and biochemical parameters recorded under conditions of storage in a controlled atmosphere for as long as possible.

MATERIALS AND METHODS

Sample preparation

The main objective is to keep table grapes in fruit storages with a controlled atmosphere by correlating different parameters (temperature, relative humidity and different fractions of CO_2, N_2 and O_2) with a dual purpose: to extend the storage period and ensure quality products for consumers. Although shelf life can be extended at a low temperature of about 0°C, this is not enough to control the senescence of the rachis, berry abscission or fungal attack mainly caused by *Botrytis cinerea*, which is able to grow at low temperatures. For this study, three table grapes varieties were selected: ‘Coarnă Neagră Selecționată’, ‘Muscat Hamburg’ and ‘Muscat d’Adda’. The varieties are located in the experimental field of the ampelography collection from the Research and Development Station for Viticulture and Oenology Pietroasa - University of Agronomic Sciences and Veterinary Medicine of Bucharest, with the code “ROM 13” in www.vivc.de.

For the proposed objective, the standard technology was applied throughout the technological flow (pre-harvest and post-harvest), in terms of obtaining and handling the grapes to be stored under controlled atmosphere conditions. Harvesting was done manually with the peduncle and wax layer intact and with rigorous control, ensuring sorting and calibration directly from the field, according to the quality standard. Their transport took place on the harvest day. In parallel – the storage space preparation (sanitation) and the intermediate temperature of 8-10°C regulation were established. Storage and analysis were done in the Postharvest Technology Laboratory of the Research Cener for Studies of Food Quality and Agricultural Products. In this experiment, two conditions with different CO_2 levels were chosen - 2.5% CO_2 and 5% CO_2 and the analyses were performed after 4-5 months, in terms of the parameters that interest those who intend to keep them in a controlled atmosphere (weight loss mainly) and parameters that make them attractive to consumers: commercial appearance, fermity and consistency of berries, taste (acidometric index), etc. Experimental variants are: **variant 1** (2.5% CO_2, 3% O_2 (oxygen content), RH (relative humidity) = 90%, T (temperature) = 0.5°C and **variant 2** - 5% CO_2, 3% O_2 (oxygen content), RH (relative humidity) = 90%, T (temperature) = 0.5°C.

Biochemical analysis

Because the main objective is to track weight loss (in the event of a technology transfer) after the introduction in the storage rooms, the samples were weighted every two weeks, and after 4.5 months were removed from the storage. Prior to storage, as well as after the storage period, sensory analysis of grapes was performed according to the tasting sheet (O.I.V. 2008b), as following: commercial appearance, size, uniformity and compactness (Notes: 1-3), appearance of berries to correspond to the variet, detachement of the pedicel (notes: 1-3), skin thickness, color, amber spots, itching (notes: 1-5), taste/ aroma of the stalks (notes: 1-5), core consistency (notes: 1-3), succulence (notes: 1-3). The maximum and total score on the set of assessed grades is 22 points. The tasting event was attended by 52 people, as follows: 44.23% students, 38.46% active in work, 17.30% pensionary. In order to assess the descriptive parameters of the quality, determinations of biochemical parameters of the grapes was made, as follows: to determine the dry matter and water content from samples, approximately 1 g of the average sample in the crucibles was introduced at 105°C until constant weight (Bezdadea-Cătuneanu, 2017; Moura, 2005; Skupień, 2006; Delian, 2011; Corollaro, 2014; Mureşan, 2014; Ticha, 2015, Iliescu, 2019). For the determination of fruit firmness, the electronic penetrometer TR was used, with a piston of 3 mm diameter, the results being expressed in N/cm^2. The contents of total soluble solids, glucose and fructose were determined from 5 grape berries for each sample: with refractive device Kruss DR301-95 (% Brix) for total soluble solids (TSS) (Mureşan, 2014; Oltenacu, 2015; Saei, 2011; Tolić, 2015; Yoon, 2005), with refractive device Milwaukee MA873 (%) for glucose and with refractive device Milwaukee MA872 (%) for fructose (Enciu (Bunicelu) et al., 2021). The total titratable acidity (TA) was determined by titration with 0.1N NaOH to pH 8.1, and the results were expressed in g tartaric acid/100 g. Titratable acidity calculation was done using the formula: Titratable acidity (%) = (V x N x C x 100)/m where V = volume of NaOH consumed; N = NaOH normality; C = tartaric acid equivalent; m = sample mass, C has values: 0.0075 to express acidity in tartaric acid (grapes, shoots, bananas). The maturity index, known also like acidometric equilibrium index, was calculated using the formula: TSS/TA.

All determinations were performed in triplicate. Statistical analyses were performed using Excel, including: average, standard deviation, ANOVA single factor, T Test and correlations (Pomohaci, 2017).

RESULTS AND DISCUSSIONS

Analyzing the data entered in Tables 1 and 2, for the evaluation of weight loss in case of storage, it is observed that the losses for both experimental variants are very similar, but higher for variant 1 (2.5% CO_2, 3% O_2, RH = 90%, T = 0.5°C), regardless of the time of observations.

It is observed that the 'Coarnă Neagră Selecționată' variety has the lowest weight loss, which proves that it has an increased storage capacity on a genetic basis (it is a selection of the local variety 'Coarnă Neagră'), followed by the 'Muscat d'Adda' variety and then 'Muscat de Hamburg' variety, which has proven to be the most sensitive, even in storage, not only in field conditions (Stroe, 2012).

Losses after one month of storage range from 3.07% ('Coarnă Neagră Selecționată' in variant 2, with 5% CO_2, to 5.32% ('Muscat Hamburg' - in variant 1, with 2.5% CO_2. The percentage for weight loss after 2 months is between 10.20% ('Coarnă Neagră Selecționată' in variant 2, with 5% CO_2 and 15.96% ('Muscat Hamburg' variant 1, with 2.5% CO_2.

When removed from storage (4.5 months - 145 days), the sum of the losses shows values between 17.11% ('Coarnă Neagră Selecționată' variant 1) and the highest loss in storage was recorded by 'Muscat Hamburg' - 25.90% in variant 1 and 20.64% in variant 2.

Immediately after removal from the storage, the descriptive quality parameters were assessed and the data in Tables 3 and 4 show a greater balance between samples in terms of dry matter content (DM%), the data being statistically verified.

For dry matter content (DM %) significant differences ($p<0.05$) has registered between all varieties, in both conditions.

For 'Coarnă Neagră Selecționată' has registered insignificant differences ($p>0.05$) between conditions, while for 'Muscat Hamburg' and 'Muscat d´Adda' varieties, were registered significant differences ($p<0.05$), between both conditions.

The total soluble solids (Brix) values show that they remain within the normal limits of the varieties for both experimental variants, but on the background of an accentuated dehydration in variant 1, they seem significantly higher - 23.8 Brix% (soluble solids content) - 'Muscat d' Adda'.

The same trend is observed in terms of glucose (%) and fructose (%) values - confirming a visible dehydration in the variant CO_2 - 2.5%, O_2 - 3%, RH 90%, T - 0.5°C, observed also by the values berry firmness.

The total soluble solids content (TSS), glucose and fructose content (Table 3 and Table 4) have registered significant differences ($p<0.05$) between 'Coarnă Neagră Selecționată' and 'Muscat Hamburg' and 'Muscat d´Adda' varieties, for variant 1 (2.5% CO_2), and also for variant 2 (5% CO_2). For TSS, total glusose and fructose contents, between 'Muscat Hamburg' and 'Muscat d´Adda' varieties, for both conditions, were registered insignificant differences ($p>0.05$).

For TSS and glucose content, between both conditions, for all varieties, were registered insignificant differences ($p>0.05$), while for fructose content significant differences ($p<0.05$) has registered for 'Muscat d´Adda' variety, between variant 1 (2.5% CO_2), and variant 2 (5% CO_2). For fructose content, 'Coarnă Neagră Selecționată' and 'Muscat Hamburg' were registered insignificant differences ($p>0.05$) between conditions.

Between TSS and glucose of the fruit, for 'Coarnă Neagră Selecționată' cv. it was observed a very strong significant positive correlation $R^2 = 0.9815$, with linear regression equation $y = 0.9035x + 2.5383$, for variant 1 (2.5% CO_2) and a very strong significant positive correlation $R^2 = 0.8581$, with linear regression equation $y = 1.2046x - 3.1955$, for variant 2 (5% CO_2).

Between TSS and glucose of the fruit, for 'Muscat Hamburg' variety it was observed a very strong significant positive correlation $R^2 = 0.847$, with linear regression equation $y = 0.7863x + 6.3777$, and for 'Muscat d´Adda' variety it was observed a very strong significant positive correlation $R^2 = 0.9704$, with linear regression equation $y = 1.2432x - 4.6534$, for variant 1 (2.5% CO_2)

Between TSS and fructose content of the fruit, for 'Coarnă Neagră Selecționată' cv. it was observed a very strong significant positive correlation $R^2 = 0.9479$, with linear regression equation $y = 0.9098x + 2.8251$, for 'Muscat Hamburg' variety it was observed a very strong significant positive correlation $R^2 = 0.9316$, with linear regression equation $y = 0.8189x + 6.1492$, and for 'Muscat d´Adda' variety it was observed a very strong significant positive correlation $R^2 = 0.9668$, with linear regression equation $y = 0.9706x + 2.3809$, for variant 1 (2.5% CO_2).

Between glucose and fructose of the fruit, for 'Muscat Hamburg' variety it was observed a very strong significant positive correlation $R^2 = 0.978$, with linear regression equation $y = 0.9821x + 0.8994$, for variant 1 (2.5% CO_2) and a very strong significant positive correlation $R^2 = 0.9323$, with linear regression equation $y = 0.9569x + 1.4624$, for variant 2 (5% CO_2).

Table 1. Variation of weight losses during storage in 2.5% CO_2

Samples/ Date	04.X.	18.X.	1.XI	15.XI	6.XII	1 month	2 months	4.5 months
Coarnă Neagră Selecționată	-1.42	-2.71	-4.01	-4.45	-4.45	-4.13	-12.65	-17.11
Muscat Hamburg	-2.11	-3.21	-4.54	-6.10	-9.94	-5.32	-15.96	-25.90
Muscat d´Adda	-1.71	-3.10	-4.36	-6.06	-9.41	-4.80	-15.22	-24.64

Table 2. Variation of weight losses during storage in 5% CO_2

Samples/ Date	04.X.	18.X.	1.XI	15.XI	6.XII	1 month	2 months	4.5 months
Coarnă Neagră Selecționată	-1.13	-1.94	-2.88	-4.26	-7.82	-3.07	-10.20	-18.02
Muscat Hamburg	-1.46	-2.63	-3.43	-4.81	-8.32	-4.08	-12.33	-20.64
Muscat d´Adda	-1.12	-2.11	-3.02	-4.21	-7.37	-3.23	-10.46	-17.84

[1]RH % = relative umidity

Table 3. Variation of: DM (%), TSS (%), glucose (%), fructose (%), TA and fermity during storage in 2.5% CO_2

	DM[1] (%)		TSS[2](%)		Glucose (%)		Fructose (%)		TA[3](mg/100g fw)		Fermity (N/cm^2)	
Samples	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.
Coarnă Neagră Selecționată	19.28	±0.6	17.8	±2.1	18.6	±1,9	19.0	±1.9	0.82	±0.02	6.0	±0.4
Muscat Hamburg	23.93	±0.3	21.7	±2.8	23.4	±2.4	23.9	±2.3	0.45	±0.00	5.1	±1.7
Muscat d´Adda	24.82	±0.2	23.8	±1.6	25.0	±2.0	25.5	±1.6	0.46	±0.01	7.0	±1.5

Table 4. Variation of: DM (%), TSS (%), glucose (%), fructose (%), TA and fermity during storage in 5% CO_2

	DM[1] (%)		TSS[2](%)		Glucose (%)		Fructose (%)		TA[3](mg/100g fw)		Fermity (N/cm^2)	
Samples	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.	Average	Std. dev.
Coarnă Neagră Selecționată	20.36	±1.0	18.9	±0.8	19.6	±1.0	19.9	±0.7	0.51	±0.0	4.1	±1.4
Muscat Hamburg	22.8	±0.2	21.0	±1.0	22.3	±1.3	22.8	±1.3	0.45	±0.0	4.2	±0.4
Muscat d´Adda	23.26	±0.1	21.1	±1.1	22.6	±0.7	23.1	±0.8	0.46	±0.0	5.3	±1.1

[1]DM% = dry matter content
[2]TSS% = total soluble solids (Brix)
[3]TA% Titrable acidity

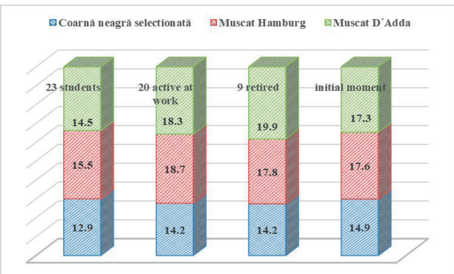

Figure 1. Qualitative scores before storage

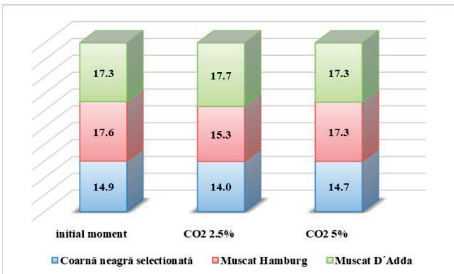

Figure 2. Qualitative scores for the stored fruits

Between glucose and fructose of the fruit, for 'Coarnă Neagră Selecționată' variety it was observed a very strong significant positive correlation $R^2 = 0.9857$, with linear regression equation $y = 1.0174x + 0.0765$, and for 'Muscat d´Adda' variety it was observed a very strong significant positive correlation $R^2 = 0.9771$, with linear regression equation $y = 0.7732x + 6.2021$, for variant 1 (2.5% CO_2).

In essence, the grapes of experimental variant 1 seem firmer due to the elasticity of the skin, and for variant 2, with 5% CO_2, the varieties have firm berries, being a particular variety, on a turgid background. This indicator of organoleptic appreciation also included the acidity expressed in tartaric acid, which together with the accumulated sugars give the The maturity index, recorded the following values: for 'Coarnă Neagră Selecționată' variety in 2.5%, CO_2, 2.18 g tartric acid/L for an acidity of 0.84 tartaric acid g/100 g FW and 3.73 g tartric acid/L at 5% CO_2, on the background of an acidity of 0.51 tartaric acid g/ 100 g FW. For 'Muscat d'Adda' variety, in 2.5% CO_2, the maturity index registered a value of 5.18 g tartric acid/L for an acidity of 0.46 tartaric acid g/100 g FW and 4.56 g tartric acid/L in 5% CO_2, against a background of an acidity of 0.46 tartaric acid g/100 g FW. For 'Muscat Hamburg' variety, the data are very similar, regardless of the CO_2 content, respectively 4.86 g tartric acid/L for variant 1 (0.45 tartaric acid g/100 g FW) and 4.69 g tartric acid /L for variant 2 (0.45 tartaric acid g/100 g FW)

The titrable acidity (TA) (Tables 3 and 4) has registered significant differences ($p<0.05$) between 'Coarnă Neagră Selecționată' and 'Muscat Hamburg' and 'Muscat d´Adda' varieties, for variant 1 (2.5% CO_2), and significant differences ($p<0.05$) between all varieties for variant 2 (5% CO_2). For 'Coarnă Neagră Selecționată' has registered significant differences ($p<0.05$) between conditions, while for 'Muscat Hamburg' and 'Muscat d´Adda' varieties, were registered insignificant differences ($p>0.05$), between conditions.

The firmness (Tables 3 and 4) has registered insignificant differences ($p>0.05$) between all varieties, for variant 1 (2.5% CO_2), and also for variant 2 (5% CO_2). For 'Coarnă Neagră Selecționată' and 'Muscat d´Adda' varieties, were registered significant differences ($p<0.05$) between conditions, while for 'Muscat Hamburg' variety, were registered insignificant differences ($p>0.05$), between conditions. Regarding the tasting of the samples (Figure 1), before storage - it is observed that the values are very close, and the most appreciated by the three categories of people participating in the tasting is the 'Muscat Hamburg' variety with a total score of 17.6 out of 22. The organoleptic assessment of the grapes after storage, it seems that, in general, consumers (active people at work) appreciated more the varieties stored in the conditions of variant 2, giving a significantly higher score, with small exceptions. The most appreciated was the 'Muscat d'Adda' variety (notes: 17.3 out of a maximum of 22), followed by the 'Muscat Hamburg' variety (17.3 out of a maximum of 22).

CONCLUSIONS

The 'Coarnă Neagră Selecționată' variety has registered the lowest weight losses, which shows that has an increased genetic storage capacity, followed by the 'Muscat d'Adda' variety and then the 'Muscat Hamburg' variety, regardless of the experimental variant.

The values of total soluble solids (Brix %) show that they remain within the limits of the varieties for both experimental variants, on the background of a more accentuated dehydration in variant 1, they seem significantly higher for 'Muscat d'Adda'.

Tasting of samples, before storage, shows a very close score, the most appreciated being the variety 'Muscat Hamburg'. For TSS and glucose content, between both conditions, for all varieties, were registered insignificant differences. After storage, it is observed that the samples stored in the conditions of variant 2 have a score closer to the initial moment, with small exceptions. It turns out that the samples stored in controlled atmosphere variant 2 - 5% CO_2, 3% O_2 (oxygen content), RH (relative humidity) = 90%, T (temperature) = 0.5°C, show lower weight loss (regardless of variety) and, in terms of taste, they are much more balanced.

The firmness has registered insignificant differences between all varieties, for both variants.

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EFFECT OF FERTILIZATION WITH *Azotobacter* AND HORNWORT (*Ceratophyllum demersum* L.) ON GROWTH PARAMETERS OF EGGPLANT (*Solanum melongena* L.)

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Abstract

Field experiment was conducted in a farm of Najaf governorate during 2018-2019 season, to study the effect of different concentrations of Azotobacter and hornwort on growth parameters of Eggplant (Solanum melongena L.). The experiment included 6 treatments concerning in three different concentrations of Azotobacter (0, 5 and 10 g·100 ml^{-1} d·w) and two levels of hornwort (0 and 5 T·Ha^{-1}).
The use of Azotobacter had an increasing effect with concentration 10g·100ml $^{-1}$ on all vegetative growth parameters and carbohydrate (plant length, leaves number, shoot dry weight, stem diameter, total chlorophyll in leaves and total soluble carbohydrate in leaves) compared with control treatment (sprays with distilled water) which gave the least means values. Interaction between factors reveal a significant effect in all vegetative growth parameters and carbohydrate.
Hornwort addition with level 5 T·Ha^{-1} had a significant effect on all vegetative growth parameters compared with the control treatment. Interaction between two factors gave a significant effect on all vegetative growth and carbohydrate.
10g·100 ml^{-1} of Azotobacter had a significant effect on all fruit growth parameters (fruit numbers, diameter and length) compared with control treatment which gave the least means. Hornwort addition with level 5 T·Ha^{-1} had a significant effect on all fruit growth parameters compared with control treatment. Interaction between two factors gave a significant effect on all fruit growth parameters.

Key words: *Eggplant, Azotobacter, Hornwort.*

INTRODUCTION

Eggplant (*Solanum melongena* L.) is a warm season crop. It requires a long and warm growing season for successful production (Sukprakarn et al., 2005). It is nutritious, being low in calories, fat, sodium and is a non-starchy. It contains a large volume of water. It is good for balancing diets that are heavy in protein and starches. It is high in fiber and provides additional nutrients such as potassium, magnesium, folic acid, vitamin B6 and A (Praça et al., 2004). Well-drained, sandy loam soils are ideal for eggplant production (Bliss et al., 2004). The United Nations FAO reported that the total areas cultivated by eggplant in the world reached 1,847,787 ha with a productivity of 55,197,878 tons, while, the total cultivated areas in Iraq reached 8,660 hectares with a productivity of 136,749 tons (FAO, 2021).
In recent years, bio-fertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yield through environmentally better nutrient supplies (Marozsán et al., 2005).
Azotobacter belongs to family Azotobacteriaceae, aerobic, free living, and heterotrophic bacteria. *Azotobacter* are present in neutral or alkaline soils and *A. chroococcum* is the most commonly occurring species in arable soils. *A. vinelandii, A. beijerinckii, A. insignis* and *A. macrocytogenes* are other reported species, the amount of *Azotobacter* rarely exceeds of 10^4-10^5 CFU·g^{-1} of soil due to lack of organic matter and presence of antagonistic microorganisms in soil (Subba, 2001).
Azotobacter sp. has “Nitrogenase” activity, which mediates the conversion of atmospheric nitrogen (N_2) into ammonium. Bio fertilizers improve the quantitative and qualitative features of many plants (Yosefi et al., 2011).
Ceratophyllum demersum L. (coontail or hornwort) is a completely submersed plant and commonly seen in ponds, lakes, ditches, and quiet streams with moderate to high nutrient

levels (Johnson et al., 1995). It does not produce roots, instead it absorbs all the nutrients it requires from the surrounding water. If it is growing near the lake bottom, it will form modified leaves, which it uses to anchor to the sediment. However, it can float free in the water column and sometimes forms dense mats just below the surface. Les (1986) indicated that the habitat preferences of *C. demersum* are at pH 7.4, with a range extending from 5.9 to 9.4, and in alkaline nutrient-rich sites. In recent years, the use of natural seaweed as fertilizer has allowed for partial substitution of conventional synthetic fertilizer (Zodape et al., 2010).

Thus, the aim of the experiment was to demonstrate the effect of addition of *Azotobacter*, hornwort and their interactions to determine the best focus on improving plant growth of eggplant plant.

MATERIALS AND METHODS

An experiment was conducted during the growing season of 2018/2019 in Najaf governorate. The experiment design was factorial within randomized complete block design (R.C.B.D.) The experiment included 6 treatments i.e. three concentrations of *Azotobacter* (0, 5 and 10 $g \cdot 100\ ml^{-1}$ d.w.) and two concentrations of Hornwort (0 and 5 $T \cdot Ha^{-1}$) The aquatic plant (*C. demersum*) biomass used in this study was collected from the Kufa river. Samples were washed with tap water to remove soils and other impurities.

Planting seedlings in 1/4/2018, distance between plants (50) cm. Irrigation was done by dripping system.

Cultural practices were done equally and when it is considered necessary e.g. cultivation, weeding, etc. as mentioned in (Matlob, 1989).

Least significant difference (L.S.D.) test was used to compare means when it is considered significant at probability of 0.05 (Al-Rawi & Khalaf-Allah, 2000).

Studied parameters

Growth measurements were taken as follows:

1. Vegetative growth parameters included the following:

1.1. Plant length (cm);

1.2. Leaves number ($leaf \cdot plant^{-1}$);

1.3. Shoot dry weight (g). Shoot was taken and weighed, then dried in the electric oven at a degree of 65-70°C for a period of 48-72 hours till the weight remains constant (Al-Sahaf, 1989);

1.4. Stem diameter;

1.5. Total chlorophyll in leaves ($mg \cdot 100\ g^{-1}$). Acetone was used to extract chlorophyll pigment, according to (Mackinney, 1941);

1.6. Total soluble carbohydrates in leaves ($mg \cdot g^{-1}$). These were detected according to (Duboies, 1956).

2. Fruit growth parameters included the following:

2.1. Fruits number ($fruit \cdot plant^{-1}$);

2.2. Fruit diameter (cm);

2.3. Fruit length (cm).

RESULTS AND DISCUSSIONS

Results in Table 1 showed that there was a significant difference between the treatments of hornwort on all vegetative growth parameters (plant length, leaves number, shoot dry weight, stem diameter, total chlorophyll in leaves and total soluble carbohydrate in leaves) were 74.1cm, 41.03 $leaf \cdot plant^{-1}$, 86.5 g, 2.166 cm, 19.976 $mg \cdot 100\ g^{-1}$ and 2.86 $mg \cdot g^{-1}$, respectively compared with control treatment (without addition of hornwort) which gave the least means were 62.01 cm, 54.416 $leaf \cdot plant^{-1}$, 86.5 g, 2.166 cm, 19.976 $mg \cdot 100\ g^{-1}$ and 2.86 $mg\ g^{-1}$, respectively.

Azotobacter had an increasing effect with con. 10 $g.100\ ml^{-1}$ on all vegetative growth parameters (plant length, leaves number, shoot dry weight, stem diameter, total chlorophyll in leaves and total soluble carbohydrate in leaves) which gave the highest values 74.41cm, 40.05 $leaf \cdot plant^{-1}$, 88.35 g, 2.135 cm, 19.33 $mg \cdot 100\ g^{-1}$ and 2.705 $mg \cdot g^{-1}$, respectively compared with treatment (without addition of hornwort) that gave the lowest values 59.59 cm, 27 $leaf \cdot plant^{-1}$, 55.335 g, 1.87 cm, 18.8 $mg \cdot 100\ g^{-1}$ and 2.555 $mg \cdot g^{-1}$, respectively.

The interaction between hornwort and *Azotobacter* treatments showed significant differences on all vegetative growth parameters (plant length, leaves number, shoot dry weight, stem diameter, total chlorophyll in leaves and total soluble carbohydrate in leaves). Treatment of 5 $T \cdot Ha^{-1}$ hornwort and 10 $g \cdot 100\ mL^{-1}$ of

Azotobacter gave the largest values, 80.67 cm, 47.9 leaf·plant^{-1}, 113.3 g, 2.32 cm, 20.21 mg·100 g^{-1} and 2.898 mg· g^{-1}, respectively, while the treatment of 0 hornwort and 0 g·100 mL^{-1} of *Azotobacter* gave the lowest values, 50.61 cm, 20 leaf·plant^{-1}, 39.67 g, 1.72 cm, 17.81 mg·100 g^{-1} and 2.301 mg· g^{-1}, respectively.

Table 1. Effect of fertilization with *Azotobacter* and hornwort on vegetative growth parameters and carbohydrate

Treatments		Concentration	Plant length (cm)	Leaves number (leaf·plant^{1})	Shoot dry weight (g)	Stem diameter (m)	Total chlorophyll in leaves (mg·100 g^{-1})	Total soluble carbohydrate in leaves(mg·g^{-1})
Hornwort (T.Ha^{-1})		0	62.01	27.1	54.416	1.863	18.04	2.438
		5	74.1	41.03	86.5	2.166	19.97	2.86
L.S.D A (0.05)			1.487	1.581	3.94	0.095	0.03	0.01
Azotobacter (g·100 mL^{-1})		0	59.59	27	55.335	1.87	18.8	2.555
		5	70.61	35.15	67.69	2.04	18.9	2.688
		10	74.41	40.05	88.35	2.135	19.33	2.705
L.S.D B (0.05)			1.821	1.937	4.83	0.117	0.084	0.012
Hornwort (T·Ha^{-1} × *Azotobacter* (g·100 mL^{-1})	0	0	50.61	20	39.67	1.72	17.81	2.301
		5	67.26	29.1	60.18	1.92	17.87	2.502
		10	68.16	32.2	63.4	1.95	18.45	2.512
	5	0	68.57	34	71	2.02	19.79	2.81
		5	73.06	41.2	75.2	2.16	19.93	2.874
		10	80.67	47.9	113.3	2.32	20.21	2.898
L.S.DAB (0.05)			2.576	2.739	6.83	0.166	0.119	0.018

Results in Table 2 appeared that there was a significant difference between the treatments of Hornwort on all fruit growth parameters (fruit numbers, diameter and length) were 23.013 fruit·plant^{-1}, 7.436 cm and 15.133 cm, respectively compared with control treatment (without addition of hornwort) which gave the lowest means were 16.773 fruit·plant^{-1}, 6.483 cm and 13.413 cm, respectively.

Table 2. Effect of Fertilization with *Azotobacter* and Hornwort on fruits growth parameters

Treatments		Concentration	Fruit number (fruit·plant^{-1})	Fruit diameter (cm)	Fruit length (cm)
Hornwort (T·Ha^{-1})		0	16.773	6.483	13.413
		5	23.013	7.436	15.133
L.S.D A (0.05)			1.769	0.086	0.141
Azotobacter (g·100 mL^{-1})		0	16.83	6.535	13.965
		5	20.445	6.535	14.28
		10	22.405	7.235	14.575
L.S.D B (0.05)			2.166	0.105	0.172
Hornwort (T·Ha^{-1}) × *Azotobacter* (g·100 mL^{-1})	0	0	13.96	5.90	12.98
		5	17.42	6.73	13.57
		10	18.94	6.82	13.69
	5	0	19.7	7.17	14.95
		5	23.47	7.49	14.99
		10	25.87	7.65	15.46
L.S.D AB (0.05)			3.064	0.149	0.244

Azotobacter had an increasing effect with con. 10 g·100 ml^{-1} on all fruit growth parameters (Fruit numbers, diameter and length) which gave the highest values 22.405 fruit·plant^{-1}, 22.405 cm and 14.575 cm, respectively compared with treatment (without addition of hornwort) that gave the least values 16.83 fruit.plant^{-1}, 6.535 cm and 13.965 cm, respectively.

The interaction between hornwort and *Azotobacter* treatments declared significant differences on all fruit growth parameters (Fruit numbers, diameter and length) treatment of (5 T.Ha^{-1} hornwort and 10 g.100 mL^{-1} of *Azotobacter*) gave the highest values, 25.87 fruit.plant^{-1}, 7.65 cm and 15.46 cm respect-tively, while the treatment of (0 hornwort and 0 g.100 mL^{-1} of *Azotobacter*) gave the lowest values, 13.96 fruit.plant^{-1}, 5.90 cm and 12.98 cm, respectively.

The improvement in vegetative characteristics (plant length , leaves number , shoot dry weight, stem diameter, total chlorophyll in leaves and Total soluble carbohydrate in leaves) might be due to the ability of *Azotobacter* to fix atmospheric N which may share its role in increasing the percentage of mineral nutrient in soil. In addition, it increases the surface area of the root hairs followed by increase in average absorption of mineral nutrients (Farida et al., 2003). These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis. This cell protein is then mineralized in soil after the death of the *Azotobacter* cells thereby contributing towards the nitrogen availability of the crop plants thus resulting in a strong symbiotic relationship (Haller & Stople, 1985).

The bio-fertilizer (*Azotobacter*) caused a significant increase in all the fruit growth parameters (fruit numbers, diameter and length) compared with plants having received no *Azotobacter*. The improvement in fruit growth parameters as a result of using *Azotobacter* might be due to the improvement of vegetative growth (plant length , leaves number , shoot dry weight, stem diameter, total chlorophyll in leaves and total soluble carbohydrate in leaves)in the treated plots. Having done the similar work on potato plants, Kumar et al. (2001) reported that increase in yield was due the treatment of soil with *Azotobacter*. Therefore, this treatment caused a high improvement in plant hormones production like auxin, IAA and gibberellins in addition to the vitamins (biotin, folic acid and vitamin B groups).

The improvement in vegetative parameters (plant length, leaves number, shoot dry weight, stem diameter, total chlorophyll in leaves and total soluble carbohydrate in leaves) might be due to use of macrophytes as fertilizer such hornwort and aquaculture purposes is based on their high nutritive value arising from the richness of biochemical constituents such as proteins, carbohydrates and lipids (Rather & Nazir, 2015). Seaweeds contain high macroelement levels (Ca, K, P), especially those from the Phaeophyta (Hong et al., 2007). The content of minerals in the seaweeds used in this research was in general agreement with the typical values for these marine algae from other countries (Kalaivanan & Venkatesalu, 2012; Sivasangari et al., 2010).

The hornwort as fertilizer caused a significant increase in all the fruit growth parameters (fruit numbers, diameter and length) compared with plants non addition hornwort. Adding APB (aquatic plant biomass) derived compost to soil following dewatering and shredding improves soil quality and increases crop yields (Balasubramanian et al., 2013), although the success of the compost in this capacity depends on both the concentration of plant available nutrients (i.e. the C:N:P ratio).

CONCLUSIONS

The addition of *Azotobacter* with concentration 10 g.100 mL^{-1} and hornwort addition with level 5 T.Ha^{-1} had an increasing effect $^{-1}$of eggplant and improving the vegetative, chemical and Fruit growth parameters characteristic. The double interference treatments between *Azotobacter* and hornwort addition had a significant effect on increasing all the studied traits more than single factors.

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RESPONSE OF SOME VEGETATIVE INDICATORS OF FABA BEANS TO ORGANIC RESIDUE SOLUTION AND SPRAYING WITH PROLINE

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Abstract

An experiment was conducted during the fall season of 2020 in one of the protected facilities (plastic house) in the Faculty of Agriculture, University of Kufa/Iraq. This research aimed to study the effect of applying three types of decomposing organic residue solution to soil (cow, sheep, and chicken), and foliar spraying of proline with three concentrations (0, 40 and 80) mg·L^{-1} on some indicators of the vegetative growth of a local variety of faba bean. A factorial experiment was conducted according to R.C.B.D with three replications. The results revealed significant effects of organic residue solution of chicken for plant height 46.03 cm, leaf number 26.10 (leaf·plant^{-1}), branch number 4.32 (branch·plant^{-1}), and chlorophyll content 44.04 (SPAD unit) in comparison with cow residue solution. Moreover, spraying with proline at 80 mg·L^{-1} realized a significant increase in leaf number 25.96 (leaf·plant^{-1}), branch number 3.73 (branch·plant^{-1}), and relative chlorophyll content 34.68 (SPAD unit) compared to the control treatment. Consequently, the combination of the two factors was the most efficient treatment for faba bean plant growth indicators studied aforementioned.

Key words: *faba bean, organic residues, proline, vegetative growth.*

INTRODUCTION

Faba bean *Vicia faba* is one of the main leguminous crops grown in the winter season in order to obtain green pods or soft or dry seeds, which are rich in proteins, carbohydrates, and vitamin B complex, which makes this crop of good significance to compensate as well for the high price of animal protein (Abbas, 2013). It is used to treat many conditions such as kidney stones, liver impairment, and eye diseases (Khalil et al., 2015). Farmers prefer using animal organic fertilizer over other synthetic chemical fertilizers in fertilizing and caring for the soil for several benefits that benefit the soil and crops. Therefore, animal organic fertilizers can reduce the losses that may be caused to plants and the environment as a result of the use of synthetic fertilizers. Human kinds realized in the past the importance of organic fertilizer in soil fertilization, as they noticed better growth of plants in the lands where their livestock graze, and thus it became common to add organic fertilizers and disseminate the remains of previous crops before each new planting (Laboski et al., 2003). Recently, the focus on the use of mineral fertilizers has led to many problems, the most important of which is the pollution of groundwater with the residuals of those fertilizers, in addition to the increase in the content of nitrates in vegetable products and their negative effects on human and animal health. It is reported that 80% of nitrates are sourced from consuming vegetables, and if they are not assimilated in the formation of proteins, they are stored in the cells (Al-Redhaiman, 2004). Supporting crops with decomposed organic fertilizers which are not available in large quantities in most countries due to the cost and slowness of their decomposition processes, necessitates adding them in the form of solutions in order to save the added quantities per unit area and homogenize the fertilizer distribution and facilitate its availability to the plant root area (Radhi, 2010). Proline, an amino acid, is widely found in higher plants and greater quantities than the rest of the amino acids (Abraham et al., 2003). Further, it is a very osmotic active compound and increases the stability of cell membranes and reduces their breakdown in case of exposure to sodium and chloride ions

(Mansour, 1998), through its interaction with the phospholipids in the membranes and protects the protein complexes (protein structures) against denaturation (Samaras et al., 1995). Moreover, proline plays a protective factor for enzymes (Solomen et al., 1994) and other cytosolic organelles (Van Rensburg et al., 1993). In addition, it is a key factor to solve the problem of flower dropping of bean plants as a result of exposure to some environmental stresses (Fang et al., 2010), and accordingly, this work was achieved to investigate the response of some vegetative indicators of faba bean plants to proline spray and the addition of animal organic residue solution.

MATERIALS AND METHODS

Experiment site. The field trial was carried out for the fall season 2020 in one of the protected facilities (plastic house) belonging to the Faculty of Agriculture, University of Kufa, Najaf city, Iraq.

Experiment material. This research aimed to study some vegetative growth indicators of a local variety of faba bean plants in response to different types of organic residue solution and different concentrations of spraying with proline. Cow and chicken residues were collected from the fields of the Faculty of Agriculture - University of Kufa, while sheep residues were prepared from one of the private sector fields in Najaf region. A water solution was made from these three residues in a volumetric ratio of 3:1 as to (organic fertilizer: water) respectively, while proline and faba bean seeds were purchased from the local market. Prior to planting, random soil samples were collected from the field site with a depth of between 0-30 cm, in order to test some of the soil physical and chemical properties (Table 1).

Table 1. Chemical and physical properties of the field soil

Electrical Conductivity E.C. ($dS \cdot m^{-1}$)	pH	Organic Matter ($g \cdot kg^{-1}$)	Nitrogen ($mg \cdot kg^{-1}$)	Phosphorus ($mg \cdot kg^{-1}$)
1.26	7.55	3.20	2.35	1.60
Potassium ($mg \cdot kg^{-1}$)	**Clay %**	**Silt %**	**Sand %**	**Soil Texture**
0.50	6.90	7.60	85.5	Sandy soil

The seeds of faba bean were planted after soaking them in water for 24 hours to accelerate their germination, then seeds were planted on 24/11/2020, with a rate of 10 plants in each experimental unit, with a distance of 20 cm between one plant and another. Organic residue solution was added to each experimental unit accordingly. Proline was sprayed three times during the season on the vegetative growth of bean plants and the first spray was applied when seedlings reached 3-4 true leaves then leaving 14 days between other sprays (Alhasnawi et al., 2020). All the operations of the recommended agricultural management were implemented as needed.

Experiment design. This field trial included two factors; the first factor was three types of decomposing animal organic residue solution (cow, sheep, and chicken) and the second factor was three concentrations of spraying with proline (0 without spraying, 40, and 80) $mg \cdot L^{-1}$. A factorial experiment was conducted according to a Randomized Complete Block Design (RCBD) with two factors as aforementioned with three replicates.

Vegetative growth indicators. Five plants were randomly selected from each experimental unit to measure the vegetative indicators which were plant height (cm), average number of leaves per plant, average number of lateral branches per plant, and relative content of total chlorophyll in leaves using SPAD field device.

Statistical procedure. Data in this study were analyzed according to the analysis of variance (two-way ANOVA), then least significant difference (L.S.D) procedure at $p < 0.05$ was employed for the mean separation of the studied indicators (Montgomery, 2020). Statistix 10 (Analytical Software, 2013) was used for all statistical analyses.

RESULTS AND DISCUSSIONS

Results in Table 2 indicated that all the treatments of the organic residue solution of different types (cow, sheep, chicken) resulted in a significant improvement in all studied vegetative growth indicators. The organic residue solution of chicken realized the maximum average for plant height 46.03 cm, leaf number 26.10 ($leaf \cdot plant^{-1}$), branch number 4.32 ($branch \cdot plant^{-1}$), and relative chlorophyll content 44.04 (SPAD unit) in comparison with cow residue solution. This growth increment of bean plants may be due to the influence of the animal organic residue in improving the chemical, physical, and biological characteristics of the medium soil (Abbas, 2013). Furthermore, the process of fermentation of organic residues before use may have contributed to the availability of many nutrients. Subsequently, these elements have an important role as they play many biological and physiological processes, stimulating nutrient contents in plant tissues, cell division and elongation, or forming cell membranes that increase plant growth such as plant height, lateral branch number, leaf number, and leaf chlorophyll (Al-Sahaf, 1989).

Faba bean growth indicators were affected significantly by foliar application of proline at the 0.05 probability except for plant height. Increasing concentration of the applied proline caused increases in plant vegetative indicators (Table 2). The highest concentration of proline at (80 mg·L^{-1}) recorded the highest average for leaf number 25.96 (leaf·plant^{-1}), lateral branch number 3.73 (branch·plant^{-1}), and relative chlorophyll content 34.68 (SPAD unit) compared to the control treatment (without spraying). This may be due to the fact that spraying with proline boosts the growth and elongation of the roots and thus enhance the growth of plant vegetative group. Moreover, proline acts as an enzymatic preserver under the action of hormones and enzymes necessary for growth and helps the plant cells to absorb water in turn leading to an increase in the number of leaves and branches of the plant. These results are in accordance with (Amin et al., 2014) and (Ismail and Halmy, 2018). In addition, the increase in the chlorophyll content in leaves when sprayed with proline may be due to the fact that proline helped in the activity of many enzymes, especially the enzymes responsible for the formation and construction of chlorophyll molecule or increasing the plant photosynthesis capacity, and consequently increasing the content of chlorophyll in leaves.

The interaction between animal organic residue solution and foliar spray of proline (Table 2) referred to significant effects for the bean vegetative parameters. The combination of chicken solution with the spraying of proline at a concentration of (80 mg.L^{-1}) notably realized the highest average for plant height 46.94 cm, leaf number 29.10 (leaf·plant^{-1}), lateral branch number 4.52 (branch·plant^{-1}), and relative chlorophyll content 49.74 (SPAD unit). However, the interaction treatment of cow solution without proline spray gave the lowest average for plant height 35.80 cm, leaf number 18.90 (leaf·plant^{-1}), lateral branch number 1.84 (branch·plant^{-1}), and relative chlorophyll content 14.45 (SPAD unit). These results are consistent with Amanullah et al. (2010) and Reda et al. (2014).

Table 2: Influence of the Organic Residue Solution and proline spray on some phenotypic indicators of faba bean

Treatments	**Plant Height (cm)**	**Leaf No. (leaf·plant^{-1})**	**Branch No. (branch·plant^{-1})**	**Relative Chlorophyll Content (SPAD unit)**
Effect of Organic Residue Solution (3:1) as volume				
Cow	36.33	20.50	2.44	17.21
Sheep	39.78	22.69	3.22	29.42
Chicken	46.03	26.10	4.32	44.04
L.S.D 0.05	**1.78**	**2.06**	**0.21**	**1.75**
Effect of Proline (mg·L^{-1})				
0	39.59	19.70	2.86	26.00
40	41.24	23.63	3.39	29.99
80	41.32	25.96	3.73	34.68
L.S.D 0.05	**N.S.**	**2.06**	**0.21**	**1.75**

		Effect of the interaction				
Cow	**0**	35.80	18.90	1.84	14.45
Sheep		38.73	18.70	2.62	24.52
Chicken		44.23	21.50	4.12	39.04
Cow	**40**	35.91	20.50	2.44	17.21
Sheep		40.88	22.70	3.42	29.42
Chicken		46.92	27.70	4.32	43.34
Cow	**80**	37.28	22.10	3.04	19.98
Sheep		39.74	26.68	3.62	34.32
Chicken		46.94	29.10	4.52	49.74
L.S.D 0.05		**3.08**	**3.57**	**0.36**	**3.03**

N.S. means non-significant.

CONCLUSIONS

It is concluded from the research that adding animal organic residues and spraying with proline had a significant impact on improving the bean vegetative growth parameters, especially the addition of chicken solution with proline spray at 80 mg·L^{-1}.

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Lophantus anisatus (Nett.) Benth. USED AS DRIED AROMATIC INGREDIENT

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Abstract

Lophantus anisatus Nett. belongs to the Lamiaceae (Labiatae) family, and it is an aromatic, medicinal, and honey plant. Due to its high content of substances with nutritional and sensory value, the powder obtained from the naturally dried plants can be used as aromatic and nutraceutic ingredients for vegetable chips. In addition to its sensory quality, this powder also stands out by having high antioxidant activity, with beneficial effects on the human body. The aim of this study was to obtain fine powder from natural dried lophantus leaves, which can be further used as aromatic ingredient. The lophantus powder was characterized in terms of dry matter content, total polyphenols, antioxidant activity using DPPH method, ascorbic acid, total pigments, and volatile compounds. The main compounds identified in the volatile oil were: methyl chavicole, methyleugenol, caryophyllene, and germacrene D.

Key words: *aromatic ingredients, bioactive compounds, natural drying, powder.*

INTRODUCTION

Lophantus anisathus (Nett.) Benth. (*Agastache foeniculum* (Pursh) Kuntze), with the common names "Blue Giant Hyssop", "Giant Hyssop", "Fragrant Giant Hyssop", "Anise Hyssop", "Wild Anise", and "Lofant popular", is a member of the family Lamiaceae (Luchian et al., 2020). It is an herbaceous, perennial plant (Ivanov et al., 2019; Kormosh et al., 2020), native to Asia (Vînătoru et al., 2019).

Due to its adaptability to environmental conditions, the plant is spreadaround the world. In Romania it was acclimatized and analyzed at the Research and Development Station for Vegetables Buzău (Vînătoru et al., 2019). The *Lophantus anisatus* species is considered to be an aromatic, medicinal, ornamental, and melliferous plant (Zhekova et al., 2010; Kormosh et al., 2020).

The chemical composition of this plant (caffeic acid, alkaloids, phenolic compounds, estragole, anethole, vitamins, minerals and other useful compounds) gives it antibacterial (Zielinska & Matkowski, 2014), antifungal (Ownagh et al., 2010; Zielinska & Matkowski, 2014; Hashemi et al., 2017), antiemetic, antiviral, anti-inflammatory (Duda et al., 2013; Zielinska & Matkowski, 2014; Costache & Vînătoru, 2017) and antipyretic properties (Shanaida et al., 2020), being used in cardiovascular, neurological, digestive disorders (Duda et al., 2013; Costache & Vînătoru, 2017).

Due to their aroma, the plants are used in gastronomy as a popular seasoning in culinary products. Fresh and dried young shoots are used for baked products and as spices for various dishes. It is an irreplaceable aromatic plant in many cuisines around the world (Kormosh et al., 2020). It has also proved to have great potential in the cosmetic and pharmaceutical industries (Kormosh et al., 2020, Luchian et al., 2020).

Plants of the Lamiaceae family contain triter-penoids, flavonoids, essential oils, and other bioactive compounds. One of characteristic of this family is the higher amounts of phenolic compounds (flavonoids, tannins, hydroxycinnamic acids) (Benedec et al., 2015).

The aim of this paper was to highlight the benefits of the lophantus powder for nutrition, in a healthy lifestyle.

MATERIALS AND METHODS

Samples consisted of organic natural dried *Lophantus anisathus* from Sălcuța, Dâmbovița County. After natural drying the samples were crushed for approx. 30 sec at 9000 rpm using a GrindoMix mill. The samples were analyzed for various nutritional attributes (dry matter, ascorbic acid, polyphenols, antioxidant activity and volatile oil composition) in the Research Center for Studies of Food Quality and Agricultural Products from USAMV of Bucharest.

Determination of the dry matter (DM) was achieved using the MAC 50 PARTNER thermo balance by drying 1 g of sample at 105 °C (Mitu et al., 2021).

Ascorbic acid content was determined after extraction of 1 g of raw material, grinded with 2 mL of orthophosphoric acid (2%, v/v) for 1 minute at room temperature. The mixture was quantitatively passed into a 15 mL centrifuge tubes, and brought to a final volume of 10 mL with orthophosphoric acid (2%, v/v). After extraction, all samples were centrifuged and filtered for HPLC analysis (Stan et al. 2020). Ascorbic acid quantification was realized using HPLC-DAD equipment (Agilent Technologies 1200 Chromatograph). Chromatographic separation of compounds was performed using an ZORBAX XDB-C18 (4.6 x 50 mm, 1.8 μm i.d.) column. The following conditions were used for analysis: column temperature was 30°C, injection volume was 2 μL, isocratic flow rate of 0.5 mL/min using 0.05% formic acid in water as mobile phase (Chanforan et al., 2012).

Extraction of polyphenols from lophantus samples was based on the method described by Bădulescu et al. (2019) using Folin-Ciocâlteu method. To 0.2 g of dried sample, 10 mL of 70% aqueous methanol were added and the samples were incubated in dark at room temperature (approx. 21 °C). Then the extracts were homogenized at 500 rpm for 1 h, centrifuged at 5000 rpm, 4°C, for 5 min, followed by supernatant recovering and re-extracting the residue for two more times, with a final volume of 30 mL. Sample preparation for spectrophotometric measurements was performed after the method described by Stan et al., 2021.

The antioxidant activity of methanolic extracts was performed by the DPPH assay after the method described by (Bujor et al., 2016, Alberti et al., 2017) with minor modifications. Briefly, 0.2 mL of the sample extract was added to 2 mL of 0.2 mM solution of DPPH in methanol and incubated in dark with continuous homogenising. Then the absorbance was measured at 515 nm after 30 minutes. Methanol was used as a blank reference. The results were expressed as mg Trolox eq./100 g sample. Triplicates of independent extract solutions were analyzed.

Determination of assimilating pigments content

Extraction of chlorophyll content was made based on Lichtenthaler & Wellburn (1983) method, using acetone 80% as solvent. Successive extractions were performed until the residue has become colourless and then was adjusted to 50 ml fixed volume (Bujdei et al., 2019). The absorbance of extracts was measured at 663, 646 and 470 nm against blank (acetone 80%). Formula used for calculation of chlorophyll a (Ca), b (Cb), and total carotenoids (Cx+c) content were:

$$\text{Ca } \mu g/ml \text{ extract} = 12.21A663 - 2.81A646$$

$$\text{Cb} \mu g/ml \text{ extract} = 20.13A646 - 5.03A663$$

$$\text{Cx+c } \mu g/ml \text{ extract} = \frac{1000A470 - 3.27Ca - 104Cb}{229}$$

The results were further calculated for mass and final extraction volume and were expressed as mg/100g sample.

Essential oil extraction and GC-MS analysis

Around 70 g of dried leaves samples and 180 g of dried flower samples were hydro-distilled for 3 h in a Clevenger-type apparatus, and the essential oil was collected for further analysis using GC-MS (Agilent 6890 GC coupled with a 5973 Network MS and a 7673 injector). For the separation of volatile compounds a HP-5MS capillary column (30 m × 0.25 mm id, 0.25 μm film thicknesses) was used. The following GC oven operating conditions were employed: 50°C for 8 min, then a 4°C/min ramp to 280°C. Helium had a constant flow of 1.0 mL/min, and the injection volume was 3 μL with a split ratio 50:1. The GC column was coupled directly to the spectrometer in EI mode at 70 eV with the mass range of 50-550 amu at 2 scan/s (Ion et al., 2020).

RESULTS AND DISCUSIONS

The industrialization of the last century has brought many advantages for the mankind, but also several environmental and health related issues. Consequently, there was a significant increase in compounds potentially harmful for the human health in food products. In this regard, studies are being conducted to replace these compounds with different natural substances that bring no harm to the human health and, in addition, contain substances capable of contributing to restoring the balance in terms of the proper functioning of the body. The use of plants in different forms has contributed to the interest regarding diet renewal, used alone or combined, fresh or dry, aromatic plants are a common component of the daily diet (Kwaśniewska-Karolak & Mostowski, 2021). Plants are a valuable source of nutrients that efficiently influence the human body.

Lophantus anisatus species shows high concentrations in different bioactive compounds: 98.79 mg/100 g for ascorbic acid, 7048.13 mg GAE/100 g for total polyphenols, and an antioxidant activity of 3982.99 mg Trolox equivalent/100 g (Table 1). It also has high content of assimilating pigments as depicted in Table 2: 14.93 mg/100 g chlorophyll a, 6.8 mg/100 g chlorophyll b, 21.73 mg/100g total chlorophyll, and 4.08 mg/100 g carotenes.

Vitamin C is an important antioxidant substance, used by both plants and humans for neutralizing the harmful effects of the free radicals. It is also involved in the regeneration of antioxidant pigments (carotenes, xanthophylls), of vitamin E and of glutathione (Burzo, 2018). With regard to chlorophyll pigments, they have a vital role in performing the photosynthesis process in plants, and they also have favourable effects on the human body helping to fight anemia and stimulate breathing (Dumbravă et al., 2012). Carotenes are precursors for the synthesis of vitamin A, provide photo-protection (Stahl & Sies, 2007) and are involved in neutralizing the singlet oxygen and peroxyl radicals (Fiedor et al., 2005).

Due to their antioxidant role, carotenes prevent cardiovascular diseases, disorders of the eyeball, and inhibit the development of cancer (Bertram, 1993; Takyi, 2001). Carotenoids are essential in performing various functions during the development of plants and animals (Cazzonelli, 2011). The synthesis by plants of substances such as vitamin C, E, phenols, polyphenols, and carotenes that have a strong antioxidant action prevents or inhibits the occurrence of various diseases. Therefore, knowing the antioxidant capacity of the substances that are used in the diet has a particular practical importance (Burzo, 2018).

Aromatic plants, along with fruits and vegetables, are the most important sources of antioxidants. Phenolic compounds that increase the antioxidant defence play also a special role. The analysis of the polyphenols from the *Lophantus anisatus* plants has shown vast amounts of rosmarinic acid and flavonoid apigenin-7-O-glucoside possessing significant therapeutical properties (Shanaida et al., 2020).

Table 1. Nutrient content of *Lophantus anisatus*

Compounds	*Results*
Dry matter (%)	89.27 ±0.4
Ascorbic acid (mg/100 g)	98.79 ±2.50
Total polyphenols (mg GAE/100 g)	7048.13 ±474.37
Antioxidant activity (mg equiv Trolox/100 g)	3982.99 ±230.15

Table 2. Pigment content of *Lophantus anisatus*

Compounds	*Results*
Chlorophyll a (mg/100 g)	14.93 ± 0.77
Chlorophyll b (mg/100 g)	6.8 ± 1.76
Total chlorophyll (mg/100 g	21.73 ± 2.53
Carotenoids (mg/100 g)	4.08 ± 0.04

The phenols included in the human diet have a beneficial effect due to their antioxidant, anti-inflammatory and anticancer capacity (Lambert et al., 2005). Studies carried out by certain scientists indicate also the presence of phenylpropanoids in the chemical analysis of the lophantus plants. These substances include caffeic acid and rosmarinic acid. Caffeic acid has antioxidant, anti-inflammatory and anticarcinogenetic action (Hirose et al., 1997). Rosmarinic acid has antiallergic, antiviral, anti-inflammatory, anti-Alzheimer effect and helps to prevent cancer. Phenylpropanoids also protect low-density lipoproteins (LDL) from oxidation, thus preventing cardiovascular diseases and atherosclerosis (Anderson et al., 2001).

The way plants are being processed is very important because they can have an influence on the quality of the food products. Some studies have showed that the drying processes in aromatic plants and medicinal plants have led to a decrease in vitamin C (*Salvia officinalis*) and chlorophyll (*Rosmarinus officinalis*) content compared to the fresh material. While the content of polyphenols increased having a positive impact on the antioxidant activity of the medicinal plants (Kwaśniewska-Karolak & Mostowski, 2021).
The obtained oil yield for lophantus dried leaves was of 1.87%, and 0.89% for dried flower samples. With regard to the composition of the volatile oil, 40 chemical compounds (Table 3) have been determined, both in the leaves and flowers. The major common chemical compounds were as follows: methyl chavicol (73.56%-84.29%), limonene (6.64%-3.26%), methyl eugenol (5.93%-3.48%), caryophyllene (4.49%- 2.79%) and germacrene D (2.17%-1.53%), the weight being held by methyl chavicol which had a higher value for the volatile oil extracted from flowers (Figure 1). Methyl chavicol (estragole) was also the main chemical compound which prevailed in the composition of the volatile oil analyzed by Najafi et al. (2022), Ivanov et al. (2019); Ebadolbahi (2011), Mallavarapu et al. (2004), Omidbaigi & Sefidkon (2003) and Mazza & Kiehn (1992). In the *Agastache* genus the amount of methyl chavicol varies depending on the species, for example: *Agastache rugosa* species, contain more than 90% methyl chavicol (Weyerstahe et al., 1992) and the *Agastache mexicana* species may contain up to 0.51% methyl chavichol (Jadczak et al., 2017).
This chemical compound is uses in the food industry as a flavoring agent (Martins et al., 2012), giving an anise-like flavor (Mallavarapu et al., 2004; Zhekova et al., 2010). It is also of interest to the pharmaceutical and cosmetics industries (Martins et al., 2012).
Limonene has an antibacterial, antiviral, antitumor effect and contributes to the characteristic aroma (Burzo et al., 2005; Vieira et al., 2018; Yingjie et al., 2020).
Methyl eugenol is used as a flavoring agent in foods such as: baked goods, jellies, candy, nonalcoholic beverages, ice cream, and pudding. It is also used in the cosmetics industry (NTP, 2000). Caryophyllene has a role as a non-steroidal anti-inflammatory drug, a fragrance, and an insect attractant (Web 1).
It also has an anticancer and antibacterial effect (Dahham et al., 2015). In studies conducted by by Rather et al. (2012), both caryophyllene and germacrene D showed antioxidant activity.
Other constituents had a value below 1 % (from 0.01% borneol - leaves, 0.01% α-pinene - flowers, to 0.87% elixene - flowers, Table 3).

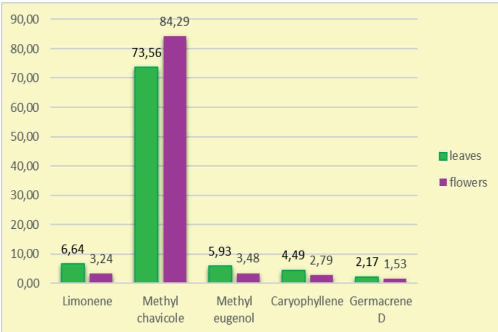

Figure 1. Major chemical compounds in *Lophantus*

Table 3. Chemical compounds in the volatile oil of *Lophantus anisatus*

			Leaves	Flowers
	Chemical composition	RT (min)	%	%
1	α-Pinene	9.596	0.02	0.01
2	β-Phellandrene	11.755	0.03	0.02
3	1-Octen-3-ol	12.243	0.10	0.20
4	3-Octanone	12.597	0.07	0.05
5	β-Pinene	12.801	0.09	0.05
6	3-Octanol	13.080	0.02	0.02
7	p-Cymene	14.353	0.02	0.02
8	**Limonene**	**14.548**	**6.64**	**3.26**
9	Cineole	14.680	0.05	0.11
10	trans-β-Ocimene	15.147	0.08	0.06
11	β-Ocimene	15.616	0.04	0.02
12	γ-Terpinene	16.060	0.02	0.01
13	Terpinolene	17.346	0.04	0.03

14	**Linalool**	**17.967**	0.61	**1.12**
15	1-Octen-3-yl-acetate	18.566	0.49	0.20
16	cis-p-Mentha-2,8-dien-1-ol	18.775	0.05	0.04
17	3-Octyl acetate	19.073	0.05	0.02
18	Camphor	19.689	0.06	0.13
19	Borneol	20.622	0.01	0.02
20	(-)-Terpinen-4-ol	21.103	0.11	0.17
21	α-Terpineol	21.713	0.17	0.12
22	**Methyl chavicole**	**22.101**	**73.56**	**84.29**
23	Linalyl acetate	24.249	0.39	0.23
24	*Unidentified*	25.236	0.08	0.04
25	(±)-Lavandulyl acetate	25.473	0.14	0.05
26	Isoeugenol	27.756	0.08	0.05
27	Eugenol	28.185	0.46	0.40
28	b-Bourbonene	28.656	0.77	0.27
29	**Methyleugenol**	**29.361**	**5.93**	**3.48**
30	**Caryophyllene**	**29.804**	**4.49**	**2.79**
31	trans-α-Bergamotene	30.369	0.12	0.06
32	Humulene	30.895	0.25	0.13
33	**Germacrene D**	**31.776**	**2.17**	**1.53**
34	Elixene	32.259	0.87	0.40
35	δ-Guaiene/α-Bulnesene	32.537	0.29	0.12
36	γ-Cadinene	32.797	0.07	0.05
37	δ-Cadinene	33.080	0.29	0.14
38	Spathulenol	34.714	0.47	0.06
39	Caryophyllene oxide	34.876	0.51	0.14
40	τ-Cadinol	36.932	0.29	0.05

CONCLUSIONS

The variation in the chemical composition makes the study of the *Lophantus anisathus* species of particular interest regarding the value of this plant both for medicine and gastronomy. Products which have in their composition powders obtained from lophantus plants may thus contribute to improving lifestyle.

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NUTRITIONAL COMPOSITION OF FRESH ORGANIC VEGETABLES

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Abstract

Vegetables are important elements for rational and healthy diet due to active compounds and their nutritive values. Currently, technical progress in food industry offer new solution for processing vegetables. The purpose of this study was to determine the nutritional composition of different organic vegetables which can be used as raw materials for healthy chips. Several vegetables (beets, carrot, sweet potato, and turnip) were purchased from organic certified farms and further analysed in terms of dry matter content, total phenolic content, antioxidant activity, and ascorbic acid. When comparing the vegetables in terms of total phenolic content and antioxidant activity, 'Detroit' beets variety registered the highest values, followed by sweet potato and turnip. The ascorbic acid content of sweet potato registered the highest values, but the 'Detroit' and 'Albino' beets varieties registered values below limit of quantification. The total phenolic content and antioxidant activity registered similar trend for all analysed samples. All studied organic vegetables can be successfully used for healthy crispy snacks.

***Key words**: bioactive compounds; chips; crispy snacks; organic vegetables.*

INTRODUCTION

Vegetables represent a major category of foods for rational and healthy diet due to active compounds and their nutritive values. All over the world, vegetables play a significant role in human nutrition especially nutrients as vitamins sources (C, A, B1, B6, B9, E), minerals, dietary fiber, and phytochemicals (Dias & Ryder, 2011). Vegetables supply these nutrients in forms that are generally low in energy and fat, making them more "nutrient dense" than most other food sources (Titchenal & Dobbs, 2006). Especially roots and tubers, can also possess significant caloric value, serving as staple crops in many parts of the world (Radovich, 2011).
Daily vegetable consuming was correlated with reduced risk of heart disease, improvement of gastrointestinal health, stroke, chronic diseases such as diabetes, and some forms of cancer (Cruz et al., 2006; Dias, 2012). Because in each vegetable is available a unique combination of nutrients, humans should daily consume different and various vegetables to get a balanced and varied diet.
Most often vegetables are consumed raw or processed (Aguero et al., 2008). Fresh, raw vegetables are the major vegetable types that consumers purchase for consumption, while processed vegetables in the dried, frozen, and canned forms are also readily available (Ong & Liu, 2011).
With the growing world population (Vicente, 2022) and consumer awareness for nutritious and balanced diet (Kumar et al., 2021), the production and processing of horticultural crops, especially fruits and vegetables, have increased significantly to fulfil the demands (Ciurzyńska et al., 2020). Significant losses and waste in the fresh and processing industries are becoming a serious nutritional, economical, and environmental problem (Sagar et al., 2018; Ciurzyńska et al., 2020). Food and Agriculture Organization (FAO) has estimated the fruit and vegetable losses and waste up to 60% of the total horticulture production. The by-product generated during processing of fruit and vegetable industry alone account for the 25-30% of the horticulture product loss (Sagar et al., 2018).

Currently, technical progress in food industry offer a number of innovative processing methods to meet the requirements of both consumers and producers (Miteluț et al., 2021) for processing horticulture products as “healthy snacks” made from ingredients as dried fruit, vegetable, fruit and vegetable bars, cereal products in the form of bars or cakes. Such products are often sold in a certain amount to suggest the portion size of a single snack that should be eaten (Mielmann & Brunner, 2018). Freeze-dried fruit and fruit and vegetable bars are gaining more and more popularity (Ciurzyńska et al., 2020). As compared to fruit, vegetables are less attractive in terms of taste due to their small content of sugars, but are richer in fibre, vitamins and some minerals (Ciurzyńska et al., 2019). In order to make vegetable snacks more pleasant and tastier it can be added spices, giving them unconventional form which may be a freeze-dried vegetable bar (Ciurzyńska et al., 2019; Ciurzyńska et al., 2020) or chips. The aim of study was to determine the nutritional composition of different organic vegetables which can be used as base for healthy chips.

MATERIALS AND METHODS

Chemicals

Chemicals were purchased from many producers: acetonitrile and methanol from Honeywell (Riedel-de Haën, Seelze, Germany), formic acid from Sigma-Aldrich (GmbH, Germany); ascorbic acid, Folin-Ciocâlteu’s reagent and DPPH (1,1-diphenyl-2-picrylhydrazyl) from Sigma-Aldrich Chemie GmbH (Riedstrasse, Steinheim); Trolox from Acros Organics, Fisher Scientific (Geel, Belgium); Gallic acid from Carl Roth; anhydrous sodium carbonate from Lach-Ner, (Neratovice, Czech Republic); sodium hydroxide 0.1 N from Cristal R Chim S.R.L. (Bucharest, Romania); and ultrapure water was obtained with Milli-Q water equipment (Millipore, Bedford, MA).

Samples

Vegetables such as beets ‘Detroit’, ‘Albino’, and ‘Chioggia’, carrot ‘Flakkee’ and turnip ‘Purple top white globe’ were purchased in 2021 from organic certified farm Grădina Corbilor S.R.L., and sweet potato ‘Rok 1’ from organic certified farm Beleza Store S.R.L. All vegetables were stored at 1±0.5°C and 85±5 % RH until analysis.

Dry matter determination. Dry matter content was performed by drying 1 g of sample in oven (UN110 Memmert) at 105°C (Stan et al., 2021; Ticha et al., 2015) until constant weight.

Ascorbic acid determination was realized through Agilent Technologies 1200 chromatograph equipped with an UV-DAD detector and Agilent ChemStation B.04.03 software (Agilent, USA). Extraction was performed by triturate 1 g of fresh sample in 2% o-phosphoric acid in water (v/v), then homogenized for 15 min at 500 rpm (Chanforan et al., 2012; Stan et al., 2020). Obtained extract was filtered through 0.45µm RC syringe filters. Compounds separation was performed through ZORBAX XDB-C18 (4.6 x 50 mm, 1.8 µm i.d.) column with Rapid Resolution HT and analytical guard column XDB-C18 (4.6 x 12.5 mm, 5 µm i.d.) (Agilent, USA). Column temperature was set up at 30°C, injection volume at 2 µl, flow rate at 0.5 mL/min, using isocratic elution (Chanforan et al., 2012; Hoza et al., 2020) and wavelength at 244 nm. Mobile phase consist in 0.05% (v/v) formic acid in water. Calibration curve was obtained by ascorbic acid standard injection of known and different concentration.

Total polyphenol content (TPC) through Folin-Ciocâlteu (Bădulescu et al., 2019) consist in extraction of 1 g fresh sample with 10 mL of methanol (70%), incubated in dark and room temperature (approx. 21°C), then homogenized for 1 h at 500 rpm, followed by centrifugation for 5 min, 4°C and 7000 rpm. Supernatant was recovered, residue was re-extracted two more times and the final volume was 30 mL. Sample preparation for spectrophotometric measurements were realised by 2 minutes room temperature (approx. 21°C) incubation of 0.5 mL of extract mixed with 2.5 mL of Folin-Ciocâlteu reagent. After 2 mL of 7.5% Na_2CO_3 were added samples were incubated for 15 min and 50°C. Spectrophotometric measurements were performed with Specord 210 Plus UV-VIS spectrophotometer (Analytik Jena, Jena, Germany) at the 760 nm wavelength. Results were calculated and expressed in mg GAE/ 100 g fresh weight.

Antioxidant activity determination using the DPPH (2,2-diphenyl- 1-picrylhydrazyl) method (Bujor et al., 2016) consist in mixing 0.2 mL of extract with 2 mL of 0.2 mM DPPH solution in methanol and incubated for 30 minutes in dark and continuous homogenising. Also the Specord 210 Plus UV-VIS spectrophotometer (Analytik Jena, Jena, Germany) was used for spectrophotometric measurements at 515 nm wavelength. Results were calculated and expressed as mg Trolox eq./100 g fresh weight.

Statistical analysis

Obtained data are results of three independent replicates and standard deviation was calculated using Microsoft Excel.

RESULTS AND DISCUSSIONS

Quality indicators and bioactive compounds were represented by dry matter content, ascorbic acid, total phenolic content, and antioxidant activity and performed for organic vegetables as beets, carrot, sweet potato, and turnip.

Dry matter content

The dry matter results (Table 1) of fresh organic sweet potato registered 26.70% which was the highest value compared with the other analyzed vegetables. Organic beets 'Albino', 'Chioggia', and 'Detroit' obtained 23.33%, 20.46, respectively 19.55%. Organic carrot registered the lowest dry matter content.

Table 1. Dry matter content of organic vegetables

Sample	Dry matter content (%)
Sweet potato	26.70 ±0.40
Beet 'Detroit'	19.55 ±0.39
Beet 'Albino'	23.33 ±0.18
Beet 'Chioggia'	20.46 ±0.20
Carrot 'Flakkee'	13.32 ±0.18
Turnip 'Purple top white globe'	11.73 ±0.24

Ascorbic acid content

When ascorbic acid content of organic vegetables (Table 2) was performed it was determined in sweet potato, 'Detroit' beet and 'Purple top white globe' turnip. The highest amount was observed in organic sweet potato 13.48±1.17 mg ascorbic acid/100 g FW, when in 'Albino' and 'Chioggia' beets and 'Flakkee' carrot the ascorbic acid content was below limit of quantification.

Table 2. Ascorbic acid content of organic vegetables

Sample	Ascorbic acid (mg/100 g)
Sweet potato	13.48 ±1.17
Beet 'Detroit'	1.49 ±0.32
Beet 'Albino'	< LOQ
Beet 'Chioggia'	< LOQ
Carrot 'Flakkee'	< LOQ
Turnip 'Purple top white globe'	10.01 ±1.59

Total polyphenol content (TPC)

The total phenolic content and antioxidant activity present similar trend for all fresh organic vegetables analyzed. The exception was for sweet potato where TPC shown higher concentration in comparison with antioxidant activity.

In this case the highest amount of phenolic content (Figure 1) was observed in 'Detroit' beet which obtained 167.31 mg GAE/100 g FW and the lowest value was obtained by 'Albino' beet (29.17 mg GAE/100 g FW).

Sweet potato obtained 62.96 mg GAE/100 g FW which was the second best value obtained for total phenolic content comparing with other organic vegetables.

Antioxidant activity

The highest antioxidant activity (Figure 2) was obtained when 'Detroit' beet was analysed with 1694.83 mg equiv Trolox/ 100 g FW, followed by 'Purple top white globe' turnip with 382.75 mg equiv Trolox/100 g FW.

The lowest antioxidant activity was registered by sweet potato 186.29 mg equiv Trolox/ 100 g FW.

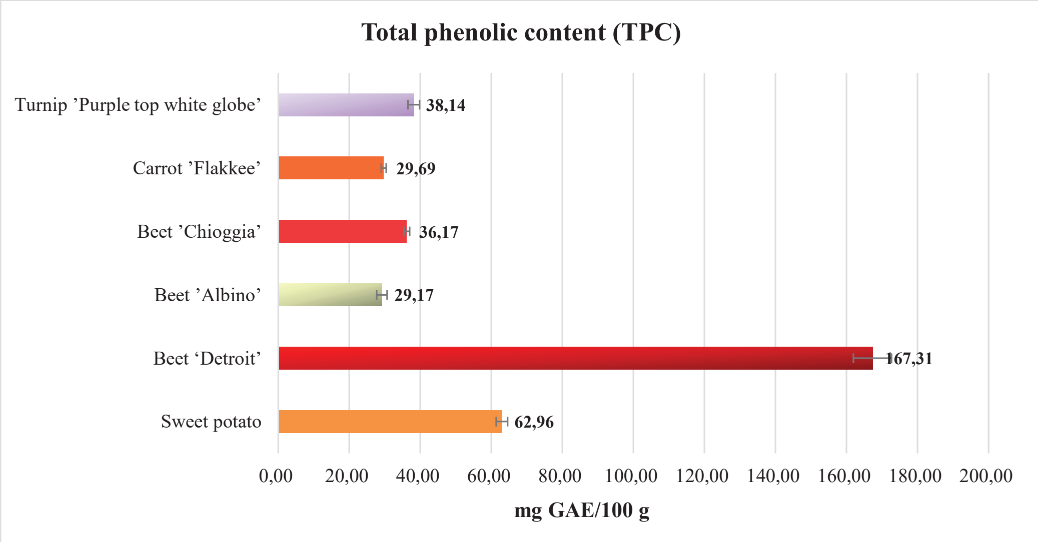

Figure 1. Total phenolic content in organic vegetables

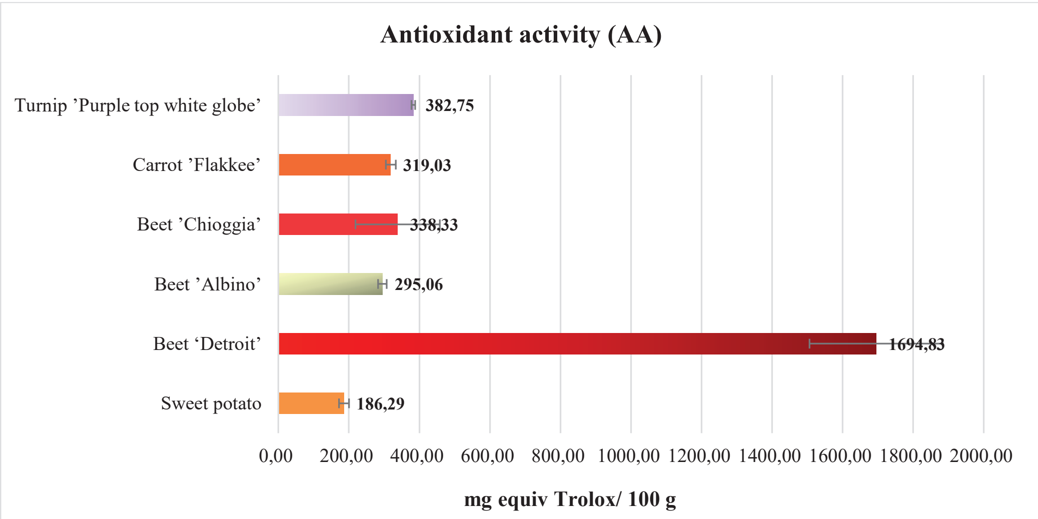

Figure 2. Antioxidant activity values in organic vegetables

CONCLUSIONS

When comparing the vegetables in terms of total phenolic content and antioxidant activity, ‘Detroit’ beets variety registered the highest values, followed by sweet potato and turnip.
The ascorbic acid content of sweet potato registered the highest values, but the ‘Detroit’ and ‘Albino’ beets varieties registered values below limit of quantification.
The total phenolic content and antioxidant activity registered similar trend for all analysed samples. All studied organic vegetables can be successfully used for healthy crispy snacks.

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PHYSICO-CHEMICAL PROPERTIES OF TWO CHERRY TOMATO VARIETIES IN RELATION TO THE GREENHOUSE ENVIRONMENTAL FACTORS

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Abstract

To prevail on the agricultural market, greenhouse grown cherry tomatoes need to meet certain quality standards. The quality can be assessed by determining several chemical and physical characteristics. The aim of the current study is to analyse such parameters of two cherry tomato varieties (Cheramy and Flaviola) in relation to their flowering stage and to the growing conditions within the greenhouse: air temperature, relative humidity and CO_2 concentration. The soluble solids, titratable acidity and dry matter were determined. Furthermore, the β-carotene, lycopene and ascorbic acid contents were analysed. Tomato fruit size and firmness were also assessed. The current study illustrates a limited correlation between the environmental factors and the quality parameters. Thus, future research should be pursued for a better understanding of the effect of these climatic conditions on greenhouse grown tomatoes.

Key words: *tomato, cherry tomato, quality, greenhouse*

INTRODUCTION

Today's horticultural market imposes high quality standards for its products (Coyago-Cruz et al., 2019). These quality standards refer to a series of physiological and chemical properties often described in literature (Schwarz et al., 2017).

Controlling the environmental conditions helps in achieving the commercial and nutritional quality parameters (Doan & Tanaka, 2022). Thus, indoor cultivation is the standard for obtaining both high yield and high-quality tomatoes. Manipulation of these climatic conditions requires proper space and equipment, however. The use of greenhouses is a viable solution to ensure higher yields and high-quality crops (He et al., 2022).

The aim of the current paper is to study the effect of three environmental conditions (air temperature, relative humidity and CO_2 concentration) on the quality parameters of two greenhouse-grown cherry tomatoes (*Solanum lycopersicum*). For this, several physico-chemical properties will be assessed: fruit weight, longitudinal and equatorial diameter and firmness. Furthermore, the total soluble solids and titratable acidity will be measured. These will also help identify the taste and maturity indices. Moreover, the dry matter and ascorbic acid will be assessed. Pigment synthesis is important for both the ripening and red coloring of the fruit (Felföldi et al., 2022). Thus, β-carotene and lycopene contents were analysed, both pigments having important nutritional properties (Junior et al., 2022). This also translates to several health benefits (Lazzarini et al., 2022; Meng et al., 2022).

The current study addresses whether the environmental factors influence the quality parameters of the greenhouse cherry tomatoes.

MATERIALS AND METHODS

The research was conducted in the Research Greenhouse and the Research Center for Studies of Food and Agricultural Products Quality within the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV) campus. Two cherry tomato varieties ('Cheramy' and 'Flaviola') were selected for the current study. Cheramy RZ F1 is a Dutch hybrid from Rijk Zwaan (Rijk Zwaan, 2022). Flaviola F1 is a Romanian hybrid developed within the Buzău Vegetable Research Station (Jerca et al., 2021). The two varieties were sown on September 15th, 2021. The plants were transplanted to coconut-based Jiffy growbag, each variety being cultivated in a separate greenhouse compartment. Throughout the study, the plants were watered and fertilized daily according to a standard recipe. The 'Cheramy' varieties are illustrated in Figure 1. The analyses were performed on tomatoes harvested starting with the fourth inflorescence (fourth harvesting week) - February 28th, 2022. Tomatoes were randomly harvested from the compartments and analysed once every two weeks, with the last harvesting week being the fourteenth - May 3rd, 2022.

Figure 1. 'Cheramy' tomato plants in the Research Greenhouse at USAMV of Bucharest

Equatorial and longitudinal diameter (cm) were used as metrics for evaluating the marketable tomato fruit size, being assessed using a digital calibre and the weight (g) measured with a digital scale. Furthermore, the firmness (N/cm^2*9.84, where 9.84 is the firmness factor) was analysed using a digital penetrometer, TR Turoni equipped with 3 mm diameter tip (Massantini et al., 2021). Soluble solids (% °Brix) were assessed using the Krüss DR301-95 refractometer (Bezdadea-Cătuneanu et al., 2017).
The titratable acidity was determined by homogenising the samples with distilled water and titrated with 0.1 N NaOH until they reached a pH of 8.1. The results were calculated according to the method proposed by Saad et al., 2014.
The ascorbic acid content was determined following the method of Stan et al., 2020.
Taste and maturity indexes were calculated after Mendez (2011).
Dry matter was determined by drying the fresh tomatoes at 105°C for 24 hours, with results expressed in % fresh weight (FW).
The carotenoids pigments content was quantified according to the petroleum ether extraction method as follows: fresh sample was ground using mortar and a small quantity of sea sand. The sample was washed repetitively with the extraction solvent until the residue was colorless. The absorbance of the etheric extract was measured at 452 and 472 nm against a petroleum ether blank, using Specord 210 Plus UV/VIS spectrophotometer. The total carotenoids content was calculated following Rodriguez-Amaya et al. (2004) and lycopene content following Pelissari et al. (2016). The results were expressed as mg/100 g of FW.
To determine the influence of the environmental factors on the quality parameters, correlation analyses were performed using Microsoft Excel. This software was also used to determine the standard deviation of the analysed samples. Five repetitions per sample were used to determine the average weight, longitudinal and equatorial diameter, the firmness and the total soluble solids. Furthermore, three repetitions were used to obtain the average titratable acidity, ascorbic acid, β-carotene and lycopene contents of the tomato varieties.

RESULTS AND DISCUSSIONS

The average weekly temperature before harvesting the tomatoes varied between 15.6°C and 23°C (Figure 2).
Optimal growing conditions are obtained at temperatures between 15.5 and 28°C (Tahery et al., 2021). While this threshold was surpassed a few times during the day in the later harvesting weeks, the air temperature only reached values above 30°C a few times. Therefore, while not achieving the optimal temperature at all times, the plants did not suffer from cold or heat stress.

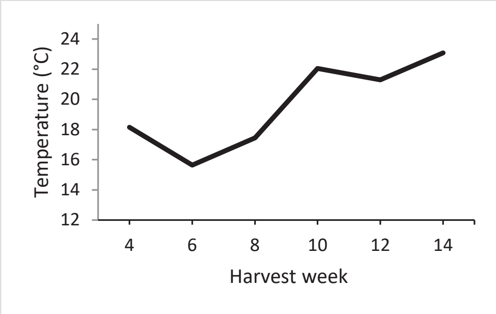

Figure 2. Average weekly greenhouse air temperature prior to harvesting

The seven-day average relative humidity prior to the harvest of the tomatoes varied between 44% and 78.2% (Figure 3), correlated with the indoor greenhouse air temperature.

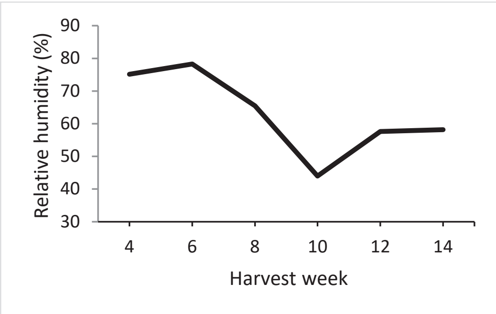

Figure 3. Average weekly greenhouse relative humidity prior to harvesting

While the optimal relative humidity is considered to be around 65%, the growing conditions were favourable for the tomato plants (Tahery et al., 2021).

The average weekly CO_2 concentration prior to harvesting the tomatoes varied between 373 ppm and 594 ppm (Figure 4).

Figure 4. Average weekly greenhouse CO_2 concentration prior to harvesting

For greenhouse crops, research shows optimal CO_2 enrichment values of up to 1000 ppm (Li et al., 2018). The average CO_2 concentration recorded through the current study was lower than optimal, plant growth being negatively affected.

Given the phenotypical characteristics of the two varieties, larger weights were recorded for the Cheramy tomatoes throughout the study (Figure 5).

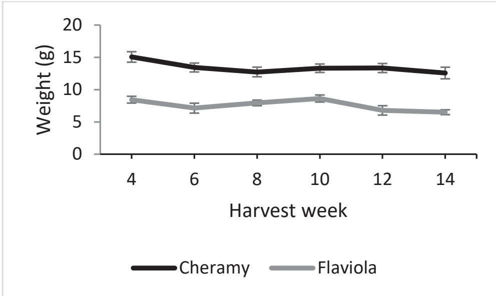

Figure 5. Average weight of the two varieties. Vertical bars represent the standard error of the mean

The lowest average weights for both varieties were measured in the fourteenth harvesting week: 12.57 g for 'Cheramy' and 6.5 g for 'Flaviola'. On the other hand, while the largest weight was recorded at 15.06 g in the first week for 'Cheramy', an average of 8.62 g per fruit was measured in the tenth week for 'Flaviola'.

The average longitudinal and equatorial diameters were relatively consistent throughout the study. Thus, the larger differences in longitudinal diameter between the harvesting weeks were registered for 'Flaviola' (Figure 6).

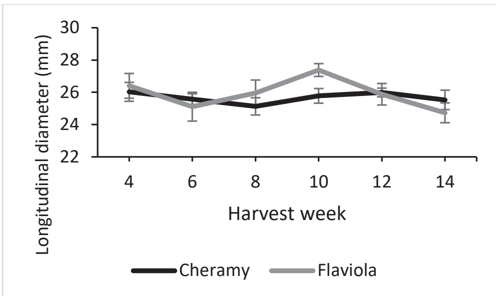

Figure 6. Average longitudinal diameter of the two varieties. Vertical bars represent the standard error of the mean

Similarly, a significant dip within the equatorial diameter was recorded for 'Flaviola' in the twelfth and fourteenth harvesting week (Figure 7).

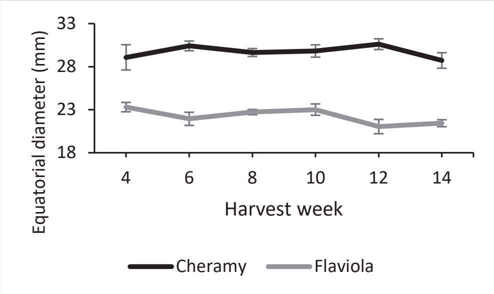

Figure 7. Average equatorial diameter of the two varieties. Vertical bars represent the standard error of the mean

This might be due to several factors, among which might be the variety’s phenotype or the greenhouse whitefly infestation which affected 'Flaviola’s' plant vigour in a more significant manner compared to 'Cheramy'.

The tomatoes’ firmness varied between the harvesting weeks for both varieties (Figure 8).

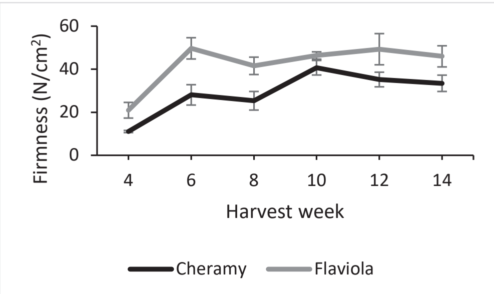

Figure 8. Average firmness of the two varieties. Vertical bars represent the standard error of the mean

Overall, due to the intrinsic characteristics, the 'Flaviola' tomatoes were firmer compared to the 'Cheramy' variety due to the higher resistance recorded.

The total soluble solids were found in higher percentage within the 'Flaviola' tomatoes (Figure 9). However, the analysis performed in the fourteenth harvesting week revealed a higher percentage for 'Cheramy', peaking at 7.84%.

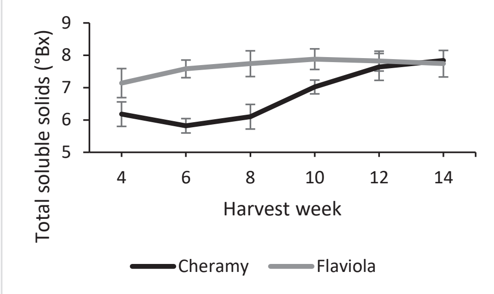

Figure 9. Average total soluble solids of the two varieties. Vertical bars represent the standard error of the mean

For 'Cheramy', the results reveal slightly higher values compared to the findings of Dobrin et al., 2019. However, total soluble solids values are in line with the determinations of Khan et al., 2017.

A significant positive correlation $R^2 = 0.8864$, with the linear regression $y = 0.2737x + 1.4007$ was identified between temperature and total soluble solids for the 'Cheramy' variety (Figure 10).

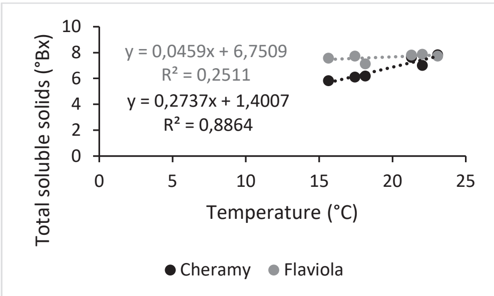

Figure 10. Correlation between the greenhouse temperature and total soluble solids for both varieties

This illustrates the direct influence of air temperature on the sugar content of the tomatoes. Thus, a higher temperature is synonymous with a higher concentration of soluble solids.

The lowest titratable acidity was recorded for 'Flaviola' in the fourth harvesting week (0.29% citric acid), closely followed by 'Cheramy' with 0.34%. While the largest value for 'Cheramy' was recorded in the eighth week (0.95%), 'Flaviola' registered the two largest values within the sixth and eighth weeks at 0.7% (Figure 11).

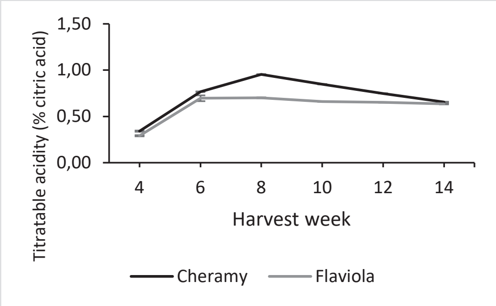

Figure 11. Average titratable acidity of the two varieties. Vertical bars represent the standard error of the mean

Using the previous two quality parameters, the taste and maturity indices were calculated. Because of the low titratable acidity in the fourth week, the largest taste index registered for 'Flaviola' was 1.52. 'Cheramy' tomatoes had a more consistent taste index, averaging at 1.24 throughout the whole study (Figure 12). This is lower than the 1.29 average taste index assessed for 'Flaviola'.

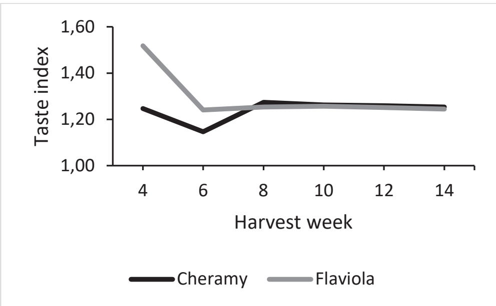

Figure 12. Taste index of the two varieties

The maturity index varies more between the varieties in the first harvesting weeks, reaching a similar value in the fourteenth week: 11.97 for 'Cheramy' and 12.19 for 'Flaviola' (Figure 13).

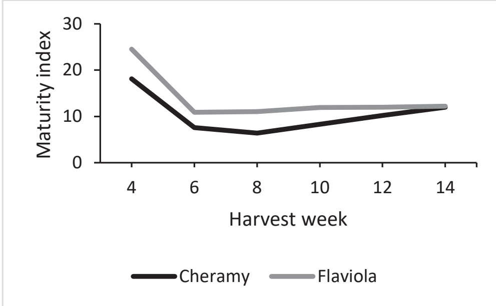

Figure 13. Maturity index of the two varieties

Both the taste and maturity indices are in line with those determined in other studies (Dobrin et al., 2019; Figàs et al., 2018).

The dry matter percentage varied slightly between the varieties and the harvest weeks (Figure 14).

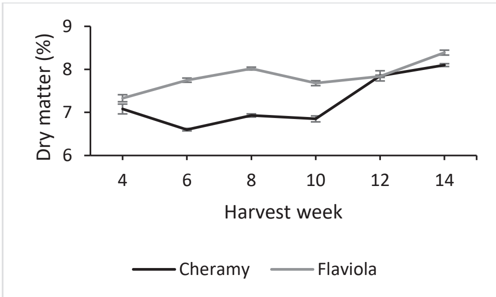

Figure 14. Average dry matter of the two varieties. Vertical bars represent the standard error of the mean

The findings are in line with the values determined through previous studies for the 'Cheramy' variety (Dobrin et al., 2019). Other studies reveal similar values for the tomato dry matter percentage (Felföldi et al., 2022).

No significant correlation was identified between temperature and dry matter (Figure 15).

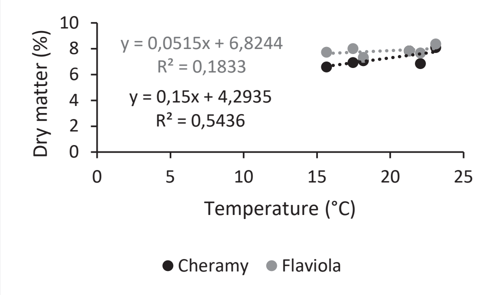

Figure 15. Correlation between greenhouse temperature and dry matter for both varieties

As such, the largest percentage for both varieties were registered in the fourteenth week: 7.97% for 'Cheramy' and 8.3% for 'Flaviola'.

The antioxidant value of tomato (rendered by the content of vitamin C, carotene and lycopene) could be significantly influenced by the crop system and variety (Murariu et al., 2021).

The ascorbic acid varied significantly between the harvesting weeks (Figure 16). For 'Cheramy', the ascorbic acid content varied between 10.23 and 27.56 mg/100 g FW. These represent larger variations compared to 'Flaviola' which ranged from 13.47 to 23.5 mg/100 g FW. The average ascorbic acid is comparable with the results found in the literature (Khan et al., 2017).

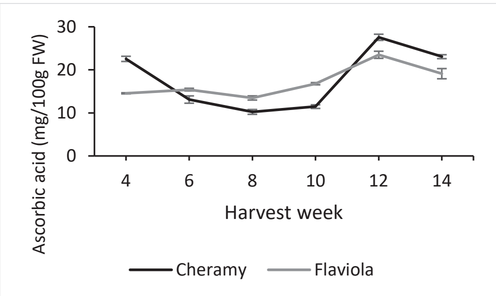

Figure 16. Average ascorbic acid content of the two varieties. Vertical bars represent the standard error of the mean

β-carotene also varied between harvesting weeks, the values being inconsistent with the development stage (Figure 17).

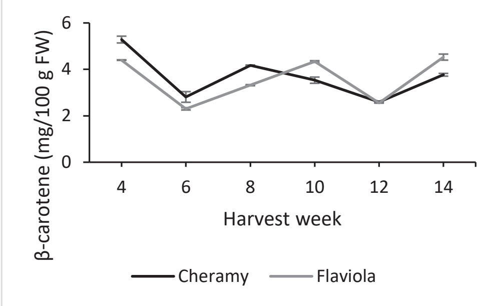

Figure 17. Average β-carotene content of the two varieties. Vertical bars represent the standard error of the mean

The β-carotene content is higher compared to the results of previous studies (Felföldi et al., 2022; Junior et al., 2022).

Analysing the correlation between the temperature and the β-carotene content, no significant interaction was determined (Figure 18).

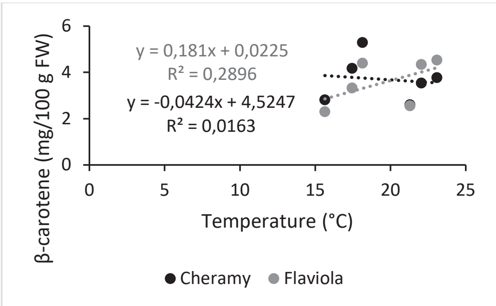

Figure 18. Correlation between the greenhouse temperature and the β-carotene content for both varieties

Similarly, large variations between the harvest weeks could be observed for the lycopene content (Figure 19).

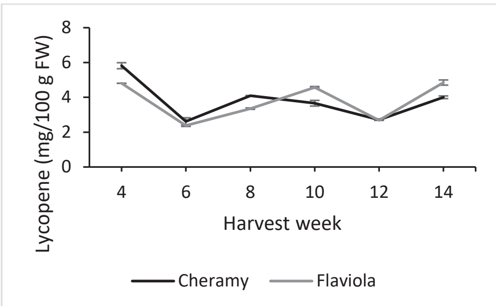

Figure 19. Average lycopene content of the two varieties. Vertical bars represent the standard error of the mean

The tomato lycopene content is lower compared to previous research results (Felföldi et al., 2022), but also higher compared to other studies (Junior et al., 2022). Furthermore, an in depth look at β-carotene and lycopene contents reveals similar values between both the varieties and the harvesting weeks. This is in line with previous findings (Dobrin et al., 2019).
Similarly to β-carotene, no significant correlation was identified between the air temperature and the lycopene content (Figure 20).

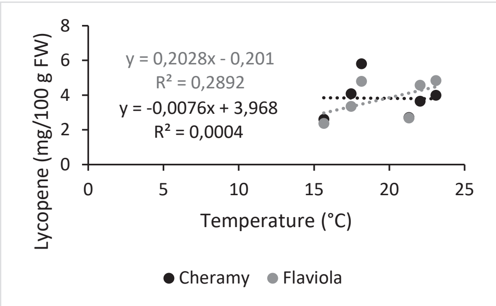

Figure 20. Correlation between the greenhouse temperature and the lycopene content of both varieties

The studied nutritional parameters are in close relationship with the commercial quality of tomatoes (Marti et al., 2016). Monitoring the environmental conditions is essential to reach optimal quality values for greenhouse grown tomatoes. This is further demonstrated by previous studies which concluded that there is no significant difference within the nutritional parameters between organically and conventionally grown tomatoes (Vélez-Terreros et al., 2021).
A well-coordinated cultivation technique is necessary in order to achieve the optimal phytochemical content in tomatoes (Lima et al., 2022). Thus, tomato quality directly translates to the health benefits obtained by consuming the fruits (Meng et al., 2022).

CONCLUSIONS

Environmental factors play a significant role within the plants' growth and development. While the current study only illustrates a particular correlation between the climatic conditions and quality parameters, the interaction between them is proved through previous studies. Future research should focus on a more in-depth review of the environmental factors' effect on the nutritional quality parameters. Furthermore, the differences between plant varieties should be considered for further research.

ACKNOWLEDGEMENTS

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STUDY REGARDING THE PHYSIOLOGICAL CHARACTERISTICS OF SOME VARIETIES OF BASIL CULTIVATED IN THE NUTRIENT FILM TECHNIQUE SYSTEM

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Abstract

The study presents aspects regarding the main physiological processes in the species of Ocimum basilicum, the varieties: 'Aromatic de Buzău', 'Crispum', 'Macedon' and 'Bulatum' cultivated in hydroponic system. Physiological indicators were analysed such as: photosynthesis, transpiration, respiration, dry matter content and water in the basal, middle, and apical leaves of the plants. The LCPro+ automatic analyzer was used to measure the intensity of the photosynthesis, transpiration, and respiration processes. The results obtained varied depending on the variety and the position of the leaves on the plant. The 'Macedon' variety stood out in terms of photosynthesis intensity, dry matter content, water and chlorophyll. The apical leaves showed a higher intensity of physiological processes, compared to the basal and middle leaves.

Key words: *photosynthesis, transpiration, respiration, dry matter.*

INTRODUCTION

Ocimum basilicum belongs to the genus Ocimum, the Lamiaceae family and is a herbaceous plant, native to Africa, Asia, Central and South America (Snežana, 2017). Basil plants have been used since ancient times with a wide use in various industries, such as: food industry as an aromatic plant (Ion et al., 2020) being often used in gastronomy, pharmaceutical industry, as a medicinal plant used in various diseases like: headaches, intestinal parasites, cough and diarrhea (Labra et al., 2004). It has also been widely used in perfumery as well as in dental and oral products (Simon et al., 1990).

The therapeutic action of the plants of the Lamiaceae family is due to the varied composition of the chemical compounds present in the volatile oil (Jailawi et al., 2019). The properties of basil are provided by the content of active substances it contains, having an effect in preventing and treating digestive disorders, cardiovascular disorders, menstrual cramps, diabetes and cancer (Purushothaman et al., 2018). The anti-inflammatory (Raina et al., 2016), antioxidant (Pandey et al., 2016; Flanigan & Niemeyer, 2014) and antithrombotic (Tohti et al., 2006) activity are also highlighted. Over time, it has been shown that some species of *Ocimum* have insecticidal properties, and other species have ornamental qualities varying depending on the variety (Kintzios et al., 2004).

Some phytochemical components such as phenolic compounds, carotenes and essential oils are of particular interest due to their antioxidant and anti-inflammatory properties (Zlotek et al., 2016). It is also a plant used in organic farming to control pathogens (Galea-Deleanu, 2015; Hamburdă, 2016; Teliban, 2016).

Basil, also called the king of herbs, is one of the species that behaves very well in the nutrient film technique system. It can be grown all year round on a vertical farm where much higher yields can be obtained compared to the standard from greenhouse culture (Jailawi et al., 2021).

The main physiological processes involved in plant growth and development are photosynthesis, transpiration and respiration. Photosynthesis is an important indicator in the accumulation of organic substances.

Assimilating pigments (chlorophyll a, b, xanthophyll and carotene) have an essential role in achieving this process. They also have beneficial effects on the human body such as: stimulating respiratory function and combating anemia (chlorophyll), anticancer, antimicrobial, epithelializing and antioxidant effects attributed to carotenes (Dumbravă et al., 2012).
The objective of this study was to highlight the main physiological parameters with an impact on plant growth and development using the hydroponic system.

MATERIALS AND METHODS

The research were carried out within the Hortinvest greenhouses, which belong to the Research Center for Studies of Food Quality and Agricultural Products from USAMV of Bucharest, in the nutrient film technique (NFT) system.
The biological material used was represented by the basil varieties: 'Macedon' (Figure 1), 'Aromat de Buzău' (Figure 2), 'Crispum' (Figure 3) and 'Bulatum' (Figure 4) created in the Vegetable Research and Development Station, Buzău, Romania.
Different parameters (such as photosynthesis, transpiration and respiration rate, water, chlorophyll and dry substance content), have been determined during the flowering phenophase, in May 2021, on the basal, middle and apical leaves.
The physiological parameters were analysed according to the variety and the position of the leaves on the plant. The intensity of the photosynthesis, respiration and transpiration process was determined with the LCPro$^+$ automatic analyzer, directly in the field of experience (700-800 µmol $m^{-2}s^{-1}$ light intensity and 31-32^{0}C). The results were expressed in µmol of CO_2 $m^{-2}s^{-1}$ for photosynthesis and respiration, and the results for transpiration intensity were expressed in µmol H_2O $m^{-2}s^{-1}$. The amount of water and dry matter were determined by gravimetric analysis and expressed as a percentage. The quantitative analysis of the assimilating pigments was performed through the Arnon spectrophotometric method, which is based on the extraction of pigments in an organic solvent (80% acetone) and measuring the absorbance of the extract, by reading the sample extinction at a spectrophotometer at three different wavelengths: 470 nm, 646 nm and 663 nm. The obtained results were expressed as mg 100 g^{-1} FW using the formula of Lichtenthaler & Wellburn (1983).

RESULTS AND DISCUSSIONS

The intensity of the photosynthesis process in the leaves of the basil varieties

From Table 1, the data analysis showed that the value of photosynthesis intensity in the basal leaves ranged from 2.92 µmol CO_2 $m^{-2}s^{-1}$ for 'Aromat de Buzau' to 4.52 µmol CO_2 $m^{-2}s^{-1}$for 'Macedon', in the middle leaves ranged from 4.06 µmol CO_2 $m^{-2}s^{-1}$ for 'Aromat de Buzău' to 6.68 CO_2 $m^{-2}s^{-1}$ for 'Macedon' variety while for apical leaves ranged from 13.1 µmol CO_2 $m^{-2}s^{-1}$ for 'Aromat from Buzău' to 7.18 µmol CO_2 $m^{-2}s^{-1}$ for 'Crispum' variety. The results obtained by Burzo & Mihăiescu (2005) for different varieties of basil showed that the value of the photosynthesis process varied from 4.34 µmol CO_2 $m^{-2}s^{-1}$ ('Citrodorum' variety) and 6.20 µmol CO_2 $m^{-2}s^{-1}$ ('Greek' variety).
It should be noted that the value of photosynthesis intensity is higher in the apically located leaves compared to the basal and middle leaves, this being due to a higher exposure to light radiation.
The intensity with which the photosynthesis process takes place in order to biosynthesis the organic substances, ensures the growth and development of the plants, directly influencing the production.
The leaves of plants perform the process of photosynthesis according to the photosynthetic type C3 being influenced by environmental factors, especially light, temperature, but also by variety characteristics.

The intensity of the transpiration process in the leaves of the basil varieties

The value of the transpiration intensity in the basal leaves varied between 0.91 µmoles H_2O $m^{-2}s^{-1}$ for the 'Aromat de Buzau' variety and 1.84 µmoles H_2O $m^{-2}s^{-1}$ for the 'Crispum' variety. In the case of middle leaves, the value varied from 1.07 µmoles H_2O $m^{-2}s^{-1}$ for the 'Aromat de Buzău' variety to 1.65 µmoles H_2O $m^{-2}s^{-1}$ for the 'Crispum' variety. In apical leaves, the highest value was in the 'Crispum' variety (3.80 µmol H_2O $m^{-2}s^{-1}$) followed by the 'Aromat de Buzău' variety (3.08 µmol H_2O $m^{-2}s^{-1}$).

Approximately 45% of the light absorbed by plants is converted into caloric energy which is consumed in the process of transforming liquid water into steam (Burzo et al., 2004), thus ensuring thermoregulation. It also has a role in avoiding the supersaturation of the cells with water and the generation of the suction force of the leaf through which the ascending transport of the raw sap takes place.

Table 1. Results regarding some physiological parameters in basal, middle and apical leaves of basil

Variety	Photosynthesis intensity (µmol CO_2 $m^{-2}s^{-1}$) Leaves-flowering phenophase		
	basal	middle	apical
Aromat de Buzău	2.92	4.06	13.1
Macedon	4.52	6.83	8.94
Crispum	3.72	5.44	7.18
Bulatum	3.88	6.68	9.14
Transpiration intensity (µmol H_2O $m^{-2}s^{-1}$)			
Aromat de Buzău	0.91	1.07	3.08
Macedon	1.50	1.55	1.99
Crispum	1.84	1.65	3.80
Bulatum	1.27	1.54	1.62
Respiration intensity (µmol CO_2 $m^{-2}s^{-1}$)			
Aromat de Buzău	2.03	5.24	7.34
Macedon	1.83	2.95	3.28
Crispum	2.92	3.01	4.36
Bulatum	1.63	1.58	4.50

The intensity of the respiration process in the leaves of the basil varieties

The intensity of the respiration process in the basal leaves varied between 1.63 µmol of CO_2 $m^{-2}s^{-1}$ in the 'Bulatum' variety and 2.92 µmol of CO_2 $m^{-2}s^{-1}$ in the 'Crispum' variety. In the middle leaves, the intensity of respiration was higher in the 'Aromat de Buzău' variety (5.24 µmol CO_2 $m^{-2}s^{-1}$) and the lowest value was for the 'Bulatum' variety (1.58 µmol CO_2 $m^{-2}s^{-1}$). In the apical leaves, an increase in the intensity of respiration was observed in the 'Aromat de Buzău' variety (7.34 µmol CO_2 $m^{-2}s^{-1}$) compared to the other varieties studied (Table 1).

The analysed data show that the intensity of the respiration process was lower in the basal leaves, compared to the leaves located apically.

According to Brezeanu (2009), research on tomatoes has shown that the intensity of the respiration process is dependent on the degree of maturation of the leaves. Leaves with a more advanced degree of maturation had lower respiratory intensity compared to young leaves. This process provides the biochemical energy needed for plant growth and development.

Water and total dry matter content of the basil varieties

The total dry matter contains organic substances as well as soluble or insoluble inorganic substances from plant cells, their accumulation in plant organs being largely due to the process of photosynthesis.

According to the data in Table 2, it is observed that the value of the dry matter content was higher for the apical leaves (16.17%) and the lowest value was for the middle leaves (8.44%, 'Crispum' variety).

The highest water content was in the leaves of the 'Macedon' variety. The other varieties had approximately the same values (Table 2).

The analysis of the obtained data showed that the maximum value for the dry matter was obtained for the apical leaves (16.17% for 'Macedon' variety), while the minimum value was recorded at the level of the middle leaves (8.44% for 'Crispum' variety).

Table 2. Dry matter and water content of basal, middle and apical leaves of basil

Variety	Dry matter (%)		
	basal	middle	apical
Aromat de Buzău	9.75	11.31	12.89
Macedon	12.05	11.64	16.17
Crispum	8.65	8.44	10.16
Bulatum	9.51	9.35	11.41
Water (%)			
Aromat de Buzău	90.25	88.69	87.11
Macedon	99.87	99.88	99.83
Crispum	91.35	91.56	89.84
Bulatum	90.49	90.65	88.89

Chlorophyll and carotenoids content of the studied varieties

The minimum value of the total chlorophyll content was obtained for the basal leaves (56.92 mg/100 g FW, 'Crispum' variety) while the maximum value was recorded for the apical leaves (159.89 mg/100 g FW, 'Macedon' variety, Table 3).

The total chlorophyll content was higher for the 'Crispum' variety (176.01 mg/100 g FW) analyzed by Burzo & Mihăiescu (2005).

The data in Tables 4-5 showed that the minimum value of the chlorophyll a and b content was obtained for the 'Crispum' variety (42.59 mg/100 g FW, chlorophyll a, respectively 14.33 mg/100 g FW, chlorophyll b in the basal leaves) and the highest value was recorded for the 'Macedon' variety (100.59 mg/100 g FW, chlorophyll a, respectively 59.31 mg/100 g FW, chlorophyll b in the apical leaves).

The content of chlorophyll pigments varies depending on the species, the age of the leaf (Burzo et al., 2005) and the position of the leaf on the plant (Ionescu, 2011).

From the studies performed by Dumbravă et al., 2012, it appears that basil contains a higher amount of chlorophyll pigments and carotenoids compared to rosemary.

The chlorophyll a/b ratio varied between 1.70 mg/100 g FW in the apical leaves for the 'Macedon' variety and 3.06 mg/100 g FW in the middle leaves for the 'Crispum' variety (Table 6).

In terms of carotenoids pigments content, there was an increase in the 'Macedon' variety in the basal leaves 6.16 mg/100 g FW, 8.27 mg/100 g FW in the middle leaves and apical leaves 9.63 mg/100 g FW (Table 7).

The amount of carotenoids in the apical leaves of the 'Aromat de Buzău' variety had the same value as that obtained in the basal leaves of the 'Macedon' variety (6.16 mg/100 g FW, Table 7).

The chlorophyll/carotenoids ratio of the 'Macedon' variety ranged from 18.42 mg/100 g FW in the middle leaves to 22.97 mg/100 g FW in the basal leaves (Table 8).

The content of chlorophyll pigments is correlated in the case of many plants with the intensity of the photosynthesis process and the accumulation of nutrients in plants, respectively (Aelenei et al., 2020).

Table 3. Total chlorophyll content (mg/100 g FW) of basil leaves in the varieties studied

Variety	Leaf position		
	basal	middle	apical
Aromat de Buzău	77.14	99.99	130.28
Macedon	125.17	152.36	159.89
Crispum	56.92	71.90	99.36
Bulatum	78.64	90.31	112.03

Table 4.The chlorophyll a content (mg/100 g FW) of the basil varieties studied

Variety	Leaf position		
	basis	middle	apical
Aromat de Buzău	56.50	73.25	92.01
Macedon	89.77	99.07	100.59
Crispum	42.59	54.67	74.43
Bulatum	59.20	68.07	83.88

Table 5. Chlorophyll b content (mg/100 g FW) of the basil varieties studied

Variety	Leaf position		
	basal	middle	apical
Aromat de Buzău	20.65	26.74	38.27
Macedon	35.40	53.29	59.31
Crispum	14.33	17.22	24.93
Bulatum	19.44	22.23	28.15

Table 6. Clorophyll a/b content (mg/100 g FW) of the varieties studied

Variety	Leaf position		
	basal	middle	apical
Aromat de Buzău	2.74	2.74	2.40
Macedon	2.54	1.86	1.70
Crispum	2.97	3.17	2.99
Bulatum	3.05	3.06	2.98

Table 7. Carotenoids content (mg/100 g FW) of the varieties studied

Variety	Leaf position		
	basal	middle	apical
Aromat de Buzău	3.64	4.80	6.16
Macedon	6.16	8.27	9.63
Crispum	2.81	3.42	4.46
Bulatum	3.75	4.25	5.57de

Table 8. The ratio of total chlorophyll to carotenoids (mg/100 g FW) in the varieties studied

Variety	Leaf position		
	basal	middle	apical
Aromat de Buzău	21.19	20.82	21.15
Macedon	22.97	18.42	16.61
Crispum	20.25	21.05	22.27
Bulatum	20.96	21.27	20.13

Figure 1. Morphological aspects of *Ocimum basilicum* 'Macedon' variety

Figure 2. Morphological aspects of *Ocimum basilicum.* 'Aromat de Buzău' variety

Figure 3. Morphological aspects of *Ocimum basilicum* 'Crispum' variety

Figure 4. Morphological aspects of *Ocimum basilicum* 'Bulatum' variety

CONCLUSIONS

The plants under study are an essential resource for future research on the use of these varieties of basil in various industries. The results obtained in terms of the studied parameters (photosynthesis, transpiration, respiration, dry substance, and water content) varied depending on the variety and position of the leaves on the plant. The 'Macedon' variety was noted for the intensity of its physiological processes, the content of assimilating pigments, dry matter, and water. The apical leaves showed a higher intensity of physiological processes, in comparison to the basal and middle leaves.

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THE INFLUENCE OF THE CLIMATIC CONDITIONS IN THE GREENHOUSE AND OF THE CULTURE SUBSTRATE ON SOME PARAMETERS OF TOMATO GROWTH

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Abstract

The study was carried out in the greenhouses of the Research Center for the Study of the Quality of Horticultural Products, within the USAMV of Bucharest, Romania, on a tomato crop, the cultivar Cheramy F1, carried out in an unconventional system on a coconut substrate. The effect of temperature variation on the growth, development and yield of the tomato crop (Lycopersicon esculentum Mill.) was studied. The observations made on tomato plants led to the conclusion that temperatures above the optimal parameters, especially in the period from May to July, determined the formation of leaves with a smaller leaf surface but also a decrease in fruiting, therefore lower production which led when clearing the crop because it was ineffective. The aim of the study was to identify the effect of temperature variations on the vegetative mass formed and tomato production.

Key words*: greenhouse, temperature, tomatoes, soilless, production.*

INTRODUCTION

In the context of climate change, crops in protected areas are expanding more and more because a favorable microclimate can be ensured for the production of a wide variety of vegetables. Tomato crops can be grown throughout the year, both in the winter months and in the summer. If in the cold periods the temperature in the greenhouse can be controlled much more easily, in the summer, when the light radiation is very strong, inside the greenhouse they can reach values around 64ºC, an aspect also mentioned by Angmo et al. (2019) in very hot climates, however, overheating is a very serious problem in these greenhouses during the summer months, as the use of such greenhouses is limited only to crops in cycle II, from August-December or January-June.

For tomato cultivation in the greenhouse, maintaining an optimal average daily air temperature especially, during the flowering and fruiting period is particularly important for pollen development.

Numerous researchers have mentioned different values of temperature and relative air humidity for each specific growth stage Thus, Sato et al. (2001) and Sato et al. (2006) recommended for the vegetative growth period of tomatoes, an optimal air temperature of 22°C for and 22-25°C for fruit formation, and Drăghici et al. (2021), Jerca (2021) mention temperatures of 20-22°C vegetative growth during the day and 18-19°C during the night and 20-27°C for the period of fruit setting during the day and 17-18°C during the night.

Various authors (Kittas et al., 2011; Cherie, 2010; Hochmuth and Hochmuth, 2012; Altes-Buch et al., 2019) mention for tomatoes grown in the greenhouse at optimal air temperatures between 17-27°C, even up to 35°C because some varieties are adapted to high temperature values, with day/night amplitudes between 5-7ºC but also to radiation of 2.34 kWh/m^2/day).

Wei (2018) mentions that if the day/night temperature difference is less than or equal to 2°C and 5°C, the total number of inflorescences increases and that the lower temperature has a great effect on the production of tomato and Hernández (2015).

The experiments performed by Firon et al. (2006) under controlled conditions to see their effect on pollen viability and the influence on the number of seeds in the fruit, on several tomato cultivars, showed that day/night temperatures of 32/26°C) as well as day/night temperatures of 28/22°C led to thermal stress, pollen viability being affected. A similar aspect was also reported by Huang et al., (2011). Hernández (2015) who showed that 24°C to 32°C during the flowering period of tomatoes causes flower abortion and a significant decrease in both production and fruit quality.

Kittas et al. (2011), appreciated that providing shading in the greenhouse reduced solar radiation, temperature and humidity in the greenhouse with an effect of increasing the leaf surface index, the number of flowers and fruits per plant, but also the total production.

Leaves showed the highest biomass accumulation, followed by stems, petioles, roots and flowers, (Mejía de Tafur, 2009; Li et al., 2014).

MATERIALS AND METHODS

The present study was carried out at the research greenhouse, from UASMV Bucharest, Faculty of Horticulture, it belongs to the Research Center for the Study of the Quality of Horticultural Products. The biological material used in the study was represented by the Cheramy F1 variety. The seedling was produced in the greenhouse, between September 15 and October 29, 2021. Planting was carried out on October 30, 2021, in the greenhouse, under controlled conditions. At planting, the seedling was 40 days old, and the first inflorescence was formed.

The culture space was properly prepared in compliance with phytosanitary hygiene conditions.

After the preparation of the greenhouse, the coconut villages were distributed and placed on the culture troughs. After placing them, the substrate was moistened with a nutrient solution that had a pH of 5.5 and an EC of 3 mSiemens. 3 plants were planted on a mat 1 m long, at a distance of 30 cm between the plants. The distance between the rows was 1.5 m. The values of temperature in the greenhouse, atmospheric humidity, light and CO_2 content were recorded during the entire observation period, from the planting of the seedling in October to the final harvest in July.

All the plant care work was carried out, this consisted of staking the stems, pruning, removing the leaves, as the plant grew, lowering the plants. The vegetative mass of the leaves resulting from the gradual removal as the plants grew, the height of the plants, the mass of the fruits as they reached physiological maturity, their quality was determined. All inflorescences on the plant were recorded. At the same time, the temperature values in the culture substrate were monitored in relation to the values in the culture space.

RESULTS AND DISCUSSIONS

Temperatures were retrieved from the computer database, recorded by the temperature sensor in the culture compartment, throughout the culture period, for 24 hours, day and night.

Atmospheric humidity in the tomato culture compartment recorded appropriate values for the growth and fruiting period. The data recorded between October 2021 and April 2022 showed that the atmospheric humidity values were between 50% and 62.09%, with very few exceptions, when the atmospheric humidity dropped to 41.85% (Figure 1).

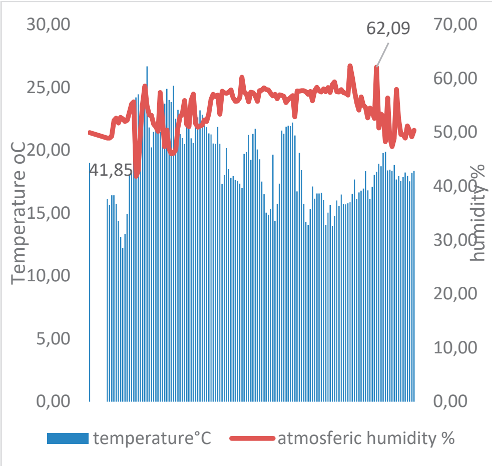


Figure 1. Temperature and atmospheric humidity values recorded in the greenhouse during October 2021-April-2022

In May, the lowest values of 22ºC were recorded on May 11, and in most days the temperatures exceeded 25°C, reaching values of 38°C towards the end of the month. During the night the temperatures were between 15°C and 21°C (Figure 2).

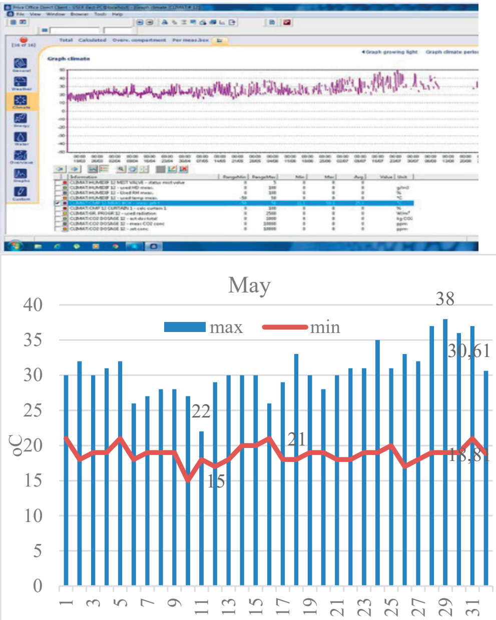


Figure 2. Minimum nighttime and maximum daytime temperature recorded in May 2022

In the month of June 2022, temperatures in the greenhouse recorded values above 26°C with the vast majority reaching values above 35° to 47°C. Temperature values during the night averaged 20°C (Figure 3).

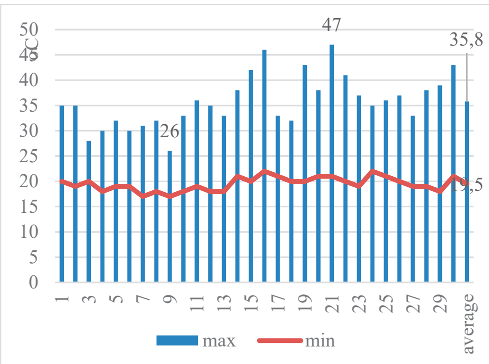


Figure 3. Minimum nighttime and maximum daytime temperature recorded in June 2022

In July 2022, temperatures in the greenhouse recorded values of over 35°C reaching values of 45 °C. Nighttime lows ranged from 17°C to 22°C (Figure 4).

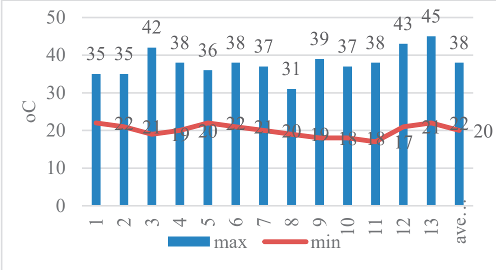


Figure 4. Minimum nighttime and maximum daytime temperature recorded in July 2022

In May the average maximum temperature was 30.61°C and in June 35.8°C with maximum values of 38 °C in July. During the night, the average temperatures were 18.81°C in May, 19.5°C in June and 20°C in July (Figure 5).

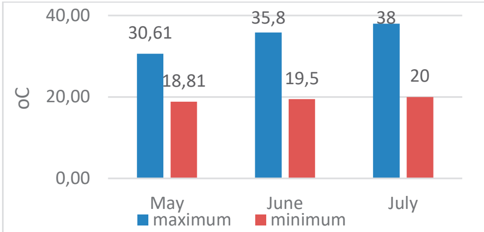


Figure 5. Average maximum and minimum temperatures recorded in May, June and July

It was noted that the temperature in the greenhouse correlated with the intensity of the light radiation also influenced the temperature increase in the culture substrate at the level of the plant's roots. The relationship between the temperature in the greenhouse, temperatures in the culture substrate, starting from the first watering, at 8 am, during the day at 10 am, at 12 pm and 3 pm, was analyzed. Temperatures were measured before the application of fertigation, after each fertigation accordingly analysis periods.

Shishido and Kumakura (1994) but also Kawasaki et al. (2013), Kawasaki et al. (2014), Kawasaki and Yon (2019) mention that an optimal temperature of 24.6°C at the root level favors nutrient assimilation by the roots.

It was found that in July, at 3 p.m., the temperature recorded in the culture space reached 46°C, the temperature of the nutrient solution when fed from the pool was 29.2°C and in the culture substrate before fertigation it was 35.5°C and after 10 minutes after watering it dropped to 35.1ºC. The lowest values were recorded in the morning, at the first watering. This aspect is important because it was found that very high values of the substrate temperature led to a difficulty in its absorption by the plants (Table 1).

Table 1. Determinations of temperatures recorded in the greenhouse and in the culture medium before and after fertigation

Temperatures	UM	Temperature values at:			
		8 am o'clock	10 am o'clock	12 pm o'clock	3 pm o'clock
In the greenhouse	ºC	24	28	36	46
the nutrient solution in the basin	ºC	25.7	26.4	27.0	29.2
In the substrate before watering	ºC	23.1	25	28.4	35.5
In the substrate after watering	ºC	23	25	28.7	35.1

Aspects of the culture compartment during the vegetative growth period are presented in Figure 6, also some aspects of the tomato culture during the fruiting period are presented in Figure 7.

Figure 6. Aspects of tomato culture

Figure 7. Aspects of tomato culture

In the greenhouse, CO2 values of over 200 ppm were maintained and throughout the culture period, they did not exceed the value of 370 ppm (Figure 8).

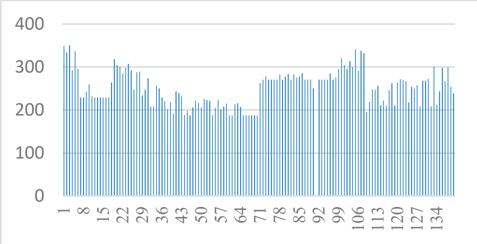
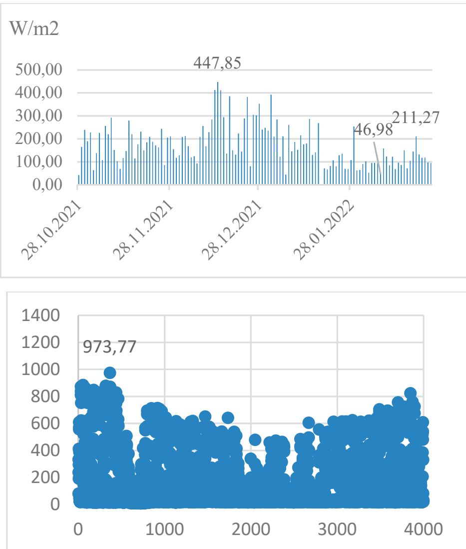


Figure 8. CO_2 content in the culture compartment during the vegetative growth and fruiting period of the plants

In October, the external light radiation showed values between 100 W/m^2 and a maximum of 300 W/m^2. In November, the values remained relatively low, reaching only on certain days the value of 250 W/m^2. In December, higher values were recorded compared to previous months, reaching for short periods maximum values of 447.85 W/m^2, an aspect observed until the end of December. At the beginning of January 2022, the light intensity was low in the first weeks of the month and towards the end of the month the values reached 211.27 W/m^2 (Figure 9 a and b).



a

b

Figure 9. Light radiation outside the greenhouse: a - during October 2021-March 2022; b - April-July 2022

At the end of the crop, in July, the total number of leaves formed per plant was 76 leaves, and the total number of inflorescences per plant was 23. The number of leaves until the first inflorescence was on average 8, and between inflorescences 1 and 2 to 4 leaves. Between inflorescence 2 and 3, an average of 4 leaves were formed, and after inflorescence 3 to inflorescence 23, an average of 3 leaves were formed (Figure 10).

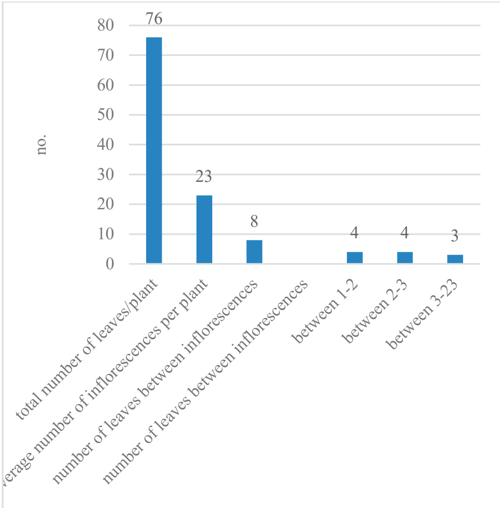


Figure 10. Total number of leaves on plant and between leaves

The Figure 11 shows in detail the harvests carried out, by stages.

At the first defoliation carried out, after 34 days from planting, carried out on 11.11.2021, a quantity of only 0.122 kg of leaves was harvested. In November 2021, the total mass of leaves harvested from the 288 plants was 13.97 kg. In December 2021, a quantity of 84.6 kg of leaves was harvested, a quantity almost similar to that of March 2022, which was 85.40 kg. In January, February and May 2022, the largest quantities of leaves were harvested, 136.4 kg in January 2022, 135.5 kg in February and 132.8 kg in May 2022. In April, an amount of only 25.27 kg of leaves. In June, no defoliation was carried out, but in July, following defoliation, a total mass of only 2.81 kg of leaves was obtained. The total mass of harvested leaves was 606.8 kg at an average number of 285.42 plants in the culture under study (Figures 11 and 12).

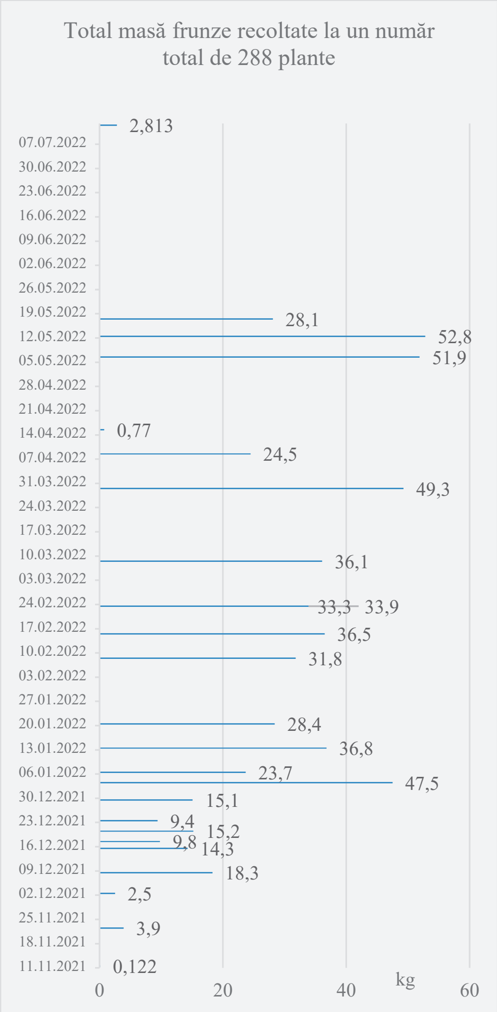


Figure 11 Total mass of harvested leaves for a total number of 288 plants per harvesting stage

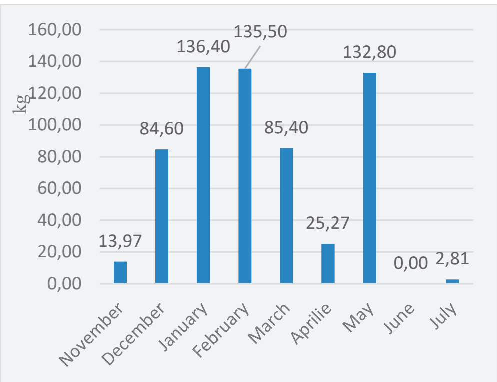


Figure 12. Total mass of leaves harvested during November-July

It was noted that at the first harvests the average mass of a leaf was lower, 48.49 g, an aspect noticed also in the leaves harvested towards the end of the crop where only 10.19 g/leaf were recorded. In the months of January, February and May, leaves were harvested that reached 473.61 g in January, 472.13 g in February and 479.42 g in May. In March, 301.23 g of leaves were harvested, and in December, 293.75 g (Figure 13).

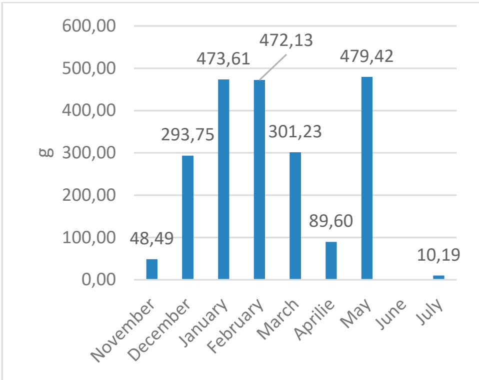


Figure 13. Mass of leaves harvested from the tomato crop - Cheramy F1

The surface of the tomato leaves varied between 240.08 cm^2 and 248.79 cm^2 for the basal and upper leaves and between 371.05 cm^2 and 393.35 cm^2 for the middle leaves.

Figure 14. Leaf surface of tomato leaves

It was noted that by the end of the crop, a total amount of vegetative mass (leaves and stems) of 926.96 kg was harvested from the tomato crop, cultivar Cheramy F1, of which 320.16 kg represented the mass of tomato plant stems and 606.8 kg total mass of leaves obtained from the 288 plants (Figure 15).

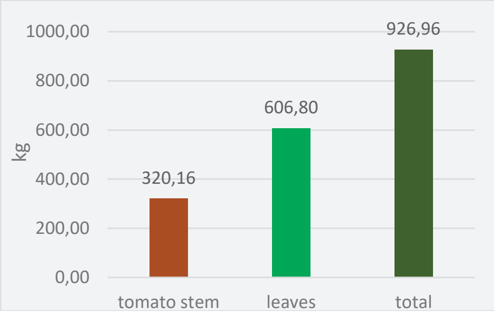


Figure 15. Vegetative mass, stems and leaves harvested from the tomato cultivar Cheramy

It was found that of the total vegetative mass, 65.46% was represented by the mass of the leaves and only 34.54% by the mass of the tomato stem (Figure 16).

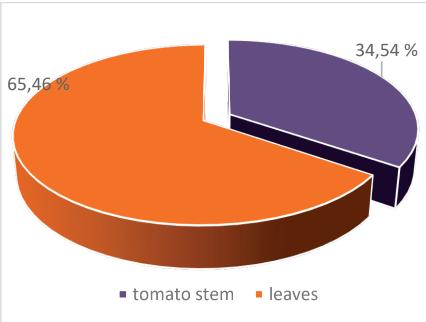


Figure 16. The percentage of the mass of leaves and the mass of tomato stems from the total vegetative mass of tomatoes - Cheramy cultivar

The first harvests were carried out in January and were 177.6 kg. In April and May, 514 kg and 397.3 kg respectively were harvested in May (Figure 17).

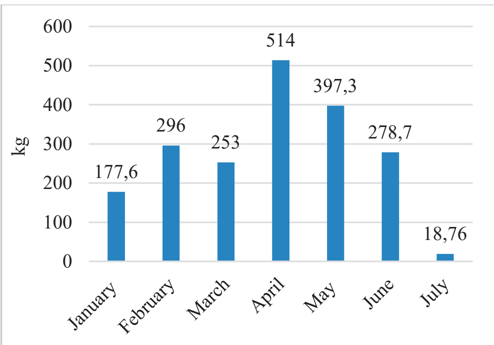


Figure 17. The harvests carried out between January and July 2022

The total vegetative mass of the plants obtained at an average number of 285.42 plants (288 plants at the beginning of the crop respectively 276 plants at the end of the crop) was 2862.32 kg, this being represented by the stem, leaves and fruits. Of this amount, 1935.36 kg were tomato fruits, 926.96 kg were leaves (320.16 kg) and stems (606.80 kg) (Figure 18).

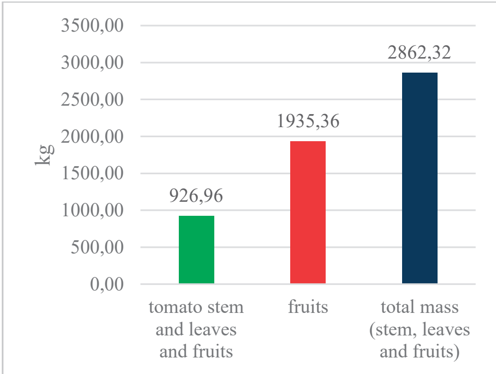


Figure 18. Total plant mass obtained on total plants in the crop, of which the total mass of fruits, leaves, and stems

It was found that, of the total vegetative mass of the plants, the leaf mass represented the largest part, 67.62%, followed by the leaf mass of 21.20% and the stem mass of 11.19% (Figure 19).

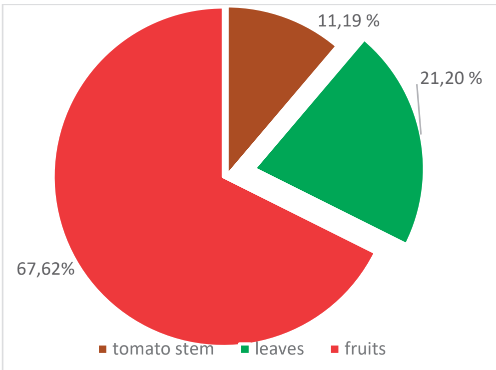


Figure 19. Percentage of fruit, leaf and stem mass in tomato plants at Cheramy F1 cultivar

CONCLUSIONS

Analyzing the data obtained in the tomato crop, Cheramy F1 cultivar, we found that in the first period of vegetative growth and fruiting, the temperature values in the greenhouse were within the normal parameters recommended by the technology. The temperature in the greenhouse did not exceed the value of 25ºC during the day and was not lower than 18ºC during the night, which led to a very good growth and development of the seedlings. Starting from May, we noticed that, during the day, the temperature values recorded values of up to 38ºC, which contributed to a decrease in production, vegetative growth, and fruiting. This aspect was accentuated in June and July when the temperature values in the greenhouse were as high as 47ºC, in July the daytime values did not drop below 35ºC.

Another aspect noted was the influence of very high temperatures on the root system.

The productions obtained were 1935.36 kg for the 288 plants taken into analysis. Total vegetative mass stems 320.16 kg and leaves 606.8 kg. It should be emphasized that the fruit production obtained represented 67.62% of the total vegetative mass, the leaves 21.20% and the stems 11.19%.

Based on these results, we can conclude that maintaining appropriate climatic conditions in the culture space ensures constancy and high production.

ACKNOWLEDGMENTS

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THE COMBINED EFFECT OF TEMPERATURE AND LIGHT VARIATION ON SOME QUALITY PARAMETERS IN CHERRY TOMATOES

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Abstract

The study was conducted under controlled conditions in a greenhouse during a crop cycle during September-April. The culture was established on a coconut substrate. All parameters of vegetation, temperature, light, humidity, carbon dioxide content in the greenhouse were monitored. Fruit mass determinations were performed on each inflorescence, as well as carbohydrate and nitrate content. The production obtained by fruiting stages was determined as well as the total production. The aim of the study was to analyze the influence of some parameters of growth and fruiting on the total production and its quality.

Key words: *tomatoes, cerise, Cheramy F1, soilless.*

INTRODUCTION

Tomatoes are plants that can be grown both in the field and in protected space. In this way, we can systematize the plantings to obtain harvests throughout the year. An important advantage for greenhouse crops is that the microclimate is ensured both during the period of vegetative growth and fruiting. Thus, by ensuring the specific climatic conditions of the plant requirements for each stage of vegetation regarding temperature and light we can count on the achievement of sustained production but also quality for fruits. The fruits are rich in vitamins, minerals, amino acids and pigments (Dinu et al., 2017) and poor in calories, being considered very healthy for the human body (Soare et al., 2015).

In the soilless system, in heated greenhouses, tomato production ensures high-quality performance. Manufacturers are constantly looking to improve their competitiveness through fine-grained control of climate parameters (Becherescu et al., 2018).

Studies conducted in the cultivation of tomatoes in the greenhouse have shown that both temperature and humidity are evenly distributed inside the greenhouses. Legast et al., (2020) note that the distribution of greenhouse temperature in tomato cultivation has a significant impact on fruit growth and development. One of the major factors affecting the growth and productivity of tomatoes is the very high temperature in the growing space. In general, very high temperatures lead to some physiological disorders but also to a decrease in production.

Temperature variations also have a negative effect on plant vegetative growth (Hurd and Graves, 1984), nutrient absorption (Kramer and Boyer, 1995), and production (Falah et al., 2010). Temperature is one of the important factors in maintaining postharvest quality and storage capacity. Kader (2002; 2013) mentions that temperatures above 30ºC speed up the fruit ripening process to the detriment of the ability to preserve the aspects mentioned by Adams et al. (2001) and Alex et al. (2021) estimate that plant density in hydroponic crops can influence the production and quality of tomatoes. Numerous authors studied the effect of temperature on plant nutrition by finding direct relationships associated with light intensity Gent et al. (1998); Giaglaras et al. (1999) and the favourable effect of CO_2, Wacquant (1990), Morison (1993).

Pokluda and Kobza (2019) analyzed the effect of climatic conditions in the greenhouse (air temperature, light intensity, relative air humidity (Redmond et al., 2018) and CO_2 level of the air), on the pH dynamics of the nutrient solution and the electrical conductivity (EC), found insignificant correlations of pH and EC with climatic conditions. However, they identified positive correlations of EC with light intensity.

The growth rate of the fruit depends on the temperature values (Li et al., 2014). Thus, Shamshiri et al. (2017), estimate that the growth rate of fruits is much lower at temperature values of 5.7ºC compared to 26ºC, this aspect was also noticed by Adams et al., 2001. Kawasaki et al. (2014), Shishido Y. & Kumakura H. (1996), Tindall et al. (1990) mention that temperatures of 25°C at the root level, determine a larger leaf area but at temperatures of about 15ºC the leaf mass decreased.

Temperatures below the optimal level, for tomatoes, determine a longer duration of vegetation but also a difficult ripening of fruits. The lycopene content is higher at higher ripening fruit temperatures of 43.5 milligrams/g at 17.8ºC and 64.77 milligrams/g at 25.6ºC (Koskitalo & Ormrod, 1972).

Studies performed on cherry tomatoes regarding the storage of the fruits at temperatures of 5ºC showed a very low percentage of fruits affected by fungi, but also a very small weight loss compared to those stored at temperatures of 10ºC. The firmness and the content in vitamin C of the fruits had also good values (Mohammad, 2012).

Tomato fruits that have reached physiological maturity have a dry matter content of about 3.0-8.88% (Ando, 2016; Dobrin et al., 2019; Kurina et al., 2021), and their content depends on genotype, growing conditions and fruit development stage (Ina et al., 2022) Mohammad et al. (2012) suggest that consumers prefer tomatoes of first quality, both in terms of appearance, nutritional quality and long shelf life. The authors also mentioned that in the 'Unicorn' cultivar at 25ºC/11ºC the carbohydrate content decreased after 28 days of storage compared to storage at 5ºC/11ºC.

Sensory characteristics of tomato fruits are a very important component of their quality and decide on a high degree of consumer's acceptance of the fruit. Azodanlou et al. (2003) mention the importance of the sensory characteristics of tomato fruits, an aspect highlighted also by other researchers (Getinet et al., 2008; Cliff et al., 2009).

MATERIALS AND METHODS

The present study was carried out at the research greenhouses, from USAMV of Bucharest, Faculty of Horticulture, this belongs to the Research Centre for Quality Control of Horticultural Products. The greenhouses have a height of 7 m and are equipped with air conditioning systems. In the experiment, we used Cheramy F_1 cultivar as biological material. Sowing for seedling production was carried out on September 12, 2021. Planting was carried out on October 30, 2021, in controlled conditions and the seedling was 40 days old at planting. At planting, the seedling already had the first inflorescence formed.

The culture space was prepared by disinfecting it and distributing the coconut hydroponic growing media on the culture gutters.

After placing the coconut substrate, they were moistened with a nutrient solution that had a pH of 5.5 and an EC of 3 mSiemens. Three plants were planted on a 1 m coconut substrate at a distance of 30 cm between plants. The distance between the rows was 1.5 m. Observations for some quality parameters on the nitrate and carbohydrate content of tomato fruits were made directly in culture, as the fruits reached physiological maturity, for each inflorescence. The dry matter content was performed in laboratory conditions using the oven, at a temperature of 105ºC (Dobrin et al., 2019).

The values of temperature, light, atmospheric humidity and CO_2 were recorded from the planting of the seedling until the end of the determinations for 5 months.

We determined the number of inflorescences as well as the number of fruits in the inflorescence.

Determinations for chlorophyll content were performed directly on the plant, with Chlorophyll Content Meter Opti-Sciences, Model CCM-200 Plus GPS, expressed in CCI = %. Measured Parameters: Optical Transmittance at 653 nm, and at 931 nm.

The Greentest ECO, a portable tester, was used to determine the nitrate content.

The sugars were determined directly in the greenhouse for the fruits from inflorescences 1-6 using the Portable Refractometer with Brix scale (Figure 1).

Figure 1 Greentest ECO and Refractometer
All data obtained were statistically processed with Microsoft Excel.

RESULTS AND DISCUSSIONS

Temperatures were taken from the computer database, and recorded for 24 hours, day and at night by the culture compartment sensor, throughout the culture period. The atmospheric humidity in the tomato culture compartment was registered for the period of growth and fruiting. The recorded data showed that the atmospheric humidity values were between 50% and 60%.

Calculating the average daily temperatures, from flower formation to fruit harvest, for each inflorescence (fruiting stage) it was found that from the formation of the first inflorescence to fruit ripening, average temperatures of 19.658°C were recorded during 30.11.2021-10.01.2022 and 19.964°C, during the formation of the second inflorescence until the fruits ripen.

It was found that from the formation of flowers in the third inflorescence to fruit ripening the average temperature was 20.139°C and until the formation of the fourth inflorescence an average of 20.574°C.

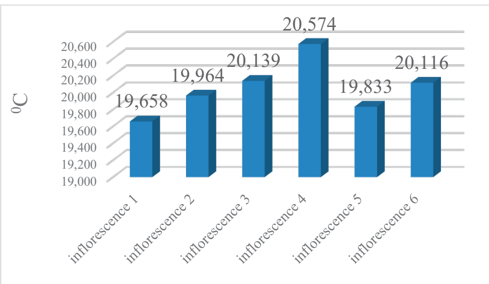

Figure 2. Average temperatures recorded from flower binding to physiological fruit maturity, on inflorescences

During the appearance of inflorescences 5 and 6 and until the ripening of the fruits, the average recorded temperatures were 19.833°C and 20.116°C, respectively. All this information was correlated with the time until the physiological ripening of the fruits (Figure 2)

Atmospheric humidity in the crop compartment was recorded during all periods of vegetation. Figure 3 shows the average atmospheric humidity data from the binding of the flowers to the physiological maturation of the fruits. The lowest value of atmospheric humidity was noticed at 53.691%, during the formation of the first inflorescence.

In the case of the following inflorescences, the values were higher from 55.28% at inflorescence 2 and up to 57.236% during the formation and physiological maturation of the fruits from the sixth inflorescence. Some authors recommend that the humidity values be about 70% during the vegetative growth and 60% during the fruiting period. It also states that the minimum values during the period of vegetative growth should not be less than 30% and during the period of flowering and fruiting less than 40% (Shamshiri et al., 2018).

Liu et al. (2006), mention as favorable the values of 55-90% for atmospheric humidity, these do not influence the photosynthetic activity. Decreasing atmospheric humidity below the threshold of 30-40% influences pollination and fruiting (Huang et al., 2011). In the case of our experiment the humidity values, correlated with the temperature values were corresponding.

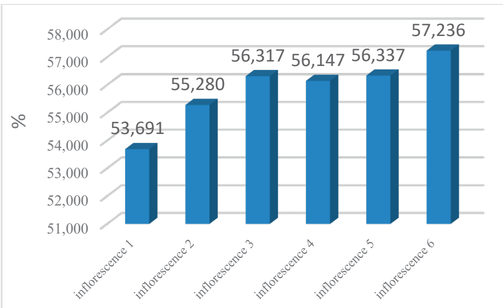

Figure 3. Atmospheric humidity recorded during fruiting stages

The lowest CO_2 values were recorded during the ripening of fruits in the first inflorescence being 435.332 ppm and the highest concentration of 551.383 ppm was recorded during the formation of the third inflorescence. In the case of the other inflorescences, values of 465.137 ppm were registered during the formation of the second inflorescence. Values of 468.827 ppm were recorded during the fruit grown in the fourth inflorescence of 476.237 ppm for the fifth inflorescence and 537.234 ppm for the period of fruit formation in the 6th inflorescence (Figure 4).

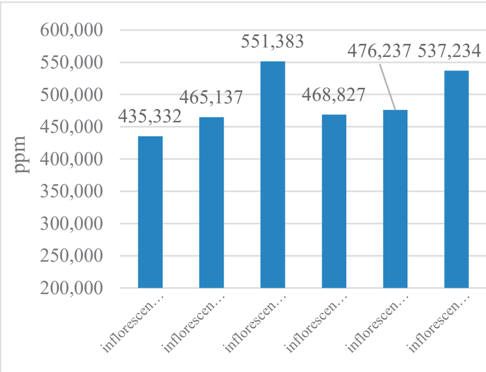

Figure 4. The average CO_2 concentration recorded for each period of fruit grown in the inflorescences

The light radiation recorded outside the greenhouse, throughout the culture period showed higher values from planting to the first harvest on 9.01.2022. The average daily values useful to plants, calculated for the period from fruit binding to physiological fruit maturity, were the lowest during the fruit growth period of inflorescence 1 (429.54 W/m^2). For the rest of the period, the average daily values recorded reached 601.05 W/m^2 in February (Figure 5).

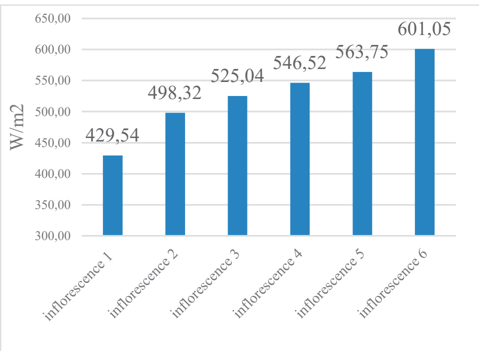

Figure 5. The average of the light radiation recorded for each period of fruit grown in the inflorescences

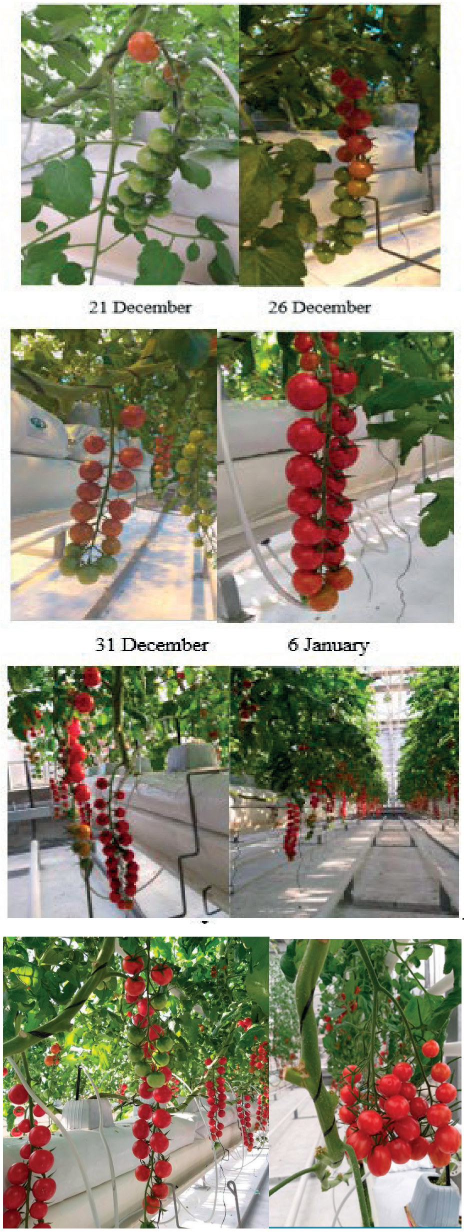

Figure 6. Aspects from culture

Figure 6 shows some aspects of tomato culture, the Cheramy variety.
In January, an average amount of 617 g of fruit per plant was harvested, and in February 1028 g/plant. The total production obtained per plant during January-February was 1645 g/plant (Figure 7).

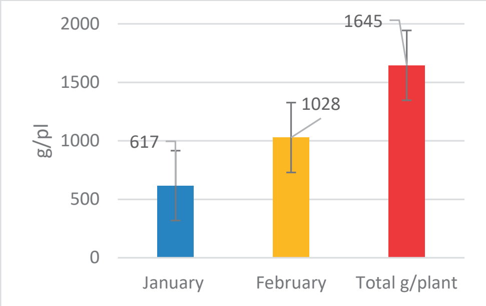

Figure 7. The average mass of fruit per inflorescence - fruits harvested in January and February and total average per plant in January-February

The firmness of the tomato fruits was performed for each fruit that reached physiological maturity, on each inflorescence, and varied between 1.54 kg/cm^2 for inflorescence 5 and 2.13 kg/cm^2 for the first inflorescence. The dry matter varied between 3.57% for inflorescence 1 and 4.30% for inflorescence 4, the values being very closed to each other (Table 1.).

Table 1. Determinations of fruit firmness and dry matter content in tomato fruit (average data on inflorescence)

Inflorescence	Firmness (kg/cm^2)	Dry matter (%)
1	2.13±0.042	3.57± 0.153
2	2.06±0.051	4.18±0.166
3	1.81±0.081	4.13±0.153
4	1.54±0.061	4.30±0.265
5	1.76±0.040	4.23±0.252
6	1.86±0.065	4.23±0.208

The average chlorophyll content in the tomato leaves was determined in the mature leaves at three points on each leaflet, at the top of the leaflet, in the middle and at the base.
It was also determined in young leaves following the same method as for mature leaves (Figures 8 and 9).

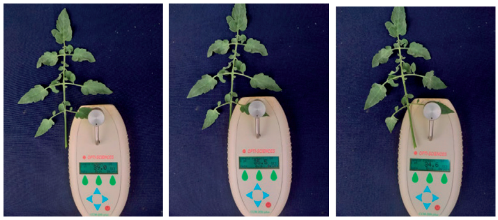

Figure 8. Determination of chlorophyll directly in tomato culture

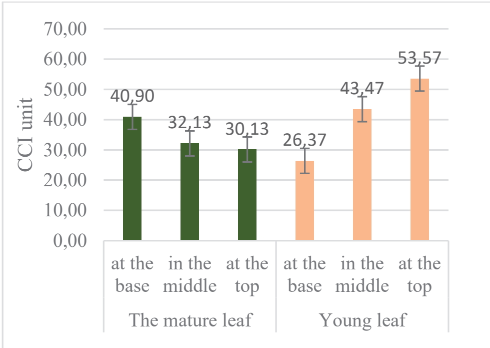

Figure 9. The average chlorophyll content in tomato leaves

The Table 2 shows the data on nitrate content in the fruits of inflorescences 1-6. The lowest nitrate content was determined in the green fruits from inflorescence no 3 (67.33 mg/kg) and the highest content in the fruits from inflorescence 6 (101.33 mg/kg). We found an increase in the level of nitrates in tomato fruits with their physiological maturity. Thus, in the case of immature fruits, the lowest content was registered for the fruits from inflorescence 1 (72.0 mg/kg) and the highest for the fruits from inflorescence 5 (132.0 mg/kg). The fruits reached physiological maturity and accumulated between 109.67 mg/kg for the fruits from inflorescence 2 and 153.0 mg/kg for the fruits from inflorescence 6.

Tabel 2. Nitrate content - mg/kg

Inflores - cence	Green fruit	Immature fruit	Fruit at physiological maturity
1	70.33±2.082	72.00±1.155	131.67±2.0817
2	72.67±1.528	78.33±2.517	109.67±1.5275
3	67.33±2.082	76.67±1.155	133.00±3.6056
4	76.33±1.528	83.67±2.082	128.67±2.3094
5	70.00±1.155	132.00±5.292	139.33±3.0551
6	101.33±2.517	91.33±3.606	153.00±4.3589
Standard norm 300 mg/kg			

Relationship between greenhouse temperature and nitrate content (mg/kg) accumulated in tomato fruits showed an insignificant influence. We can say that the temperature doesn't influence the nitrate concentration for the immature (green) fruit (R^2 = 0.0608) (Figure10).

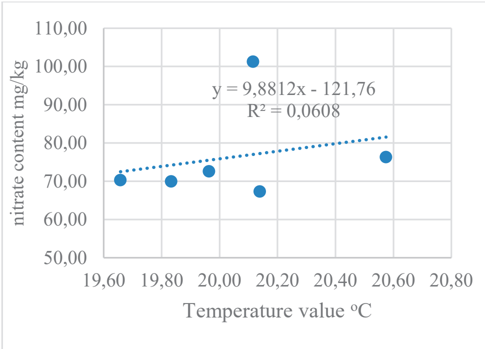

Figure 10. The influence of temperatures on the nitrate content accumulated in green fruit

In the fruits at physiological maturity, in the case of analyzing the relation between *temperature* x *content in nitrates*, (immature fruit) an insignificant influence was registered (R^2 = 0.0249), Figure 11.

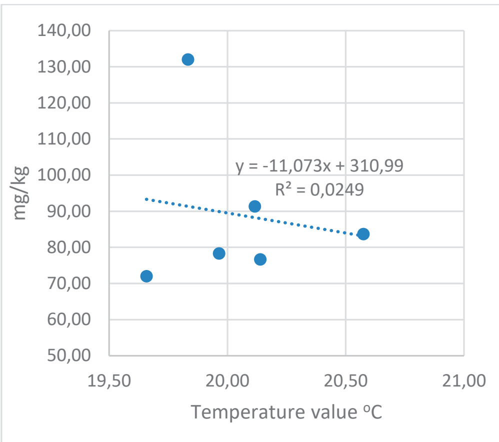

Figure 11. The influence of temperatures on the nitrate content, around physiological maturity accumulated

Determinations of total sugar content made directly in the culture showed differences depending on the stage of fruiting but also the degree of maturity of the fruit. Low sugar content was recorded for green fruit, which was between 4.40% for green fruit in the 5th inflorescence and 7.37% for fruit in the 2nd inflorescence. The fruits had a total sugar content of 5.83% for the fruits from the 6th inflorescence and 7.10% for the fruits from the 4th inflorescence. In the fruits at physiological maturity, the lowest total sugar content was registered for the fruits from the 6th inflorescence at 6.27% and the highest for the fruits from the 2nd inflorescence with 7.27% (Table 3).

Table 3. Total sugar content of tomato fruits

Inflores -cence	Green fruit (%)	Immature fruit (%)	Fruit at physiologic al maturity (%)
1	6.10±0.153	7.03±0.153	7.03±0.153
2	7.37±0.379	6.93±0.379	7.27±0.208
3	6.83±0.252	7.00±0.200	6.93±0.231
4	6.87±0.153	7.10±0.100	7.00±0.100
5	4.40±0.529	6.37±0.513	6.77±0.252
6	5.27±0.252	5.83±0.153	6.27±0.643

The relationships between *temperature x total sugar* content determined in green fruits indicated a low influence R^2 = 0.1499 (Figure 12).

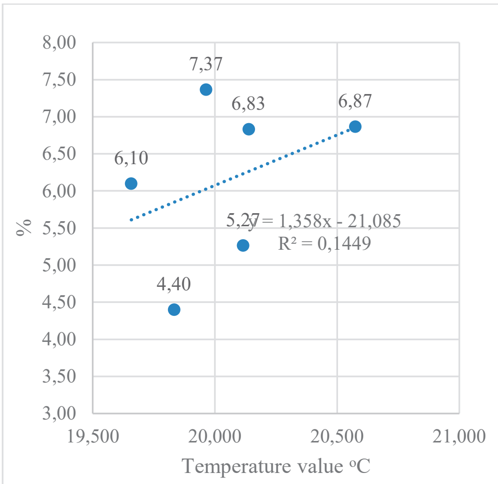

Figure 12. The influence of temperatures on the accumulation of carbohydrates in green fruit

In the case of fruits in the ripening phenophase I found that the accumulation of sugars in the fruits was not influenced by the average temperature values recorded R^2 = 0.0161 (Figure 13).

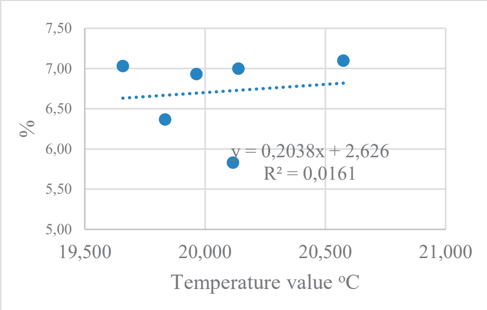

Figure 13. The influence of temperatures on the accumulation of carbohydrates in the fruit and the ripening phenophase

Analyzing the data obtained on the influence of average temperature values for each inflorescence, on the fruiting stage, on the accumulation of total sugar in the fruit, in the phenophase of physiological maturity we noticed insignificant influences $R^2 = 0.006$, which means that relatively constant temperature values led to obtaining fruits with relatively constant values in sugars (Figure 14).

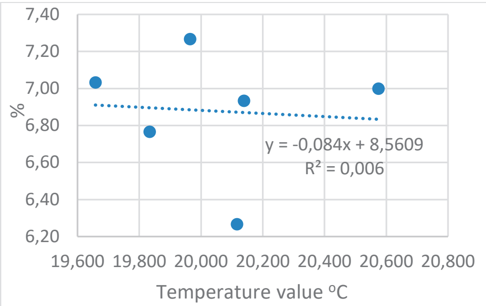

Figure 14. The influence of temperatures on the accumulation of total sugar in the fruit in the phase of physiological maturity

The correlations made to see how the light intensity influenced the accumulation of nitrates in tomato fruits, on inflorescences, showed that the light intensity influenced the accumulation of nitrates to a lesser extent, both in green fruits and immature fruits as well as in physiological maturity, the relations being $R^2 = 0.3877$, $R^2 = 0.3092$, respectively $R^2 = 0.3351$ (Figures 15-17).

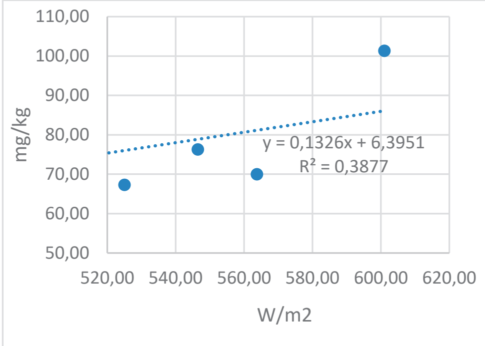

Figure 15. The influence of light intensity on the accumulation of nitrates in green fruit

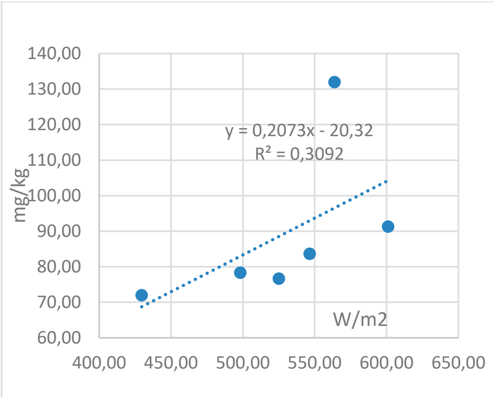

Figure 16. The influence of light intensity on the accumulation of nitrates in the fruit around physiological maturity

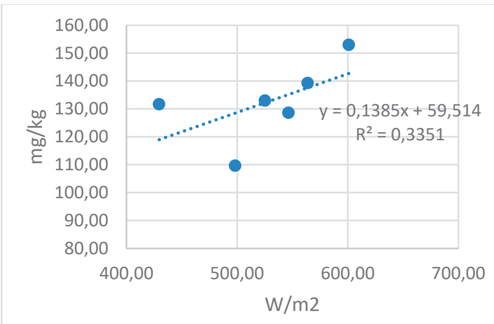

Figure 17. The influence of light intensity on the accumulation of nitrates in the fruit at physiological maturity

The light intensity did not influence the accumulation of sugars in immature (green) fruits, the relationship being insignificant $R^2 = 0.1956$, however, it showed a significant influence on fruits around physiological maturity (R^2 = 0.5299) and those that have reached physiological maturity. (R^2 = 0.5247), Figure 18.

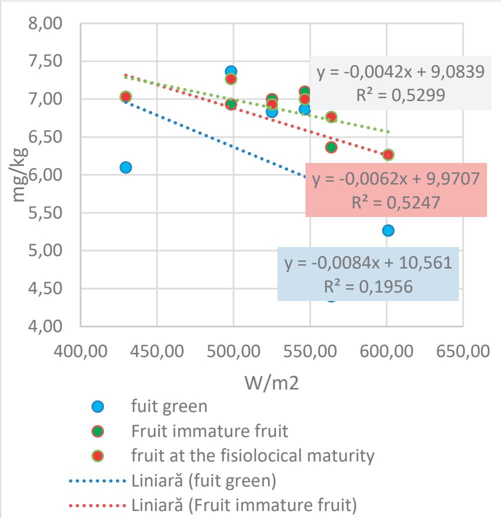

Figure 18. The influence of light intensity on the accumulation of sugars in the fruit

CONCLUSIONS

The study was carried out and, based on the analyzes performed and it was possible to highlight the influence of growth parameters, temperature, light, atmospheric humidity, CO_2 content, on the total fruit production per plant but also on the influence of dry matter content, fruit firmness, nitrate and carbohydrate content. The comparison was made taking as a constant parameter the nutrient solution for each period of growth and fruiting.

It was found that there were no significant variations between the analyzed parameters, which means that in the greenhouse the environmental factors that can be controlled, are temperature, atmospheric humidity, and CO2 content.

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EFFECT OF PESTICIDES ON THE NUTRITIONAL QUALITY OF CULTIVATED SPICE PAPRIKA

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Abstract

Spice paprika, including different species such as bell pepper and chili, is the second-largest spice commodity worldwide. It has varying significance in the European Union (EU) countries from a culinary, economic, and socio-cultural points of view. Due to the high pressure of pests and intensive plant protection, food safety aspects related to mycotoxins and pesticide residues have come to the fore. Currently, there are 41 active ingredients registered in the EU for chemical treatments in paprika cultivation. Pesticides can affect the quality of spice paprika. High dosages of pesticide treatment resulted in lowered levels of tocopherols (up to 13%) and carotenoids (up to 16%). Some of the aims of the European Green Deal are "50% reduction in use and risk of chemical pesticides", "50% reduction in the use of more hazardous pesticides" and "at least 25% of the EU's agricultural land under organic farming". The present collaboration within the Erasmus+ Hort4EUGreen project supports the dissemination of pesticide-free and organic farming knowledge, doubled by knowledge on nutritional quality of horticultural products and urban horticulture, for students, farmers and other interested citizens. This is done open access, via the hortgreen.com educational platform, that aims at becoming a long-term training hub enhancing theoretical and practical skills of horticulture specialists to better address the demands of the European Green Deal.

Key words: *spice paprika, chili, active ingredients, pesticide residues, MRL.*

INTRODUCTION

Spice paprika, a mix of different species and cultivars such as bell pepper and chili, is the second-largest spice commodity worldwide after black pepper, both in terms of production and trade value (Lakner et al., 2018). In 2019 the total production of peppers in the European Union (EU) was 2.580 thousand tons, while 176 thousand tons were imported from non-EU countries (Fruit Logistica, 2022).

Spice paprika plays different roles in the EU countries, being a market-leading commodity in Hungary and Spain (CBI, 2022). In Hungary, maintaining the quality of spice paprika as a "*Hungaricum*" product is essential. In 2020 the total production of Hungary was more than 15 thousand tons (KSH, 2022). Peppers have a special role in Turkish cuisine. The cultivation started hundreds of years before, when the ottoman Turks took the pepper from the Portuguese ships carrying peppers from Spain to Arabia. In Romania, the pepper crop ranks fourth, after cabbage, tomatoes and onion, in terms of production (INS, 2022). At world level, in 2018, Romania ranked 15 in the top of world pepper producers, with 229662 tons while Turkiye ranked third, with 2554974 tones, in the same year (Agrostandard, 2020). In Bulgaria, pepper has a significant economic and socio-cultural importance, as across the Balkan Peninsula, due to the favourable climate that favours pepper production.

Due to the high pressure of pests, food safety aspects related to mycotoxins and pesticide residues, and the influence of these contaminations on the nutritional quality of spice paprika are emphasized. The European Green Deal approved in 2020 is a set of policy initiatives by the European Commission to overcome climate change and reduce environmental degradation, phenomena which are global existential threats to Europe and the world (European Commission, 2022a). Representing the intended meaning of the "from Farm to Fork" concept, the European Green Deal envisages the reduction of the use of pesticides, fertilizers, and antimicrobials in all agricultural practices including farming within all EU-member countries (European Commission, 2022b).

In addition, an increased proportion of organic production is also targeted by the European Green Deal via an increase of the areas involved in organic farming to 25% of the total cultivated area, by 2030 (European Commission, 2022b). Thus, organic cultivation of spice paprika (with no synthetic pesticides applied and eco-friendly cultivations technologies applied) is also promoted.

Our paper overviews the status of spice paprika from the aspect of food and environmental safety. In particular, pesticide residues and other organic microcontaminants occurring in spice paprika, as well as the effect of heavy pesticide applications on spice paprika quality (contamination and levels of bioactive substances) are reviewed. Our summary relates to the Erasmus+ Hort4EUGreen project, a Strategic Partnerships between Hungary, Romania, Bulgaria, and Turkey.

PESTICIDES AUTHORIZED IN THE EUROPEAN UNION FOR SPICE PAPRIKA

The vast majority of spice paprika is cultivated under intensive agricultural practices worldwide. This is justified partly because such production is carried out under monoculture conditions, and partly because treatment with agrochemicals (plant protection products, PPPs, pesticides) is considered to be the most time- and labour-effective means of pest (mostly insect pest) control under such conditions.

Nonetheless, intensive production may result in two disadvantages: the occurrence of pesticide residues and possible alterations in the (bio) chemical composition of the produce due to the chemical stress exerted by the agrochemical treatment. Even though pesticide registration utilises increasing rigor in the authorisation of new pesticides and in the withdrawal of obsolete ones, the number of allowed pesticide active ingredients is still high, requiring complex pesticide residue and other organic microcontaminant monitoring throughout the entire production process.

Currently, 41 active ingredients are registered in the EU for pest control in paprika cultivations (European Commission, 2022c). The authorized active substances are mainly insecticides (16), and an additional 12, seven, and seven compounds as fungicides, herbicides, and soil disinfectants, respectively, have an approved status.

The control of insects in paprika cultivation is an essential part of chemical plant protection. In recent years, the application of hazardous insecticides such as chlorinated hydrocarbons, organophosphates, and neonicotinoids (except for acetamiprid) for pest control has been reduced or banned (MacBean, 2012; European Commission, 2013), but a range of pyrethroids dominantly remained in use. As in other crops, fungal diseases of paprika cultivation can also result in yield loss, so the application of fungicide formulations is required in intensive cultivation. The most used fungicides are inorganic sulphur and copper compounds (these are approved in organic farming, too), and additional systemic triazoles (e.g., tebuconazole). Various herbicide active ingredients are authorized for weed control in paprika cultivation. The most frequently used herbicide compounds are pendimethalin, napropamide, and chlomazone (MacBean, 2012). However, paprika species are relatively sensitive to herbicides, and generally, weeds are removed mechanically, although naproxamide and glyphosate are often applied against weeds in a pre-emergence setup, before the plantation.

The sustainable agricultural production and eco-friendly pest control of paprika can be supported and have been claimed to be solvable by the use of botanical insecticides (e.g., extract of neem or garlic) and jasmonic acid or with the extraction of earthworm excretory substances (e.g., Vermiwash) (George et al., 2007; Mondal & Mondal, 2012; Awang et al., 2015).

PPPs are regulated by Regulation (EC) 1107/2009 in the EU (European Parliament and

Council, 2009). Of the four mentioned and cooperating countries in the Erasmus+ Hort4EUGreen project, three are the member states of the EU, so there appear no differences in the registered active ingredients among them, because active ingredients are authorized at the level of the EU. The pesticide formulation and their uses in various crop cultures, however, are regulated by the member states on their territory under the corresponding European regulations. The authorization of pesticide active ingredients is performed based on a scientific evidence-based risk assessment conducted by the European Food Safety Authority (EFSA). If active ingredients are classified as teratogenic, carcinogenic, or persistent substances, then they cannot be approved for use in plant production products (European Parliament and Council, 2008). Also, if their occurring detrimental effects show non-monotonous dose response, the assessment is hazard-based (and not risk-based).

FOOD SAFETY ASPECTS OF PESTICIDE RESIDUES IN SPICE PAPRIKA

Nowadays, the intensive agricultural practice relies on different agrochemicals that may exert various possible hazardous effects on food safety, as their residues may appear in crops and agricultural products. Environmental and food safety of spices (e.g., spice paprika) used for culinary purposes and for flavouring in food production, receive comparatively low attention due to the low volume of spice trade networks. In general, contamination surveys of spices assess the microbial impurities or mycotoxins, although residues of pesticides are also prevalent chemical contaminants in *Capsicum* species, including spice paprika and chili (Klátyik et al., 2017). However, in pesticide monitoring programs, more attention is being given to spices and other dried foods because due to their low water content, the level of pesticides is possibly higher (Seo et al., 2013; Galani et al., 2021).

The application of pesticide active ingredients and their formulated PPPs can affect the quality of spice paprika in two ways: via the possible emergence of pesticide residues as environmental contaminants on the quality of the paprika product and also by affecting the bioactive component content of paprika, due to illegal use of pesticides (e.g., the use of not authorized pesticide formulations), incorrect application of chemical compounds to given crops or improper circumstances of harvesting and storage (e.g., pesticide treatment during storage, harvest before the end of the official waiting period of the last pesticide application). The adverse effects and even phytotoxicity of chemical PPPs on physiological parameters of plants (e.g., enzyme activities, photosynthesis) are known, particularly at high dosages of pesticides (Mitra & Raghu, 1998a; Cali & Candan, 2009; Shakir et al., 2016). DDT and its residues result in a significant reduction in the growth and yield of chili (Mitra & Raghu, 1998b). According to the results of contamination surveys, the highest level of methoxychlor, alachlor, and β-HCH in chili pepper was 7.25 mg/kg, 5.81 mg/kg, and 1.56 mg/kg, respectively, where 58.9% of the analysed chili peppers was contaminated (Galani et al., 2021), and the concentration of p,p'-DDE residues was 0.12 mg/kg in chili peppers (Oyeyiola et al., 2017).

PARALLEL ASSESSMENT OF PESTICIDE RESIDUES AND PRODUCT COMPOSITION OF SPICE PAPRIKA UNDER AGROCHEMICAL PEST CONTROL

In our semi-field modelling study (Mörtl et al., 2018), an intensively cultivated spice paprika growing site was systematically treated with pesticide premixes at several dosage levels. Exposures to pesticides were assessed partly by monitoring persisting concentrations of pesticide residues in the soil of the cultivation plot, and partly by determining occurring pesticide levels in the produce.

Possible environmental exposure to persisting pesticide residues was characterised by carrying out routine residues in agricultural soils and surface waters in Hungary. During these analyses, the presence of chlorpyrifos, tefluthrin, trifluralin, and DDT with its metabolites (DDD and DDE) was proved in the investigated soil samples. Furthermore, atrazine, diazinon, and metolachlor were detected in some soil samples, although under the limit of quantification (LOQ). In contrast, pesticide residues as soil contaminants were not detected in soil samples originated from ecological farming. In half of the collected surface water samples, trifluralin was detected in the range of 0.01-0.03 mg/l (Székács et al., 2015; Klátyik et al., 2017). According to the physical and chemical properties of chemical compounds used for plant protection, the residues of persistent compounds can be detected in various environmental matrices for a long time after the application of pesticides.

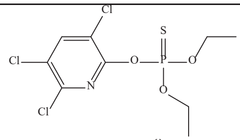
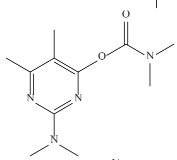
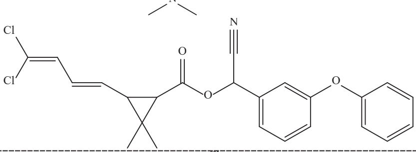
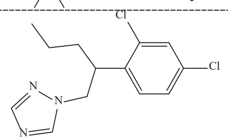
Thus, the measurement of pesticide residues was performed in various paprika fruit and environmental samples originated from fields under intensive cultivation in Hungary. During our measurements, in the investigated paprika samples, pesticide residues were not detected.

Spice paprika represents a rather difficult sample matrix for analytical measurements because it consists of a complex plant matrix, as it is rich in essential oils and red carotenoids, in addition to high lipid content and alkaloids like capsaicin (Klátyik et al., 2017).

During the food processing, treatment, and storage, bioactive components in spice paprika degraded partially. The stability of β-carotene, as well as red and yellow xanthophylls is highly affected by heat treatments (Krammer et al., 2001). Decreased levels of α-tocopherol, ascorbic acid, and red colour content were reported during the processing of red pepper (*C. annuum* L.) (Carvajal et al., 1998). The level of bioactive components, including carotenoids, is highly affected by environmental stressors, including the residues of chemical compounds (Márkus et al., 1999; Havaux, 2014).

In the modelling cultivation study (Mörtl et al., 2018), the experimental design aimed to reveal, whether treatment of spice paprika with various levels of authorized pesticides under semi-field cultivation conditions results in severe occurrence of pesticide residues, and whether it causes alterations in the composition of beneficial bioactive substance of the produce. During our investigation, the effects of pesticide treatments were investigated using a pesticide mixture (three insecticides: pirimicarb, cypermethrin, chlorpyrifos; and a fungicide: penconazole), applied 1-3 times at different dosages, where the level of pesticide residues and the bioactive component content were measured in red pepper and processed spice paprika samples. The biochemical classes of pesticide active ingredients applied in the cultivation experiment are listed in Table 1.

Table 1. The pesticide active ingredients applied in the modelling spice paprika cultivation study

Pesticide type	Active ingredient	Chemical structure	Mode of action	Formulated PPP [1]
Insecticide	chlorpyrifos		Inhibition of acetyl cholinesterase in the synaptic gaps between neurons	Cyren EC
	pirimicarb			Pyrimor 50 WG
	cypermethrin		Interaction with voltage-gated sodium channels in neurons	Cyperkill 25 EC
Fungicide	penconazole		Inhibition of ergosterol biosynthesis	Topas 100 EC

[1]PPP: plant protection product

The insecticide active ingredients belonged to biochemical classes acting on the insect nervous system by inhibiting the cleavage enzyme of the neural mediator acetylcholine (acetyl cholinesterase inhibitors: carbamate pirimicarb and organophosphate chlorpyrifos), as well as a voltage-gated sodium channel effector (pyrethroid cypermethrin). The applied fungicide is an ergosterol biosynthesis inhibitor (triazole penconazole).

Applied pesticide dosages represented low, average, high and extra high levels of PPP use compared to real agricultural field application rates. Thus, the experiments modelled realistic field situations, and the chemicals stress caused by these agrochemicals is similar or close to those that can occur in routine and common agricultural practices.

After the extraction of the samples (Figure 1), the limits of detection (LODs) of the analytical method for pesticide residues using gas chromatography coupled with mass spectrometry (GC-MS) are shown in Table 2. These LODs indicate that the target substances are detectable in the low ng/g range.

Table 2. Analytical limits of detection (LODs) of the pesticide active ingredients applied

Pesticide active ingredient	M^{+} [1]	LOD (μg/g)
chlorpyrifos	314	0.005
pirimicarb	166	0.005
cypermethrin	163 + 181	0.030
penconazole	248	0.010

[1]Characteristic quantification molecule ions in mass spectrometry

Residues of pesticide active ingredients administered to the crop readily occurred in the produce. The insecticide active ingredient chlorpyrifos was readily quantified in all spice paprika produce samples except, of course, for the untreated control. Based on our measurements, the level of pesticide residues correlated well with the number of treatments and the applied dosages, therefore the higher doses of pesticide treatments resulted in a higher level of pesticide residues and lower levels of tocopherols and carotenoids. Highest residue level of chlorpyrifos, penconazole, and cypermethrin were found to be 1.75, 0.763, and 0.339 μg/g, respectively. Levels of tocopherols (up to 13%), carotenoids (up to 16%), red and yellow pigments (up to 5%) all decreased due to the treatments. During food processing, the stability of the pesticide residues was observed.

Figure 1. The semi-field modelling spice paprika cultivation plot in Budapest, Hungary. Extract samples are shown in the insert demonstrating high variability in the colour intensity of the extracts (1: standard solution, 2-6: samples of paprika varieties ordered by colour depth of the extracts (Mörtl et al., 2001) - all varieties subjected to the same extraction procedure) (bottom right)

The results of the experiment revealed that increasing use of pesticides (both in terms of increasing dosages and multiple usage times) obviously leads to increased pesticide residue levels in the produce. Residue levels detected correlated well with pesticide treatments at increasing dosages applied. This was particularly observed for chlorpyrifos that was found even in samples treated with low dose. This justifies the ban of this active ingredient in the EU on all commodities including red pepper. Contamination occurred at high doses of active ingredients penconazole and cypermethrin, as well.

In addition to the obvious pesticide residue problem at high dosages, the experiments also clearly indicated that chemical stress by exposure to the pesticides triggered a reduction in the production of beneficial bioactive substances in the paprika produce e.g., high dosages of pesticide treatment resulted in lowered levels of endogenous tocopherols (up to 13%) and carotenoids (up to 16%) (Figure 2).

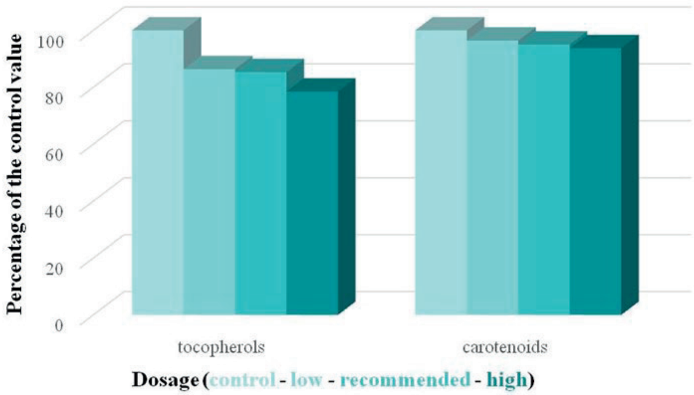


Figure 2. Endogenous levels of bioactive compounds in spice paprika at various pesticide treatment dosages

POSSIBLE EFFECTS BY PESTICIDE ADDITIVE SUBSTANCES - MAINLY THE FORMULATING AGENTS

Various pesticide formulations contain different additives (e.g., formulating agents) besides their active ingredients. The primary purpose of formulating agents used in PPPs is to enhance the efficacy and bioavailability of the formulation by increasing the water solubility and uptake, in addition to the enhancement of the adsorption capacity, environmental stability, and biological activity of the active ingredients resulting in more efficient penetration of pesticide compounds and more effective plant protection treatment (Broze, 1999; Castro et al., 2014). Previously different additives (e.g., formulating agents) in PPPs have been classified as inactive components in terms of the main biological activity of the formulations. Although, in recent years, the high individual toxicity of formulating agents or the increased combined toxic effects of the active ingredients and additives were demonstrated. In the case of glyphosate-based herbicides, the outstanding individual toxicity of the formulating agent (a mixture of polyethoxylated tallow amines; POEA) and high combined toxicity with the active ingredient was proved (Tsui & Chu, 2003; Mesnage et al., 2014), therefore the use of POEA as formulating agent in the glyphosate-based formulation has been banned (European Commission, 2016). Based on the scientific evidence, the possible adverse effects of the formulating agents in pesticide formulations and tank mixtures cannot be excluded in terms of the food safety of agricultural products and the bioactive component content of crops.

CONCLUSIONS

Due to improper use of pesticides in paprika cultivation, not correct conditions of storage and harvest, furthermore, the illegal use of chemical PPPs in commodities, residues of pesticide compounds may appear in agricultural food products, thus various physical, chemical, or biological agents may have potentially harmful effects on food safety of spice paprika. To support the promotion of appropriate food safety in the EU, an integrated concept "from farm to fork" is elaborated and applied, with risk assessment based on scientific facts and reports.

In 2019 the European Commission presented the European Green Deal and adopted a set of proposals to make Europe the first climate-neutral continent by 2050 with the improvement of the economy, human health, and life quality (European Commission, 2019; European Commission, 2022a). One of the aims of the European Green Deal is a 50% reduction in the use and risk of chemical pesticides, at least 50% and 20% reduction in nutrient losses and fertilizer use, moreover a 25% increase in organic agriculture by 2030.

Organic farming is an environmentally friendly way of agricultural practice that needs to be further developed. From 2009 the demand for organic farming in spice and herb cultivations increased in the EU to avoid the exposure of consumers to pesticide residues in spices and herbs, although the consumed quantities vary by various products and regions (CBI 2009). Moreover, the beneficial effects of organic farming on antioxidant activity, total carotenoids, the content of minerals, vitamin C, flavonoids, and color intensity of paprika were proved by several studies (Daood & Biacs, 2005; Hallmann et al., 2007; Pérez-Lopez et al., 2007; Hallmann & Rembialkowska, 2012). Emphasis is recently put on the use of pepper traditional varieties/landraces, giving their great genetic diversity, on the application of different strategies for plant protection (Sánchez-Sánchez et al, 2022), as biologic control, plants extracts, allelopathy, intercropping, (Rodino et al., 2017), use of N-fixing and K and P solubilizing microbial biostimulants bacteria (Sánchez-Sánchez et al, 2022), plant growth - promoting bacteria and arbuscular mycorrhizal fungi (Jimenez-Perez et al., 2022).

The present collaboration within the Erasmus+ Hort4EUGreen project supports the dissemination of pesticide-free and organic farming knowledge and nutritional quality of horticultural products knowledge for students and whoever other interested actors.

ACKNOWLEDGEMENTS

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PRELIMINARY STUDY REGARDING THE INFLUENCE OF NUTRIENT CONCENTRATION ON PRODUCTION AND QUALITY PARAMETERS FOR LETTUCE GROWN ON PERLITE SUBSTRATE

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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.*

INTRODUCTION

The specialized literature mentions the importance of the electrical conductivity (EC) of the nutrient solution as it can be one of the factors that can affect the lettuce production obtained in the culture in the soilless system.

Many researchers who have studied this factor has proven that prolonged use of the same nutrient solution leads to the accumulation of large amounts of trace elements such as zinc and copper (Steiner, 1980; 1984).

Abou-Hadidl et al., 1996, in the studies carried out on three varieties of lettuce grown in a soilless system tested different levels of electrical conductivity of the nutrient solution of 1.0, 1.5 and 1.8 dS/m, respectively, finding that the plant mass of salad, decreased with the increase in the EC level of the nutrient solution, an aspect also noticed by Serio et al., 2001.

Uttara et al., 2006, showed that with the increase in EC concentration from 1.4 dS/m to 2 dS/m, the concentration in plant nitrogen, phosphorus, potassium and calcium also increases without a significant content of the production.

Conversa et al., 2021, in the experiments carried out on two varieties of oak leaf lettuce grown in two seasons, spring and autumn, found that the nutrient solution with an EC of 2.5 and 3.5 dS/m influenced both the production as well as the quality of the plants.

Many researchers support soilless cultivation, as this system allows for improved lettuce plant quality in terms of both low nitrate content and product safety. On this basis, the studies conducted evaluated the effect of different EC nutrient solutions on the yield, quality and shelf life of lettuce after harvest.

Scuderi et al., 2009, showed that increasing the EC of the nutrient solution from 2.8 to 3.8 mS cm^{-1} led to a significant decrease in production from 6.1 kg/m^2 to 5.8 kg/m^2 for all varieties studied.

Enache, 2019, appreciates that the use of quantum structured water leads to a better efficiency of the use of the nutrient solution.

Quy et al., 2018, in an experimental study investigated different levels of the EC of the nutrient solution (0.5, 1.0, 1.5, 2.0 and 2.5 mS/cm) in a lettuce culture in a vertical hydroponic system and found that at the EC of

2.5 mS/cm and at a pH of 6-6.6 had obtain the maximum production

Cha et al., 2012, analyzed the influence of the electrical conductivity (EC between 0.5 and 6.0 dS/m) of the nutrient solution and the light intensity (120, 150 and 180 µmol/m²/s) on the growth and development of some lettuce varieties in a photoperiod of 16 hours/day at the temperature maintained in the range of 20~25°C, found that the best option was the one that used an EC of 2 dS/m, at an intensity of 180 µmol/m²/s.

Park and Yong, 2001, state that using an EC between 1.2 and 4.8 mS/cm led to adequate yields of lettuce, and Scuderi et al., 2009, relate that production decreases as EC increases from 2.8 mS/cm to 4.8 mS/cm, and Broadley et al., 2003, mention that nitrogen content is correlated with plant protein and nitrate levels.

Dharti et al., 2021, appreciate that the EC level but also the temperature and light conditions influence the carbohydrate content.

Al-Kinani et al., 2021, but also Asmaa et al., 2021, points out that regardless of the non-conventional cropping system used, production increases are superior to the conventional system.

The aim of the study was to see how the EC of the nutrient solution and the pH influenced the vegetative growth of lettuce grown in a non-conventional system, on mattresses filled with perlite.

MATERIALS AND METHODS

The present study was carried out at the research greenhouses, Faculty of Horticulture from UASMV Bucharest, this belongs to the Research Center for quality control of horticultural products.

The biological material used in the experiment consisted of two varieties of lettuce. The Kineta variety is of the oak leaf type, and the Lugano variety is of the Lollo Bionda type. Seedlings were obtained in the greenhouse between October 20 and November 10, 2021. Sowing was done often in pots filled with peat, and transplanting was done in Jiffy-type peat pots. Environmental factors, temperature, humidity, light was monitored during the growth of the seedlings. Determinations were made regarding the percentage of emergence, the growth of seedlings in height and the number of leaves.

The planting was carried out when the seedling was 23 days old from emergence, directly in the mattresses filled with perlite, on experimental variants. During the vegetation period, observations and measurements were made regarding the height of the plants, the number of leaves, the diameter of the plants. At the end of the culture, the height, diameter, total number of leaves, total mass of the edible part of the plants as well as root volume were determined.

Table 1. Experimental variants

Cultivars	EC value mS/cm	The pH value
Kineta Lugano	1.5	5
		6
		7
	2.5	5
		6
		7
	3.5	5
		6
		7

Correlations were performed between the analyzed parameters. Throughout the culture period, environmental factors regarding temperature and atmospheric humidity, CO2 level, as well as temperatures at the level of the culture substrate were monitored. Also, chlorophyll and nitrate content were measured directly in the greenhouse. Nitrate content was determined directly in the greenhouse, after harvest, with the apparatus Nitrate tester Greentest ECO 6 + TDS. The carbohydrate content was determined in the greenhouse after harvesting with Brix device. Data were statistically interpreted using the Duncan test (p=0.05).

RESULTS AND DISCUSSIONS

During the culture period, the temperature during the day was kept almost constant, according to the technological recommendations around the average values of 22.5ºC. The temperature values during the night period remained almost constant between 17.0ºC. The average temperature values were 19.6ºC (Figures 1 and 2).

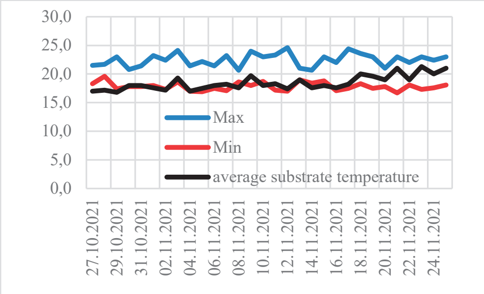

Figure 1. Temperature values during the day and night and those in the culture substrate

Figure 2. Determination of substrate temperature

In the greenhouse compartment, CO_2 values of over 237.85 ppm were maintained, reaching values of 480.43 ppm (Figure 3).

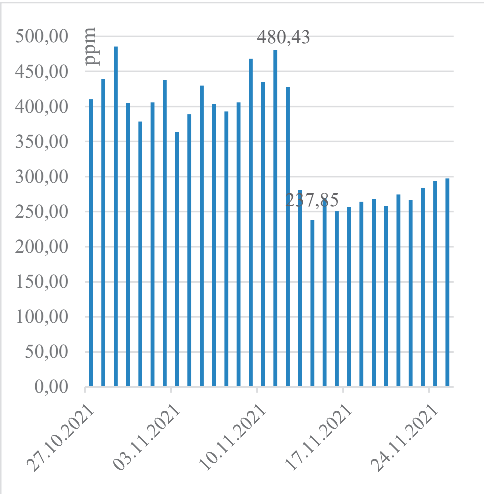

Figure 3. CO_2 content values determined in the greenhouse during the culture period

Analyzing the data regarding the height of the plants in the salad culture carried out on perlite substrate, we found that in the Kineta variety, the highest plant height of 21.67 cm was recorded in the variant cultivated at pH 6 and EC of 2.5, and the lower at 14.17 cm in the variant where we used an EC of 3.5 and a pH of 7. In the case of the Lugano variety (Lollo bionda type) the highest plant height was recorded in the variant with an EC of 1.5 and pH 7, and the lowest height was in the variant where we used EC of 3.5 and pH 7. After performing the Duncan test (p = 5), significant differences between the variants were noted (Figure 4).

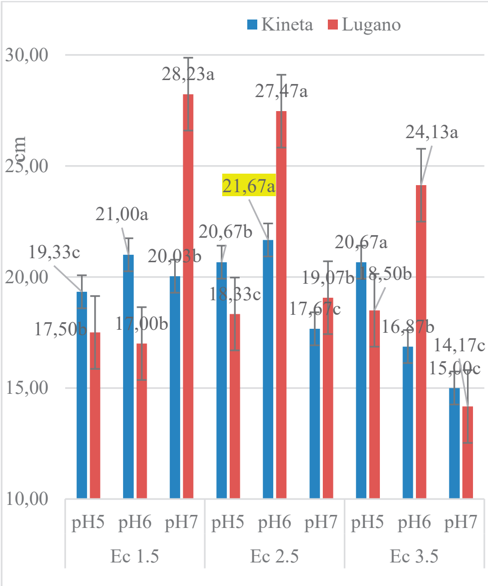

Figure 4. Height of lettuce plants 30 days after planting

In the case of the Kineta variety, a significant negative correlation (R^2 = 0.4149) was noted, which means that as EC and pH increase, plant height decreases (Figure 5).

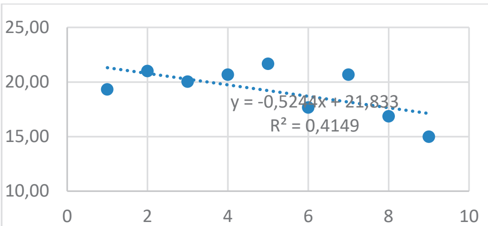

Figure 5 The influence of EC and pH values on the height of lettuce plants of the Kineta variety

The correlations carried out for the Lugano variety, regarding the influence of EC and pH on plant height growth, indicated an insignificant correlation (R^2 = 0.0098) (Figure 6.)

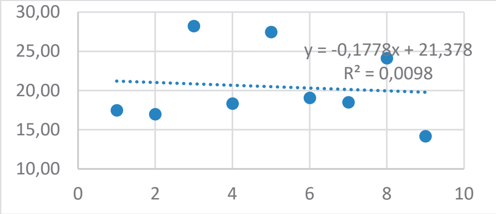

Figure 6 Influence of EC and pH values on the height of lettuce plants Lugano variety

Regarding the plant diameter, in the Kineta variety, we found that when using an EC of 2.5 mS and a pH of 6, the largest plant diameter of 37 cm and the smallest of 31.33 at an EC of 2.5 mS at pH 7. For Lugano, the largest diameter was 35.1 cm at an EC of 3.5 mS and pH 6, and the smallest 24.17 cm at an EC of 3.5 mS and a pH of 7 (Figure 7).

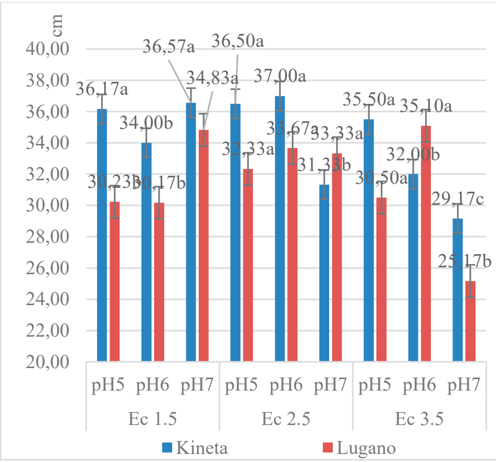

Figure 7. Diameter of lettuce plants as a function of EC and pH

We found a slightly significant negative relationship regarding the diameter of the lettuce plants of the Kineta variety (R^2 = 0.4534) (Figure 8).

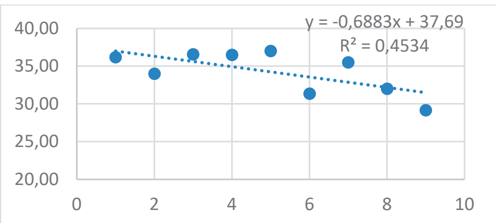

Figure 8. Influence of EC and pH values on the diameter of lettuce plants of the Kineta variety

In the case of the Lugano variety, we found an insignificant relationship, the value being R^2 = 0.0374 (Figure 9).

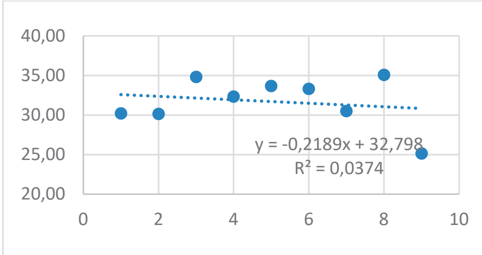

Figure 9. The influence of EC and pH values on the diameter of lettuce plants of the Lugano variety

Regarding the number of leaves formed per plant, it was found that in the Kineta variety, a number of 60.67 leaves were obtained at an EC of 1.5mS and a pH of 7. The lowest number of leaves was recorded in the plants which used an EC of 1.5 mS and a pH of 5 of 38.33 leaves. In the case of the variety Lugano, the highest number of leaves was recorded at an EC of 3.5 mS and a pH of 6 of 41 leaves/plant, and the lowest number of leaves was recorded at an EC of 1.5 mS and a pH of 5 being only 25, 67 leaves (Figure 10).

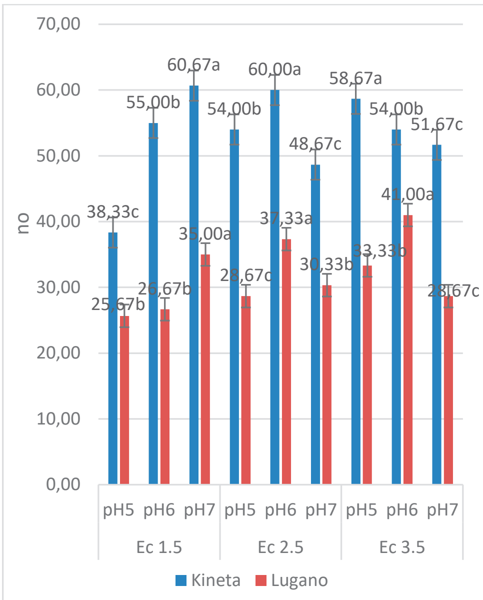

Figure 10. Number of leaves formed on lettuce plants

To see the influence of EC and pH on the formation of the number of leaves per plant, we performed a correlation and found an insignificant influence (R^2 = 0.0738) in the Kineta variety (Figure 11).

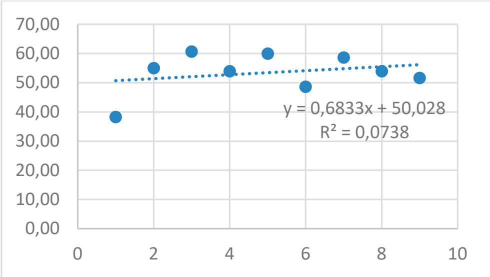

Figure 11. The influence of EC and pH values on the number of leaves in lettuce plants of the Kineta variety

In the case of the Lugano variety, we found a slightly significant positive relationship with the increase in EC (R^2 = 0.222) (Figure 12).

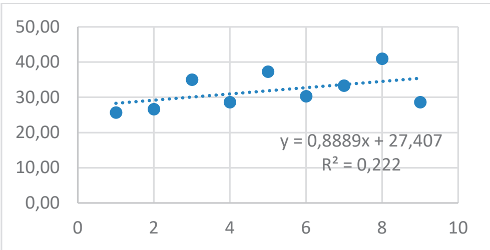

Figura 12. Influența valorilor EC-ului și pH-ului asupra numărului de frunze la plantele de salată soiul Lugano

The appearance of lettuce plants after 5 days and 20 days after planting is shown in Figures 13 and 14.

Figure 13 Appearance of lettuce plants after 5 days of planting

Figure 14. Appearance of lettuce plants 20 days after planting

Analyzing the mass of lettuce plants, we found that the highest values were recorded at an EC of 2.5mS in the case of all pH variants. The highest plant mass was recorded in the Kineta variety of 236 g at EC 2.5 mS and pH 6. The lowest value of the average mass of lettuce plants was recorded at EC 3.5 mS and pH 7, the mass being 174 g. In the case of the Lugano variety, the highest average mass of the plants was recorded in the plants grown in perlite substrate that were administered the nutrient solution with an EC of 2.5 mS and pH 6, The value being d 166.33 g per plant. The lowest value of the average mass was recorded in plants fertilized at an EC of 1.5 mS and pH 5, being only 73.33 g per plant.

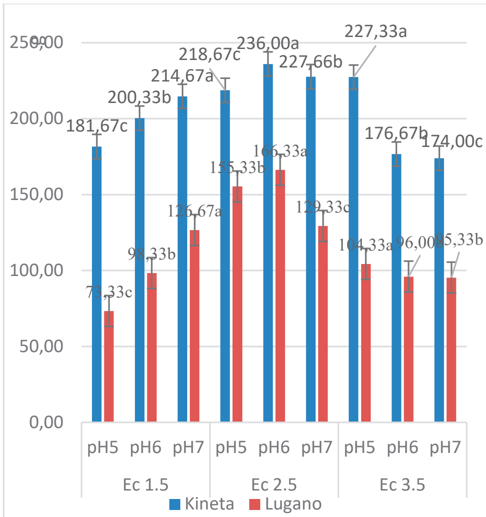

Figure 15. Total mass of lettuce plants at harvest

Figure 16. Appearance of the plants in the culture on perlite substrate after 25 days

Both in the case of Kineta and Lugano cultivars there were no significant relationships regarding the influence of EC and pH (Figures 17 and 18).

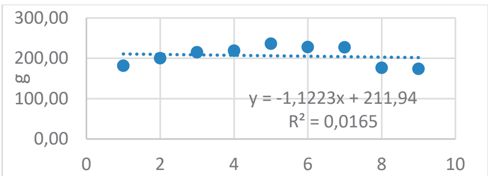

Figure 17. The influence of EC and pH values on the mass of lettuce plants of the Kineta variety

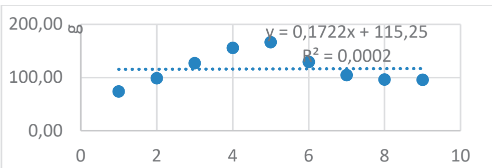

Figure 18. Influence of EC and pH values on the mass of lettuce plants Lugano variety

Regarding root volume, it was found that the highest root volume was recorded in plants grown in perlite substrate and fertilized with an EC of 2.5 mS, and pH 5, in the case of the Kineta variety (10.57 cm^3), and in the case of the Lugano variety, it was observed in the fertilized version with an EC of 3.5 and a pH of 7 (7.33 cm^3), which means that the varieties react differently to EC and pH values (Figure 19).

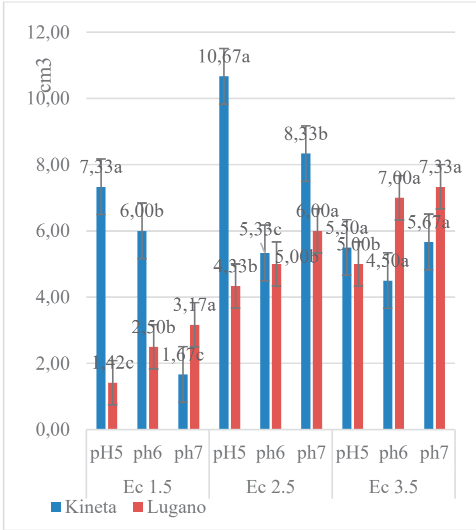

Figure 19. Root volume of lettuce plants

The appearance of lettuce plants and the distribution of the root system, in the varieties analyzed, are shown selectively in the Figures 20-21).

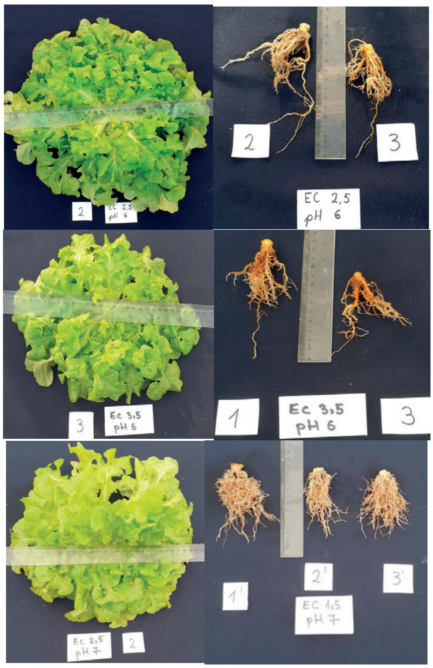

Figure 20 Appearance of plants and roots -Kineta

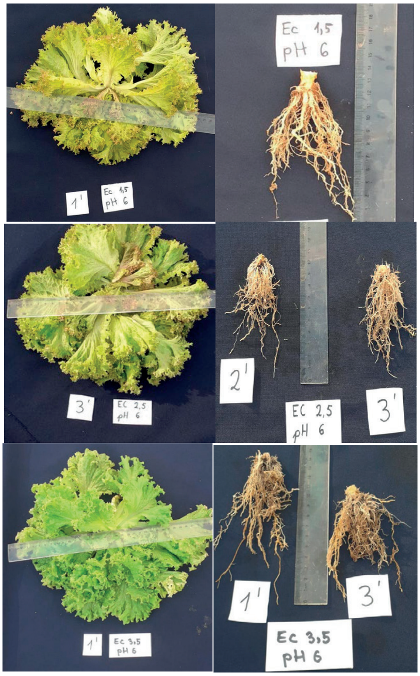

Figure 21. Appearance of plants and roots -Lugano

In the case of the Kineta variety there was no significant relationship, but in the Lugano variety a very significant relationship was found $R^2 = 0.9359$ (Figures 22 and 23).

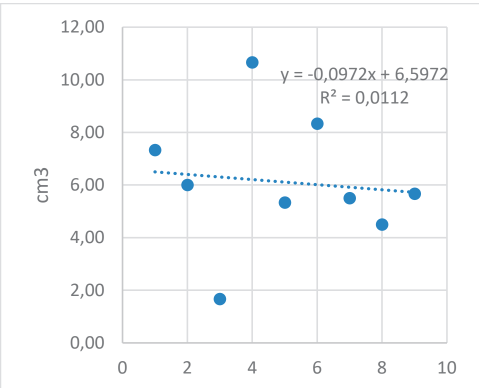

Figure 22. The influence of EC and pH values on root volume in lettuce plants of the Kineta variety

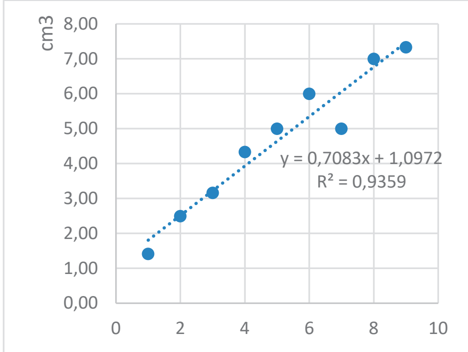

Figure 23. The influence of EC and pH values on root volume in lettuce plants of the Lugano variety

The determinations made during the test vegetation period show that on 7.12.2021, the differences are statistically insignificant at EC 1.5; 2.5 and 3.5, between variants with pH 5 and statistically significant differences at EC 1.5; 2.5 and 3.5, between the variants where a pH of 7 was used. The differences were statistically insignificant at EC 1.5 and 2.5 between the variants with pH 6 and statistically significant differences between them and the variant where an EC 3.5 was used (Figure 24).

Figure 24. Chlorophyll content Kineta

In the case of the Lugano variety on 07.12.2021 the chlorophyll content, determined during the vegetation period, presented the highest value (3.41) in the fertilized variant with an EC of 2.5 mS, an aspect also observed on 05.01. 2022, at the final harvesting of the plants (Figure 25).

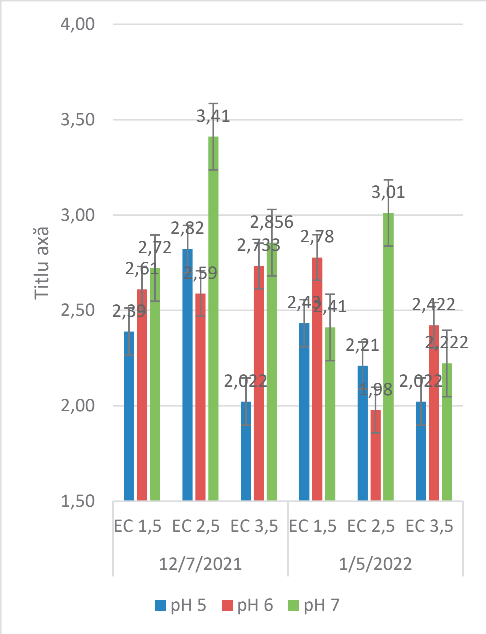

Figure 25. Chlorophyll content Lugano

In the case of the Kineta variety, we found that in the variant of the nutrient solution with pH 5, the basal leaves accumulated the highest amounts of nitrates. Thus, at an EC of 3.5 mS in the basal leaves an amount of 2833.3 mg/kg nitrites accumulated, and at an EC of 1.5 mS of 1766.7 mg/kg below the standard of 3000 mg/kg recommended for the winter period. The lowest amount was recorded in the leaves located in the middle of the plant, young, newly formed leaves. For these, the values were 520.0 mg/kg in plants to which an EC of 1.5 mS was applied. In the case of using pH 6 and at an EC of 2.5 mS, we found that the basal leaves accumulated 2100 mg/kg nitrates. In the case of using the nutrient solution with pH 7, the situation was similar, the highest amount of nitrates being determined in the basal leaves 19993.3 mg/kg at EC 3.5 (Figure 26).

The average nitrate content data showed that when using an EC of 3.5 mS and pH 5, a higher amount of nitrate accumulated at 2000 mg/kg. The lowest content in nitrates was recorded for all EC variants where pH 6 was used (Figure 27).

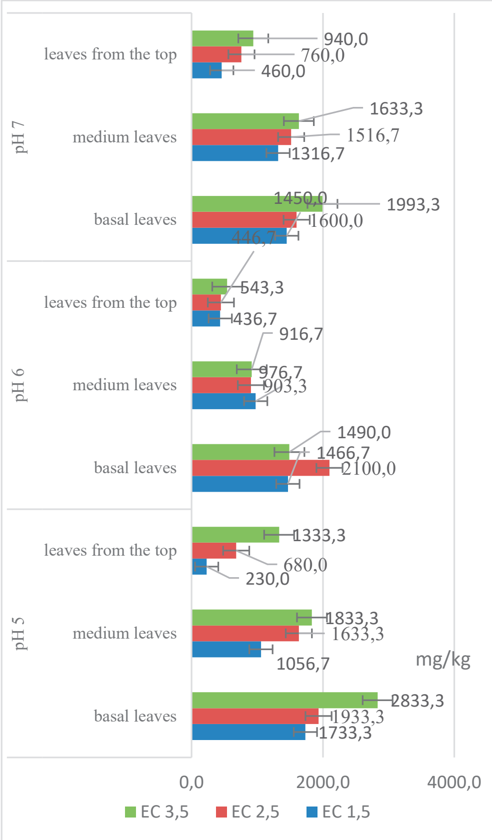

Figure 26. Nitrate content of the Kineta variety

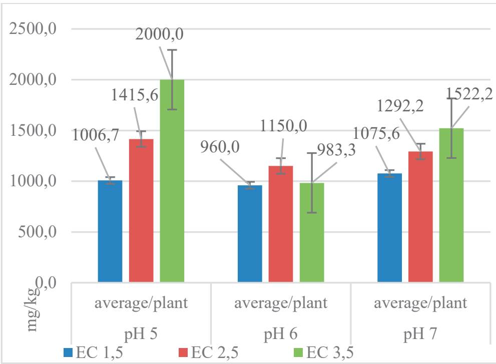

Figure 27. Nitrate content of the Kineta variety

In the case of the Lugano variety, the situation was similar to the Kineta variety, the basal leaves accumulated the highest amount of nitrates, but below the standard limit. In the case of the EC of 3.5 mS, nitrate accumulation was higher compared to the rest of the EC values (Figure 28).

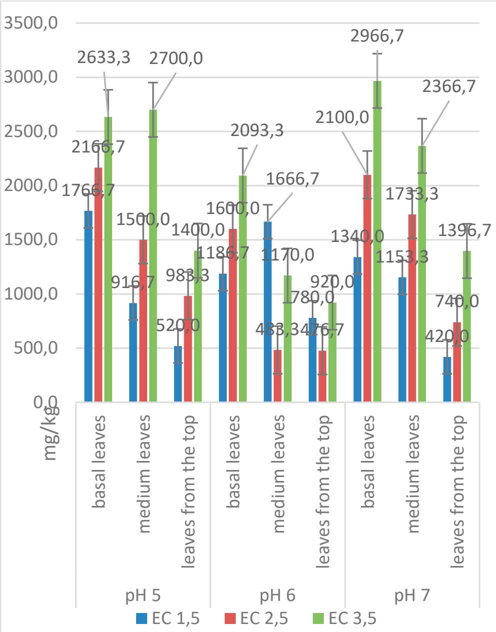

Figure 28. Nitrate content of Lugano variety

Average nitrate content data showed that the highest accumulations were found when using an EC of 3.5 and the lowest at an EC of 1.5 (Figure 29).

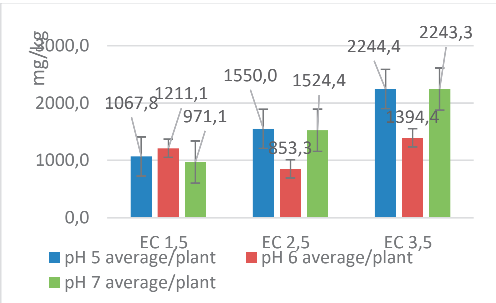

Figure 29. Average nitrate content of Lugano cultivar lettuce plants

The carbohydrate content accumulated in lettuce leaves was different depending on the position of the leaves. The basal leaves accumulated the lowest amounts and the young leaves the highest values for all pH values (Figures 30 and 31).

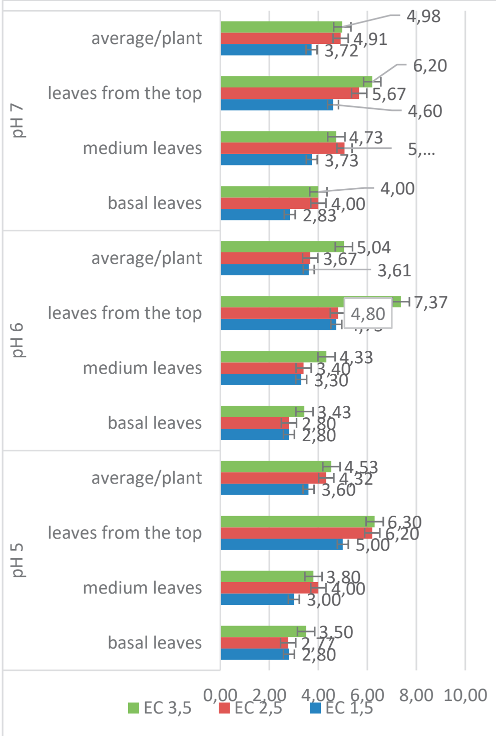

Figure 30. Carbohydrate content of the Kineta variety

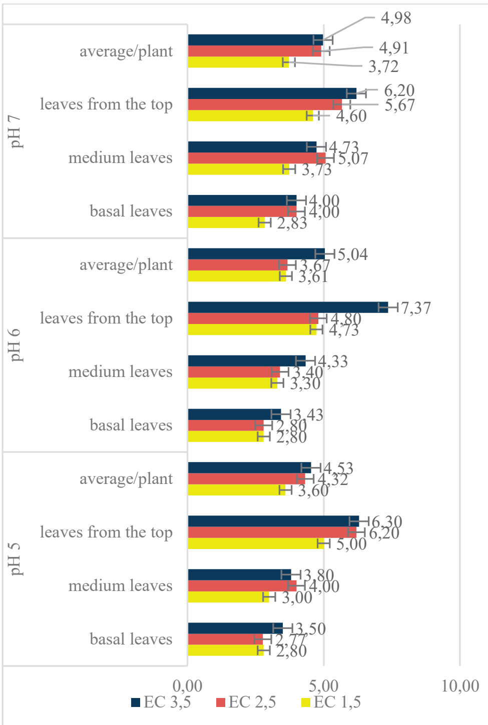

Figure 31. Lugano variety carbohydrate content

CONCLUSIONS

Soilless crops are starting to be practiced more and more because the product obtained ensures a much better productivity, a shortening of the vegetation period, commercial quality of the product, food safety and chemical composition.

Another aspect to consider is that of keeping constant of the environmental factors. The temperatures provided in the greenhouse were maintained in the optimal parameters for crop development, they were between 20ºC and 25ºC during the day and at least 17ºC at night. Also, in the culture substrate, the temperatures were within the optimal limits for the good development of the plants.

There were differences in plant height. The highest height was noted in the variety Lugano, at an EC of 1.5 and a pH of 7. In this variety, at an EC of 3.5 and a pH of 7, the height of the plants was the lowest. We noticed that, in terms of plant height, the Lugano variety showed large variations compared to the Kineta variety.

The diameter of the plants was larger in the Kineta variety compared to the Lugano variety. The root volume was greater in the case of plants grown at EC 2.5 and pH 5 and 7 respectively in the Kineta variety. In the case of the Lugano variety, the root volume increased with the increase in EC regardless of the pH value.

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VARIATION OF AUXINS AND CYTOKININES IN MICROPROPAGATION PROTOCOLS OF TWO WORLWIDE IMPORTANT SPECIES: SOLANUM TUBEROSUM AND IPOMOEA BATATAS

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Abstract

Solanum tuberosum and Ipomoea batatas are the third and the seventh world ranking species in terms of yield and consumption and they are relatively cultivated based on micropropagation techniques for limiting viruses spreading and other diseases. In this work we analyzed varieties of Ipomoea batatas, 'Ro-Ch-M', 'KSH' and 'KSP1', two varieties of Solanum tuberosum L. with purple flesh, 'Violet Queen' and 'Purple Majesty' and their response to variation of auxins and cytokinins on in vitro cultivation. The study compared the effects of basal MS medium containing various concentrations of α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) in combination with gibberellic acid (GA3) in micropropagation of those species, using material from a starter culture in vitro induced as well. For Solanum tuberosum, the shoot induction ranged between 4–5 days with variation among NAA concentrations, the longest shoots (9,8 cm), maximum number of nodes (4-5), and maximum number of leaves (10.00) were recorded on 'Purple Majesty' on variant containing 0,25 mg/L BAP + 0.03 mg/L NAA+0,05 mg/L GA_3. For Ipomoea batatas, the shoot induction ranged between 5-6 days with with variation among NAA concentrations, the longest shoots (7,8 cm), maximum number of nodes (3.8 cm), and maximum number of leaves (12.00) were recorded on 'KSP-1' on variant containing 0,25 mg/L BAP + 0.05 mg/L NAA+0,05 mg/L GA_3. The results showed that the combined effect of various concentrations of NAA between 0.01 mg/l and 0,05 mg/L, BAP between 0,25 mg/L and 1.00 mg/L and GA3 could provide solution for extend in vitro production of Solanum tuberosum tubers and potatos for Ipomoea batatas as base materials for industrial cultivation.

***Key words**: auxins, cytokinins, gibberellins, Ipomoea batatas, micropropagation, Solanum tuberosum.*

INTRODUCTION

Potato and sweet potato are major food and non-food crops among tubers and roots countries (Basera et al., 2018). Potato is cultivated in over 150 countries and they have outstanding importance for commercial purposes, the demanding on the market for those two species is higher than last century (statista.com, 2022).

Potato has high nutrition value (Devaux et al., 2021) and roots of sweet potato, behind nutraceutical value, contain a high amount of starch, used as raw material, for animal feeding and beverage industry (Danci et al., 2011; Kapinga et al., 2007). Usually vegetative propagated by shoots, roots, steam cutting, sweet potato propagated like this can be viral or bacterial contaminated and seriously impact mass production (Wondimu et al., 2012).

Propagate vegetative as well, potato can accumulate over 40 viral diseases, bacteria and systemic fungi that can impact production worldwide (Gastelo et al., 2004).

Now days, researches focuses on eradicate viruses from seed material, develop of new cultivars, new breeding lines and stabile techniques for long term storage (Gaba and Singer, 2009).

Plant tissue culture can be use in potato and sweet potato propagation in order establish a virus free material, to retain germplasm lines, offer answers regarding species resistance to all type of stress, or to promote material for breeding (Devaux et al., 2021).

Among plant tissue, micropropagation has the main role and through it is possible to develop protocols for *in vitro* culture establishment (Vinterhalter et al., 1997).
The plant hormones, mainly auxin and cytokinin, are critical for plant regeneration in micropropagation and especially cytokines playing an vital role in shoot organogenesis (Bidabadi and Jain, 2020). For potato, the success of *in vitro* multiplication depends on the presence of a balanced combination or auxins (0.01 mg/L NAA) and gibberellic acid (0.25 mg/L) (Badoni and Chauhan, 2009). Danci (2011) show that best result for regenerate shoots from meristems was achieved with auxins and Gibberellins (1 mg/L IAA, 1 mg/LIBA and 0.3 mg/L GA3) and the lowest results were obtained with cytokines (1 mg/L kinetin and 1 mg/L N6-benzyladenine). Rabbani et al., (2001) studied effects of different concentrations of GA_3 (between 1, 2, 3, 4, 5 mg/L) and BAP (between 0.5, 1, 1.5, 2, 2.5 mg/L) and maximum shoot length was obtained with 4 mg/L GA_3 and maximum number of shoots was obtained with 2 mg/L BAP.
Zang et al. (2005) and Dewir et al. (2020) suggest that increased concentration of indole-3-acetic acid (IAA) increased shoot length and the trigger of IAA action was addition of GA_3 into the medium.
In 2021, Hajare propose a full strength MS medium with variable concentration of BAP (0, 0.5, 1, 1.5 and 2 mg/L with an optimum of 1.5mg/L) combined with different concentration of NAA (0, 1, 2, 3 and 4 mg/L with an optimum of 3 mg/L) was report for shoot initiation; for shoot multiplication medium was supplemented with BAP (1, 1.5, 2, 2.5 mg/l) and kinetin (1, 1.5, 2, 2.5 mg/L). For rooting, same Hajare, combined IBA (0.5, 1, 1.5, 2 mg/L with best results on 1 mg/L) and IAA (0.5, 1, 1.5, 2 mg/l with best results on 0.5 mg/L).

MATERIALS AND METHODS

1. **Plant material and study area**
We used two *Solanum tuberosum* purple cultivars, 'Violet queen' and 'Purple majesty' and three *Ipomoea batatas* cultivars, 'RO-CH-M', 'KSP-1' and 'KSH' Results that will be discussed here refers only on *Solanum tuberosum*, both varieties, data regarding on *Ipomoea batatas* remain unpublished until confirming.
Solanum tuberosum 'Violet queen' (first name 'Hot Purple') – is a Peruvian potato variety, with deep purple skin and high concentration of anthocyanins in the flesh.
Solanum tuberosum 'Purple majesty' is a Peruvian cultivar as well, with remarkable purple flesh colour probably the most intensely colour from all purple potatoes, with exceptional flavour and texture.

2. **Micropropagation**
Solanum tuberosum 'Violet Queen'. Explants were initial cultivated from fresh biological material for 3 months. In order to obtain diseases free explants, we sub cultivativated explants for 3 times on Murashige & Skoog medium without hormones (Figure 1).
First sterilization was fungal decontamination with Aliette 80WG, 0.4% for 20 minutes. Explants were immerse in 70% ethanol for 35 seconds, rinsed with sterile distilled water, dipped in 0.2 mg/L $HgCl_2$ (mercuric chloride) for 4.5 minute. Then we washed for 4 times with sterilised distilled water.

Figure1. Fresh material and *in vitro* cultivate plant material of *Solanum tuberosum* 'Violet queen'

Solanum tuberosum 'Purple majesty'. Explants were generated from *in vitro* culture, multiplicated and mentained on MS medium without hormones for over 6 months (Figure 2).

Figure 2. Stored *in vitro* material and sub cultivate plant of *Solanum tuberosum* 'Purple majesty'

3. **Experience design**
Cultures were initiated with uninodal segments between 0.5-0.8 cm for all tested varieties from aseptic *in vitro* pre-culture on MS medium supplemented with different concentration of BAP (V variant), GA_3 (X variant) and NAA (Y variant) (Table 2) and control. Variant V has different concentration of cytokine - BAP, between 0 and 1 mg/l (Table 1) and variant Y was designed with different concentration of auxin - NAA, between 0.1-0.5 mg/l.

Table 1. Media combination in different treatments on variant V

Variant	BAP mg/l	GA_3 mg/l	ANA mg/l
control	0	0	0
V1	0	0.05	0.03
V2	0.25	0.05	0.03
V3	0.5	0.05	0.03
V4	0.75	0.05	0.03
V5	1.0	0.05	0.03

Each treatment had five repetitions and five replications. We used culture medium with pH 5.75-5.78, 30 g/l agar and 15 g/l of sucrose in 30 ml container for each.

Table 2. Media combination in different treatments on variant Y

Variant	BAP mg/l	GA_3 mg/l	ANA mg/l
control	0	0	0
Y1	0.25	0.05	0
Y2	0.25	0.05	0.01
Y3	0.25	0.05	0.02
Y4	0.25	0.05	0.03
Y5	0.25	0.05	0.05

Media was autoclaved at 121°C, for 21 minutes. Cultures were inoculated in the laminar flow bench and incubated at 24±1°C under 14h of light. All measurement was done at 7-12-19-26 and 33 days from inoculation.
The obtained experimental data were statistically processed using Jasp 0.16.1 software. To study the influence of different variants during the time of experiment we used ONE WAY ANOVA test. Also, we used POST-HOC Test to identifying the significant differences between samples (p value less 0.05)

RESULTS AND DISCUSSIONS

Results for this experiment were obtained after 33 days of culturing and dynamic metric observations and present in this paper only cytokines variant.

1. *In vitro* shoot induction and viability of explants. Cultures were initiate from uninodal segment of *Solanum tuberosum* L. 'Purple majesty' (PM) and 'Violet queen' (VQ) varieties. All experiments remain sterile after 10 days post inoculation and allow metric observation until day 33.
2. PM_VQ_V variant. Variation of BAP plus NAA and GA_3 affected shoot number and length of five variants. Significant differences ($p < 0.001$) was observed on variants V2 (0.25 mg/l), V3 (0.5 mg/l) and significant was only V4 (0.75 mg/l) with $p = 0.003$ (Figures 3 and 4).

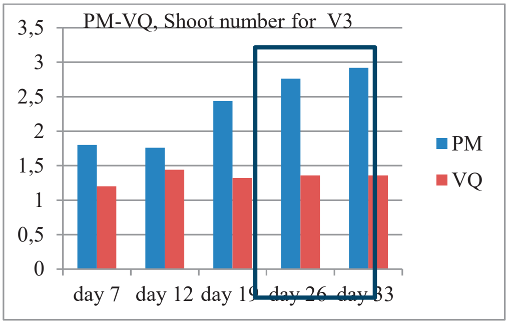

Figure 3. PM-VQ comparative shoot number on V2 (0.25 mg/l)

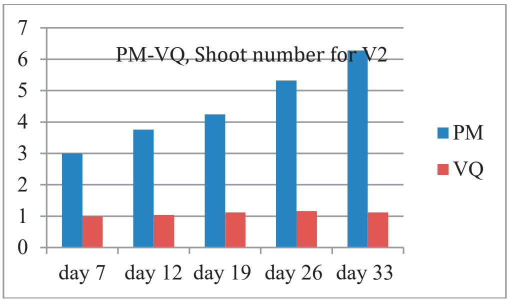

Figure 4. PM-VQ comparative shoot number on V3 (0.5 mg/l)

Regarding the height of explant exposed on BAP variation, Post Hoc test show us significant differences ($p < 0.001$***), starting with day 12: for variant V2 (0.25 mg/l) in day 26 ($p < 0.057$***), and for variant V3 (0.5 mg/l) in day 26 with $p < 0.001$*** (Figure 5).
Regarding number of leaves, ONE WAY ANOVA show us significant differences between PM and VQ cultivars on V2 variant (0.25 mg/l) with $p < 0.001$***, starting with day 19 of observation. The trend is maintained until day 33 (Figure 6).

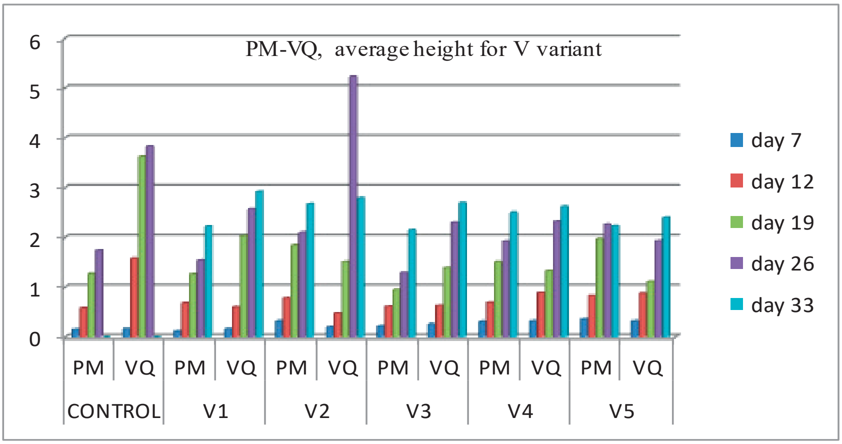

Figure 5. PM-VQ average height for V variant

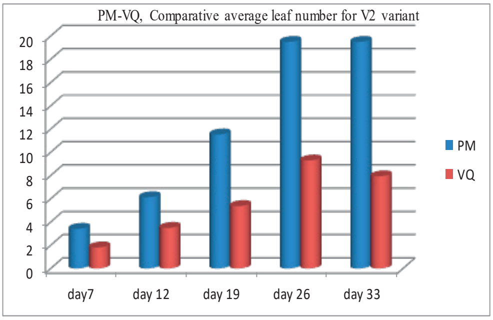

Figure 6. PM-VQ average leaf number for V2 variant

Regarding root system of explants, biometric readings show that on variant V2 (0.25 mg/l) there wasn't any differences between PM and VQ. Instead, significant differences appeared on V1, V3, V4 and V5.

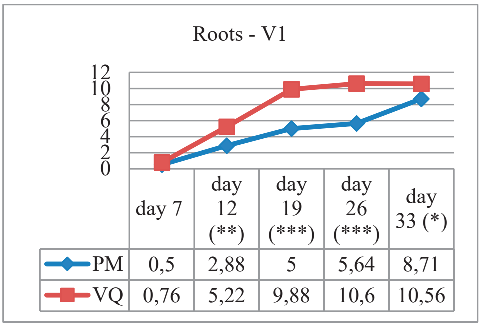

Figure 7. PM-VQ average roots number for V1 variant

Significant differences ($p < 0.001$***) was recorded starting with day 12: for V1 (0 mg/l) in days 19 and 26 (Figure 7), for V3 (0.5 mg/l) in days 12 and 19 (VQ); and for V4 (0.75 mg/l) for days 12/19/26 (VQ).

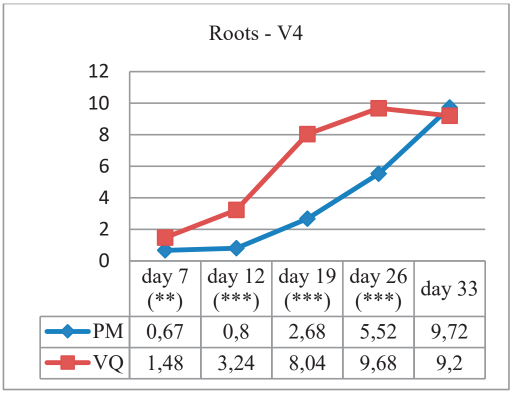

Figure 8. PM-VQ average roots number for V4 variant

Regarding auxins experience or variant Y, meta data show us as that the impact of NAA in combination with BAP and GA_3 on the development of explants is major when we compare PM and VQ varieties. Post Hoc test doesn't express any significant difference from Y1(with 0.25 mg/l BAP; 0.05 mg/l GA3; 0.0 mg/l NAA) and Y2 (with 0.25 mg/l BAP; 0.05 mg/l GA3; 0.01 mg/l NAA) on number of shoots, and for Y4 (with 0.25 mg/l BAP; 0.05 mg/l GA3; 0.03 mg/l NAA) and Y5 (with 0.25 mg/l BAP; 0.05 mg/L GA3; 0.05 mg/l NAA) for number of leaves. Instead of this, significant difference ($p < 0.001$***) appear in Y1 (with 0.25 mg/l BAP; 0.05 mg/l GA3; 0.0 mg/L NAA), Y2 (with 0.25 mg/l BAP; 0.05 mg/l GA3; 0.01 mg/l NAA) and Y3 (with

0.25 mg/l BAP; 0.05 mg/L GA3; 0.02 mg/l NAA) regarding the number of roots and biometric measurement confirm this evolution starting even with day 7 (data not show at this moment).

CONCLUSIONS

In this part of study we observe the influence of BAP and NAA on *Solanum tuberosum* cultivars development and grows though micropropagation. Parameters analysed reveal that V2 (0.25 mg/l BAP; 0.05 mg/l GA_3; 0.03 mg/l NAA) increase the number of leaves and V4 (0.25 mg/l BAP; 0.05 mg/l GA_3; 0.03 mg/l NAA) for number of shoots, but discussion need to be done only in relation with cultivar, because thre are different responses on different stimulus and in relation with control.
A discussion need to be done on variant V5 (1 mg/l BAP; 0.05 mg/l GA_3; 0.03 mg/l NAA). Comparison between PM and VQ and statistical analyses show that only on number of roots we can discuses about a significant difference on day 19 and day 26. All other measurable parameters, like number of leaves, height, or number of shoots don't show any difference between development of our cultivars.

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A COMPREHENSIVE STUDY REGARDING MULTIPLICATION OF TWO WORLDWIDE ECONOMICALY IMPORTANT SPECIES: *Solanum tuberosum* AND *Ipomoea batatas* - *IN VITRO* APPROACH

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Abstract

Solanum tuberosum is the third world ranking species in terms of yield and consumption, after rice and wheat. Ipomoea batatas is the seventh world ranking production vegetable and provides food for over 68% of population. They are growing and fructification in hard climate conditions with arid soils or desert (sweet potato), and have important role in biodiversity through culture conditions adaptability, plant diseases and pathogens resistance (potato). Despite all these advantages, infections that are combined on those species reduce production capacity up to 90% in both species and this factor can be controlled to some extent by using micropropagation techniques and thermotherapy. Scientific papers, treaties and communications were studied in order to gather the most relevant dates regarding micropropagation of those two species. The high genetic variability this two species have makes it difficult to standardize micropropagation protocols, such as disinfection, phytohormones combinations and other techniques used in micropropagation regarding the devirosation of the plant material and the production of virus free material. Solanum tuberosum and Ipomoea batatas are two species that are two of the most cultivated worldwide, in poor or in development countries based on their nutritional values and their economic importance, scientists and breeders are focusing on biotechnologies to produce new varieties with high production capacity and promising resistance to pest, diseases and viruses.

Key words: *Ipomoea batatas, micropropagation, Solanum tuberosum*, *virus free.*

INTRODUCTION

Solanum tuberosum is the one of the world's most important food crop and major food sources for humankind. Potato is asexual propagated, using the tubers, technique which allows the dissemination of pathogens to the new plants and cultures, but also threatens the maintenance of genotypes for commercial or breeding purposes (Golmirzaie and Panta, 2000). Because of its value as a food source - plant is cultivated in over 150 countries (Basera et al., 2018), its important role in biodiversity through culture conditions adaptability, potato plant is a truly studied specie regarding diseases and pathogens resistance (Bamberg et al., 2016). Because potato accumulate several systemic fungi, bacteria and viruses infection (Karyeija et al., 1998), micropropagation offers alternative methods of propagation that provide production and multiplication of plant material with high efficiently (Golmirzaie and Toledo, 1999). *In vitro* conservation of potato facilitates the availability of the material all the time, avoid transfer of major diseases and pests and make possible virus control through meristem culture (Khadiga et al., 2009). Besides clonal multiplication, biotechnology techniques make available material for breeding programs and potato certified seed, and support conservation of germplasm which is a major problem in modern world for this specie, due its high cultivation on small lands, for personal consuming or small commercial purpose. In that situation, *in vitro* multiplication, cryo conservation and storage procedures surge potato plant bioavailability for a sustainable worldwide crop.

Ipomoea batatas, (sweet potato) is the sixth most important food crop worldwide after rice, wheat, potatoes, maize and cassava. In developing countries, however, is the fifth most important food crop and its food importance is rising (Jarret and Florkowski, 1990). More than 105 million metric tons are produced globally each year and 95% of which are grown in developing countries, commonly in Sub-Saharan Africa, some countries from Asia, and the Pacific Islands (Turyagyenda et al., 2015). According to them, sweet potato will empower around 15 million resource-poor households in Asia and Africa by improving the diets nutritional status and enhance crop incomes by 15% by 2023. Despite its benefits, virus diseases have been identified as the main cause of low yield productivity and cultivars degenerate (Wambugu, 1991). *Ipomoea batatas* is usually propagated by shoots tips, stem cuttings or storage roots for asexual propagation but diseases, pests and environmental factors impede sweet potatoes from reaching their maximum potential as a food crop (Guo et al., 2001). Several studies indicate that sweet potato chlorotic stunt virus and sweet potato feathery mottle virus drastically reduced specie yields, losses may often reach 65 to 90% (CABI, 2022; Wilms et al., 2020; Clark et al., 2012; Karyeija et al., 1998). The response could be found in micopropagation and *in vitro* conservation as providing stable clonal material, full time available, fewer pathogens or diseases contamination and virus free (Micheli and Standardi, 2015).

MATERIALS AND METHODS

This review tries to summarise some relevant results regarding culture decontamination, virus eradication, carbon source and growth hormones. We try to make a true listing and we are sure that are important scientific papers that are not find here due the length limitation or missing them from websites. We review over 75 papers for this paper.

In vitro culture decontamination

For *Ipomoea batatas* and *Solanum tuberosum*, micropropagation is one of the tissue culture techniques that provide culture stability and applicability (Leva & Rinaldi, 2012). Starting with 1951 when it was wrote the first scientific paper regarding usage of 2,4D and coconut milk on *in vitro* potato tuberization, several studies tried to establish a relation between virus-meristem and conservation (Steward and Caplin, 1951), using single node cuttings or liquid shaken cultures as are described in CIP documents (Espinoza et al., 1984). Through the years, one of the extended obstacles in the development of in vitro multiplication or storage protocols for potato, remain either fungal, bacterial, or over 40 viruses contamination (Lai et al., 2022).

The first approach is disinfection of the plant material which can be done depending on the type of explant: disc from tubers-potato, tuber pieces-potato, single nodes from sprouts-potato,leaf, shooting node, lateral bud or apex, meristem. The main substance use for both species are Sodium hypochlorite (NaOCl), in concentration varying between 1%-2%-1.5%-3%-3.5%-5.2%-10%-14% and time between 8-10-15-20 minutes, Mercuric chloride ($HgCl_20$) 0.1%-1% for 4 to 8 minutes, ethanol 70% (C_2H_5OH) for 5-15-25-30-40 or 60 sec, depending on explant type and contamination. (Badoni et al., 2010; Alconero et al., 1975; Gudeva et al., 2012; Yang, 2010; Tadda et al., 2021; Zhen, 2001; Hajare et al., 2021; Fufa et al., 2013; Dewir et al., 2020). Almost all studies using fresh material add firs some systemic fungicide to get a higher percentage of sterilization, Bavistine 0.5%-0.9% or Aliette in 0.4%-0.6% concentration.

Virus eliminated methods

Number of researches on potato micropropagation or storage technologies identify and try to control over 30 virus types that are known today (Loebenstein and Gaba, 2012). For endophytic bacteria treatments some studies recommend antibiotics, but the usage of its may produce toxic chemical particles for the plant development. For potato, Schewinski-Pereira (2003) recommend tetracycline, chloramphenicol, streptomycin, and ampicillin in concentration between 32 to 256 mg/l for endophytic bacterial growth inhibit and studies revealed tetracycline and chloramphenicol interfere with *in vitro* potato plant and affect explant survival, in opposition to ampicillin witch has no toxic effect on plant growth.

Since 2004 there are techniques that could applied to potato for eradicate viruses, or limit cross infections (Khurana, 2004). Viral infection of plant base material for commercial usage, infected with one or several viruses, substantially decrease production in the field. Identifying viruses cannot be done by visual report, sequencing of PCR–amplified sections of genomes is required (C. Jeffries and Khurana, 2006).

Micropropagation through meristem culture, thermotherapy, cryotherapy, chemotherapy single but mostly combined impact the viral expression in new plants and could control seed plant material (Green et al., 1989).

Several studies identify and try to eliminate viral contamination through therapies, with successful percent between 0% and almost 100% (Table 1).

Table 1. Main potato viruses and micropropagation methods of eradication

Potato virus type	Techniques	Micropropagation method	Rate of elimination	Best results	References
PVS, PVX, PVA, PVY, PVM, PLRV	Meristems culture, shoot-tip cryotherapy by droplet vitrification, chemotherapy combined with thermotherapy	meristem, shoot tips	30-80-100%	Chemotherapy combined with thermmotherapy	Zang et al., 2019
PVM, PVS, PVX, PVY	Chemotherapy/ribavirin 100 mg/l, cryotherapy with PVS-2 vitrification protocol	shoot tips	cryotherapy alone and one virus- 38.6%; cryotherapy for 3 viruses- 0%; chemotherapy +cryotherapy and subcultivation - 100%;	Chemotherapy+ cryotherapy	Kushnarenko et al., 2017
PVX, PVS, PLRV, PVA	Chemotherapy/ribavirin 100 mg/l in concentration: 0.75, 100, 150 and 200 mg/l	shoot tips	100% for PVX, PVS, PVA; 33-66% for PLRV	Chemotherapy	Yang et al., 2014
PLRV, PVY,	Electrotherapy, electro+chemotherapy, electrotherapy+subcultivation+antiral+ASA	shoot tips	46,7%; 40%; 67,2%; 62.8%;	Electrotherapy+ subcultivation+antiviral+ASA	Naik et al., 2018
PVA, PVY	Electrotherapy	axillary buds	35 mA (8%/12.5%)	Electrotherapy+ cultivar	Meybodi D.et al., 2011
PVA, PLRV, PVM, PVS, PVX, PVY	Thermo-and/or chemotherapies (ribavirin)	shoots, apex	60%	Combined treatments	Bamberg et al., 2016
PVY, PVX, PVS, PLRV	Thermotherapy, chemotherapy (ribavirin, 5-Azacytidine, 3-Deazauridine) and combined	single nodes	PVY (83.3; 70.0 and 50.0%); PVY (30.0%)	Combined treatments	Nascimento et al. 2003

Regarding sweet potato and its viral infectious status, worldwide production is seriously affected by a range of over 30 viruses, like sweet potato virus disease (SPVD), Sweet potato pakakuy virus (SPPV) or Sweet potato feathery mottle virus (SPFMV), single or combined, the last one cause roots unmarketable (Kapinga et al., 2007). There are still made efforts to eradicate viruses in sweet potatoes to establish a standard for virus-free certificate plant material and produce plant material through *in vitro* techniques (Morais et al., 2018).

Some scientific papers indexed in this work listed meristem culture, thermotherapy, cryotherapy as potential treatment to eradicate viral disease in sweet potato, as they are summarised (Table 2).

Table 2. Main sweet potato viruses and micropropagation methods of eradication

Potato Virus type	Techniques	Micropropagation method	Rate of elimination	Best results	References
SPFMV, SPLV, SPMMV, SPVG, SPMSV, SPCFV, C-6 virus, SPCSV, SPCaLV, CMV	Meristems culture, thermotherapy	meristem, shoot tips	SPFMV-88.89% and SPCSV-100%	combined treatmens	Dugassa and Feyissa, 2011
SPFMV and SPCSV	Meristem culture and cryotherapy	meristem 0.5–1.5 mm	SPFMV- 90–93% faild in 1.5 mm meristem; SPCSV eradicate	cryotherapy	Wang et al., 2008
SPFMV, SPVC, SPMMV, SPCSV and SPLCUV	Repeteating grafting with contaminated I. setosa, controled temperaure and amended soil	meristem culture	none for SPCSV, SPFMV+ SPCSV; SPMMV (100%,20%, 60%) and SPLCUV (80% and 100%)	combined treatments	Ssamula et al., 2018
SPCSV, SPVG, SPVC, SPFMV, SPV2, SPLCV(first report), SPPV	Thermotherapy and meristem tip culture	meristem culture	over 13% for PFMV, SPV2, SPVC, SPVG, and SPLCV except SPPV	combined	Kiemo et al., 2021
SPCSV, SPFMV, SPMMV, and combinations of SPCSV + SPFMV and SPCSV + SPMMV	Thermotherapy 36°C/16 h and 32°C/8 h daily and meristem tip culture	grafted to Ipomoea setosa and micropropagated by meristem 0.5-1.00 mm	SPFMV and SPMMV 97.2%, 80.5%, 69.2%	micropropagation with thermotherapy	Rukarwa et al. , 2011
"non-persistant virus (filamentous particle of 850 nm)" and "virus with filamentous particles of about 1000 nm"	Meristem culture (0.25 to 0.4 mm) and grafting	meristem culture	80%	combined treatments	Frison et al., 1981

Carbon source and tuberization

The success of plant tissue culture is determined by culture media structure and carbon source, most likely sucrose. Sugars are required *in vitro* (they complete life circle of the plant and without them, they will not survive), they cannot be replaced by another element and their action is specific (Fufa and Diro, 2013).

Based on that, sucrose is most used carbohydrate source, among glucose, fructose, galactose, mannose, maltose, lactose, trehalose and raffinose, not all of them used in micropropagation (Yaseen et al., 2012).

Rahman, 2010 research the role of sucrose, glucose and maltose and found that maltose is preferred in terms of multiplication rate and if we are talking about unimodal segments, research results show better response with glucose.
The 30 g/l sucrose could be significant for shoot length in addition with low light and low temperature storage (Pruski et al., 2000).
Sucrose was determined as a necessary external carbon source for induction and micro tuberization and usually increased concentration act better instead of lower concentration used for slow grow techniques and conservation (Lo and Liao, 1993) but both lower or higher concentration can impair with plant development.
The explant response to carbon source vary with cultivar or genotype, presence of hormones, stocking temperature and light intensity, but, generally accepted is that sucrose is the main carbon source for plant micropropagation (Table 3).
Sugar itself or sugar less 20 g/l is not a solution when tuberization is tracked, 40 g/l gave only 75% tuberization, but in case of higher concentration, about 80 g/l, 100% of tuberization achieved (Xu et al., 1998).
Same Xu revealed the relation between sucrose and endogenous gibberellins (GA), sucrose induce the expression of tuberization genes at higher concentration.

Multiplication
Multiplication is part of micropropagation and use an initial explant like source for multiplication stage.

Table 3. Main carbon source in potato and sweet potato micropropagation

Carbon source	**Concentration**		**References**
	Solanum tuberosum	***Ipomoea batatas***	
Sucrose	20 - 25 - 30 - 40 - 50 – 60 - 80 - 100 g/l	15 - 20 - 25 - 30 - 40 - 60 g/l	AlMaarri et.al, 2012; Islam et al., 2017; Zhang et al., 2019; Wang et al., 1982; Rahman et al., 2010; Altindal & Tahsin, 2010; Yoon et al., 2004; Ibrahim, 2019; El-Far, 2007; Dugassa and Feyissa, 2011; Fadaladeen et al., 2022;
Maltose	20 - 30 - 40 - 60 – 80-120 g/l		Rahman et al., 2010; Altindal & Tahsin, 2010; Yoon et al., 2004
Glucose	30 - 80 g/l	15 - 30 - 45 - 60 g/l	Rahman et al., 2010; Fadaladeen et al., 2022
Fructose		15 - 30 - 45 - 60 g/l	Fadaladeen et al., 2022
Sorbitol		0.2 - 0.4 - 0.6 M	Smith et al., 2019
Sorbitol and manitol		20 g/l	Sriskantharajah & Ketipearachchi, 2012

Taking into account the viral infectious spectre of those two species, first recommendation for micropropagation remains meristem culture (Gudeva et al., 2012) and after that, shoot tips with discussions about optimisation of dimension (Wang et al., 2008). Danci (2011) show that even the meristem is larger and you can produce more plantlets, the presence of leaf primordia is critical for survival rate. Several works show us that meristem culture not only regenerate much rapid then other culture like shoot tips, or organogenesis, but we can manage the viral infections with this approach, both in *Ipomoea batats* and *Solanum tuberosum* (Wang & Hu, 1982; Barka and Feyissa, 2011; Smith et al., 2019; Nascimento et al., 2003).
Sprouts from potato or uninodal segments (stem cuttings from potato) from sweet potato are the next option for micropropagation, and they are used on researches about multiplication, growth rate or tuberization (Ravnikar et al.,1992; Yang et al., 2014; Abubakar et al., 2018; Vettorazzi et al., 2017; Beyene et al., 2020)
One important science direction on sweet potato is salt resistance of this specie and because of its biologically plasticity and food impact on poverty and hunger this important biotic asset need to be exploit.
Studies were made on *in vitro* plants generate through somatic embryogenesis (leaf, petiole and stem explants) and *in vitro* techniques

support plant salt resistance identification (Anwar et al., 2010; Ekanayake and Dodds, 1993).

Growth regulators

Literature suggests hormones regulate explant growth in micropropagation, can induce organogenesis (Nakajima and Kawakami, 1969), callus dedifferentiation, multiplication, rooting and plant wellbeing. Starting with MS medium of Murashige & Skoog (1962) hormones represent the necessary variables that made micropropagation and plant tissue culture possible.

For *Solanum tubeorsum* and *Ipomoea batatas* literature review the major role of hormones for micro tuberization (García-García et al., 2019), virus eradication (Gong et al., 2019; Kiemo et al., 2022) or cryoconservation (Bamberg et al., 2016; Sriskantharajah and Ketipearachchi, 2012).

Table 4. Usual concentration of major hormones used in micropropagation of *Sweet potato* and *Ipomoea batatas*

Hormone	Effect	Concentration		References
		Solanum tuberosum	***Ipomoea batatas***	
Indole-3 -butyric acid (IBA)	regulation of root apical meristem size, root elongation, lateral root development, and formation of adventitious roots;	0.01 - 0.1 - 1 mg/l	0.1 - 2 mg/l	Rabbani et al., 2001; Fadaladeen et al., 2022; Zang, Z. et al., 2019
Indole-3 -acetic acid (IAA)	inducer of cell division and elongation;	0.5 - 1 - 1.5 mg/l	0.2 - 1 - 1.2 mg/l	Alconero et al., 1975; Zhen, H R., 2001; Gudeva, K.L. et al., 2012
1-naphthaleneacetic acid (NAA)	rooting agent;	0.01- 1 mg/l	0.01 - 0.05 - 0.1 - 1 - mg/l	Fadaladeen et al., 2022; Zhen, H R., 2001; Fufa, M. and Diro, M. (2013)
2,4-Dichlorophenoxyacetic acid (2,4-D)	a dedifferentiation (callus induction) hormone	1 - 1.5 - 2.5 - 3 - 4.5 – 5 mg/l	0.01- 1.5 - 2 - 3.5 - 4.5 - 4 mg/l	El Abidine Triqui et al., 2008; García-García et al., 2019; Padmanabhan et al., 2001; Oggema, J. et al., 2007
6 -benzyladenine (BA)	induction of cell division and shoot. differentiation in plant tissue culture	0.5 - 1 - 1.5 – 2 mg/l	0.1 - 0.5 - 1 - 4 - 4.5 - 5 - 6 - 7 - 8 mg/l	Fadaladeen et al., 2022; Zhen, H R., 2001
6-furfurylaminopurine (KIN)	inducing callus (+ auxin), regenerate shoot tissues from callus (- auxins);	0.1 mg/l	0.05 - 0.1 - 0.5 - 1.5 - 2.5 - 3 - 4 - 5 mg/l	Smith, M.S. et al., 2019; Fadaladeen et al., 2022; Zhen, H R., 2001; García-García, J.A. wt al., 2019; Gudeva, K.L. et al., 2012
gibberellic acid (GA3)	essential for the induction of lateral shoots, increasing cell elongation, seed germination, dormancy, reproductive growth, tolerance against various stress types and senescence; essential in callus culture	0.25 - 0.3 - 0.5 mg/l	0.25 - 1 - 2 - 3 - 10 mg/l	Wang, Q.C., JValkonen, J.P.T., 2008; Gudeva, K.L. et al., 2012; Fufa, M. and Diro, M. (2013)
6-benzylaminopurine (BAP)	stimulates the differentiation of the cells generated in meristem and encourage the growth of side shoots, leaves apical dominance and expansion; stimulating cell division	0.1 - 0.5 - 1- 1.5 - 2 - 3 - 4 - 5 mg/l	0.1 - 0.25 - 0.5* - 0.75 - 1 - 2 - 2.5 - 3 - 5 mg/l/*callus proliferation	Wang, Q.C., JValkonen, J.P.T., 2008; Smith, M.S. et al., 2019; García-García, J.A. et al., 2019; Gudeva, K.L. et al., 2012

Micropropagation of potato depends on the genotype, nutrients in the culture medium and plant growth regulators and there is no standard recipe could be applied. Among the usual hormones, most important are Indole-3 -butyric acid - IBA, who can regulate root apical meristem size, root elongation, lateral root development, and promote formation of adventitious roots; Indole-3-acetic acid - IAA, important hormone for inducing cell division and elongation; 1-naphthaleneacetic acid - NAA, prompting rooting agent; 2,4-Dichlorophenoxyacetic acid - 2,4-D, a pesticide who can induce callus formation (dedifferentiation); 6-benzyladenine - BA, who can impact induction of cell division and shoot. differentiation in plant tissue culture; 6-furfurylaminopurine - KIN, an synergic hormone for inducing callus (+ auxin) or regenerate shoot tissues from callus (- auxins); gibberellic acid - GA_3, essential for lateral shoots induction, increasing cell elongation, dormancy seed germination, reproductive growth, supporting different stress types and senescence and essential in callus culture and the last important hormone, 6-benzylaminopurine - BAP, who can stimulates the cells differentiation, generated growth of side shoots, leaves, induce apical dominance and expansion. (El Abidine Triqui et al., 2008; Espinoza et al., 1984; Dewir et al., 2020; Rabbani et al., 2001; Gudeva et al., 2012; Bamberg et al., 2016; Steward et al., 1951). Different concentrations for some of the main hormones are present below (Table 4).

Regarding somatic embryogenesis, growth regulators are key factors for callus induction and plantlet development, studies reveal that for first stages there is a borderline for auxins presence: during initiation is necessary to inhibit auxins but for callus inducing, there is a total request of them (Rabbani et al., 2001) and for the further stages, another hormones are necessary, like 2,4-Dichlorophenoxyacetic acid - 2,4-D , GA_3 or zeatin (El Abidine Triqui et al., 2008; García-García et al., 2019; Padmanabhan et al., 2001).

CONCLUSIONS

Even potato micropropagation is studied since the early 50th, because of its large scale production, domestic or industrial, there are still issues regarding somaclonal variation and genetic stability of this specie. Similar, sweet potato present some issue regarding genetic stability and large scale mass seed production. Even species respond to somatic embryos technique, there is not enough response for this *Solanaceous* plant (potato), and somatic embryogenesis to potato still requiring studies.

Even scientific literature is much bigger than our references, *in vitro* techniques and gene conservation methods remain first option for potato, sweet potato and rest of tubers. Techniques can preserve both species on medium-term time through cryopreservation, and combined chemotherapy with thermotherapy can develop a solution for eradication viral diseases. In the low-income country, there is possible to establish a low-cost protocol for sweet potato micropropagation and that is the major goal for world issue food and hunger for African country.

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THE INFLUENCE OF THE ELECTROMAGNETIC FIELD ON GERMINATION OF OKRA SEEDS AND EFFECT USE OF SOME TREATMENTS IMPROVING GERMINATION

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Abstract

The study was carried out within the Faculty of Horticulture, UASMV Bucharest. The biological material consisted of the Acme okra variety. Seed germination was tested under two conditions, in the laboratory and in the greenhouse. The experimental variants consisted of the treatments carried out on the okra seeds, namely keeping the seeds in a magnetic field of 6.8 Hz and 10 Hz for 1 hour, 2 hours and 24 hours, respectively, then the percentage of sprouted seeds was monitored for 12 days. Differences were noted regarding the percentage of germinated seeds but also differences regarding the duration of germination.
The study addressed in this work aimed to establish some methods to improve the germination of okra seeds and at the same time identify a solution to shorten the germination period.

Key words*: Okra, germination, electromagnetic field.*

INTRODUCTION

It is known that okra seeds germinate in a very long time up to 12-15 days only when a temperature above 20°C is ensured. Also, okra seeds have a low germination of 75-90% according to vegetable quality standards and technical conditions.
Zanini, 2021, points out that the exposure of seeds and plants to a magnetic field influences the growth of seed and plant germination, an aspect also researched and confirmed by Florez et al., 2007 claiming that the greatest increases were obtained for plants continuously exposed to 125 or 250 mT but also by Carbonell et al., 2011 as well as by Dobrescu et al., 2000.
Neo et al., 2018, analyzing the importance of using plant stimulation with different values of the magnetic field, presents many researchers who have been concerned with this aspect.
In the case of the species *Pisum sativum*, it was found that the epicotyl was longer when the seeds were exposed to a magnetic field with a lower value compared to the normal geomagnetism of the Earth (Yamashita et al., 2004). Negishi et al., 1999, suggested that the cell elongation attributed to the influence of the low frequency magnetic field may be related to the increase of the osmotic pressure in the cells.
Katarzyna et al., 2022, investigated the impact of magnetic fields on the germination of cucumber seeds using Bitter magnets with a constant magnetic field.
Belyavskaya, 2001, observed that the effects of the magnetic field on the ultrastructure of the root cells while also mentioning that the weak magnetic field causes the intensification of protein synthesis in plants.
Experiments carried out on barley seedlings (Hordeum vulgare) between Helmholtz coils with an intensity of 10 nT magnetic field showed a decrease in the fresh weight of plants (by 12%) and roots (by 35%), as well as the dry matter of plants (by 19%) and roots (by 48%) compared to the controls in the gravitational magnetic field (Lebedev et al., 1977).
Abe et al., 1997, as well as Negishi, 1999, Liu et al., 2019, mentions that the impact of the magnetic field positively influences the growth and development of plants.
De Souza et al., 2010, showed that seeds and plants exposed to the magnetic field showed higher vegetative growth but also a better rate of photosynthesis.
Shine et al., 2011, showed that by exposing seeds to different magnetic field intensities from 0 to 300 mT for 30, 60 and 90 minutes

they showed that magnetic field treatment can improve seed germination parameters.

Afshan et al., 2012, investigated the effect of pre-sowing magnetic treatments on germination, growth and yield of okra (*Abelmoschus esculentus* cv. Sapz pari). They found that the seeds exposed to the sinusoidal magnetic field induced by an electromagnet of 99 mT for 3 and 11 minutes and observed a significant increase in germination percentage, number of flowers per plant, leaf area, plant height, number of fruits per plant as well as capsule mass.

Maffei, 2014, mentions that the geomagnetic field (GMF) is a natural component of our environment and plants respond differently depending on its intensity.

Besma et al., 2014, mention that the germination of okra seeds also depends on the salinity of the culture substrate.

Through the issue addressed in this paper, we aimed to establish some methods by which to improve the germination of okra seeds and at the same time identify a solution for shortening the germination period.

MATERIALS AND METHODS

The experience was carried out at the University of Agronomic Sciences and Veterinary Medicine Bucharest, Faculty of Horticulture under laboratory conditions.

The biological material was represented by the Acme okra variety.

The device used in the experiment is an electromagnetic stimulator for changing the growth behaviour of plants (Figure 1).

Figure 1. The appearance of the installation used for seed treatment

To accelerate growth and develop better vital qualities in plant organisms, they are subjected to a magnetic field.

According to this method, a pulsating polarized magnetic field is made to act correspondingly with different extents, generally correlated with each other, on cultivated land, seeds, plants, and irrigation water.

The installation refers to a method of improving seed germination and/or growth of plants or plant parts and/or plant harvest by subjecting seeds to a pulsed radio frequency electric field (PRF).

Experimental variants

In the experiment we used two variants of germination stimulation, unmoistened seeds and seeds moistened for 1 hour before being exposed to the magnetic field. Wetted and non-wetted okra seeds were exposed to the electromagnetic field of 10 Hz and 6.8 Hz for one hour, 2 hours and 24 hours, respectively at constant intensity with the value 10 times more than the natural magnetic field (Table 1). The ambient temperature was between 21-24°C.

Table 1. The experimental variants

Variants	Treatment seed	Time of exposure to the magnetic field	The frequency of the magnetic field
V1	Softened	Control	-
V2	Unmoistened	Control	-
V3	Softened	1 hour	10 Hz
V4	Unmoistened	1 hour	10 Hz
V5	Softened	1 hour	6.8 Hz
V6	Unmoistened	1 hour	6.8 Hz
V7	Softened	2 hours	10 Hz
V8	Unmoistened	2 hours	10 Hz
V9	Softened	2 hours	6.8 Hz
V10	Unmoistened	2 hours	6.8 Hz
V11	Softened	24 hours	10 Hz
V12	Unmoistened	24 hours	10 Hz
V13	Softened	24 hours	6.8 Hz
V14	Unmoistened	24 hours	6.8 Hz

The experiments were carried out under both laboratory and greenhouse conditions. The percentage of germinated seeds, the duration of the germination period, the length of the radicle, were monitored under laboratory conditions

RESULTS AND DISCUSSIONS

In the case of the experiment carried out under laboratory conditions, it was found that the lowest percentage of germinated seeds was in V2 control - unmoistened seeds, which was

70.33%, and in the case of moistened seeds not exposed to the field, 75.33%.

In the case of V3, seeds moistened for time then exposed to the 10 Hz field for one hour showed a germination percentage of 90.25%, but in the case of non-moistened seeds, the germination percentage was 85.33%. I noticed that, in the case of seeds moistened for one hour at 6.8 Hz, variant 6, the germination percentage was 78.55%, lower than the moistened variant of 85%. In the case of variant 8 unmoistened seeds exposed for 2 hours to a magnetic field frequency of 10 Hz, the germination percentage was higher, 90.33%, compared to variant 7 moistened seeds of 89.55%. In the case of the variants exposed for 24 hours to the magnetic field at the frequency of 10 Hz, the germination percentage was 95% for variant 11, moistened seeds, and 95.11% for variant 10, non-moistened seeds. In the case of variants 13 and 14 exposed to the magnetic field for 24 hours at a frequency of 6.8 Hz, the percentage of germination was higher in the moistened variants compared to the wetted ones of 95.25% and 94.05%, respectively (Figure 2).

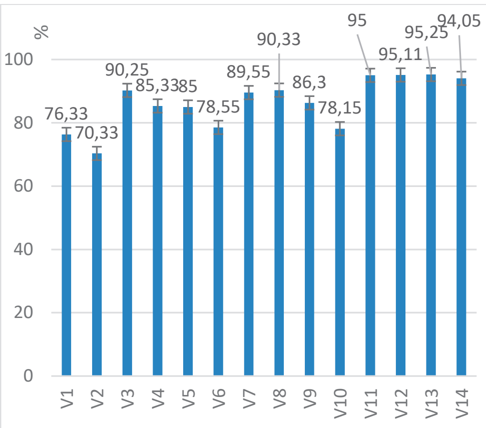

Figure 2. Percent of sprouted seeds

Analyzing the germination of the seeds from a percentage point of view, we found that, in the case of okra seeds not moistened and exposed to the field with the frequency of 10 Hz for 24 hours, the germination percentage was 35.23% higher than the non-moistened control variant, an aspect also noticed at the variant exposed to 6.8 Hz for 24 hours non-moistened seeds, this being 33.73% above the non-moistened variant (V2) taken as a control. All the experimental variants subjected to the magnetic field presented values of the percentage of emergence above 11.36 % (V5). The variants exposed for 2 hours to the magnetic field at frequencies of 10 Hz showed an increase in seed germination with values of 17.32% and 28.44%, respectively. Exposure for 24 hours resulted in an increase in the emergence percentage from 24.46% (the variant exposed to 10 hertz moistened respectively 24 79% to 6.8 kilohertz the moistened variant one. it should be noted that in the case of variants n moistened the percentage of emergence germination was higher compared to the moistened variants (Figure 3).

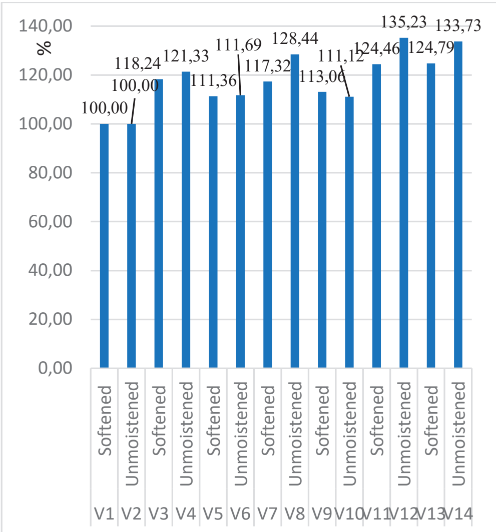

Figure 3. Percentage of germinated seeds according to control variants

In the case of the moistened variant, we found that there was a significantly positive influence on the percentage of seed germination (R^2 = 0.635) (Figure 4).

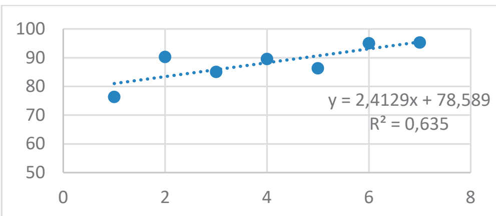

Figure 4. The influence of the magnetic treatment on percentage of germinated seeds

Also, in the case of the variant where we used the wetting of the seeds before exposure, we found that there was no significant relationship regarding the percentage of seed germination ($R^2 = 0.5658$) (Figure 5).

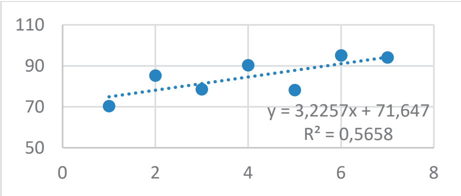

Figure 5. The influence of the treatments on the germination percentage in the case of the moistened variant

The appearance of the seedlings exposed to 6.8 Hz, is presented in the Figure 6.

Figura 6. Appearance of okra seedlings 2 days after emergence - Acme cultivar exposed to 6.8 Hz

In the case of the seeds exposed to 6.8 Hz, after 2 days from the emergence of the plants, the length of the radicle was 5.1 mm in the case of V1 - unmoistened control, and in the case of V6 - non-moistened seeds, 1.23 mm. the longest length was recorded for the moistened variant 13, of 7.55 mm, followed by V5 - moistened seeds, of 7.15 mm. In the case of variant 9, the value was 6.41 mm. In the case of non-moistened seeds, the radicle length was between 1.23 mm for variant 2 and 2.15 mm for V10 (Figure 7).

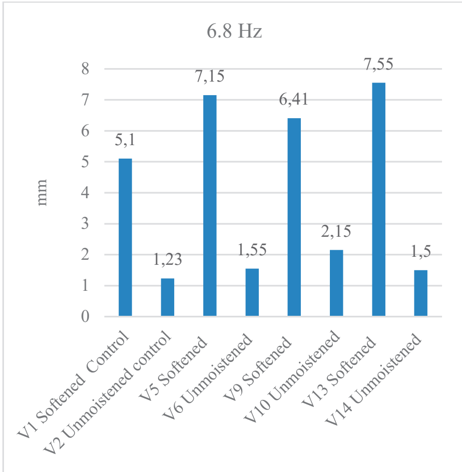

Figure 7. The influence of the treatments on the growth in the length of the radicle at 6.8 Hz

In the case of the seeds exposed to the intensity of 10 Hz, we found that the length of the radicle was greater compared to the moistened and non-moistened control variants, the values being at V 3 of 9.5 mm and at V7 of 8.3 mm. In the case of the non-moistened variants, the root length was small, regardless of the exposure time (Figure 8).

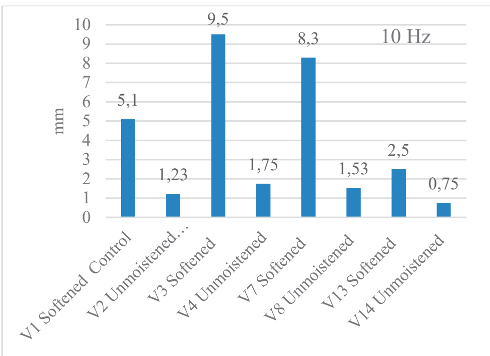

Figure 8. The influence of the treatments on the growth in the length of the radicle at 10 Hz

The appearance of the seedlings exposed to 10 Hz, is presented in the Figure 9.

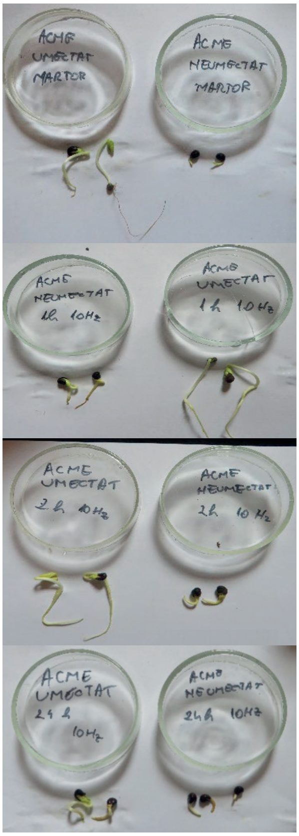

Figura 9. Appearance of okra seedlings 2 days after emergence - Acme cultivar exposed to 6.8 Hz

Analyzing the data on seed germination in greenhouse conditions, we noticed that in the case of seeds moistened and exposed to a 10 Hz magnetic field for 2 hours (V7), the seeds sprouted in greater numbers after 6 days from sowing, the percentage being 86, 2% compared to the moistened variants exposed to a 6.8 Hz field of only 16.4% (V11). In the case of the variant in which seeds moistened for one hour (V3) were used and then exposed to the 10Hz field, the percentage of sprouted seeds after 6 days was 40%, followed by V9 where the type of moistening was 24 hours, but the percentage of seeds sunrise was 68.33%. After 9 days from sowing, we found that all variants had a germination percentage between 5.18% for variant 7 seeds moistened for 2 hours and exposed to the field of 10 Hz. In the case of variant 2 control, unmoistened seeds, the germination percentage was 27% and after 12 days 52.33%. With this variant, a total germination percentage of 79.33% was recorded (Figure 10). A higher percentage of germinated seeds is noted in all the variants exposed to the magnetic field treatment with moistened seeds compared to non-moistened ones. After 12 days from sowing, in variant one, 15.8% sprouted seeds were recorded, and in variant 6, seeds n moistened for one hour exposed to 6.8 Hz, the percentage of germination was 69.66 percent.

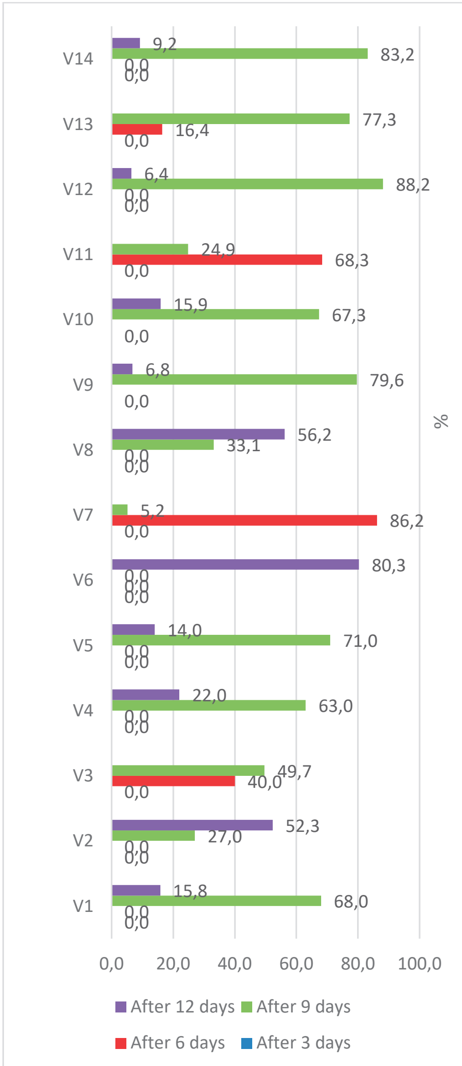

Figure 10. Emergence dynamics of okra plants in peat substrate in the greenhouse

The appearance of the seedlings in greenhouses is presented in the Figure 11.

Figure 11. Experiments performed under greenhouse conditions

Compared to the control variant, moistened and non-moistened seeds, all variants presented a higher germination percentage compared to the control variant. We noted that the highest percentage of seed germination was recorded in the variant 10 non-moistened seeds exposed for 24 hours at a frequency of 10 Hz (94.6%; Figure 12).

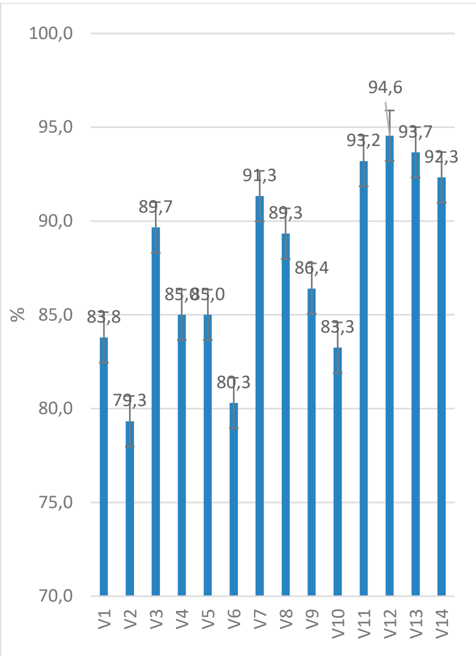

Figure 12. Percentage of seeds germinated in peat substrate in the greenhouse

Analyzing the influence of the treatment carried out, we found a positive significant relationship in the case of the germination of previously moistened seeds ($R^2 = 0.5445$) (Figure 13).

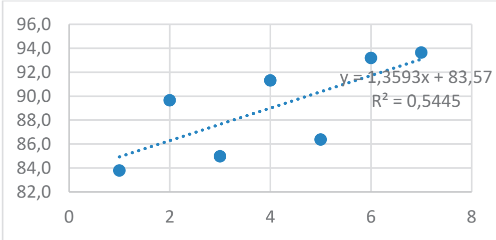

Figure 13. The influence of the treatments on the germination percentage

In the case of non-moistened seeds, a very significant relationship was also found, $R^2 = 0.6364$) (Figure 14).

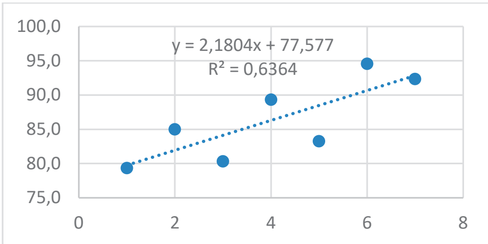

Figure 14. The influence of the treatments on the germination percentage

CONCLUSIONS

Based on the results obtained, we can conclude that, in the case of all the experimental variants moistened and exposed to the magnetic field, the percentage of germinated seeds was higher. It was found that the exposure of okra seeds to a magnetic field of 6.8 Hz and 10 Hz led to an increase in the percentage of germinated seeds but also to a shortening of the duration of germination.

In the case of testing in greenhouse conditions, on a peat substrate, it was also found that, in the case of seeds exposed to the magnetic field, the germination was higher in the case of previously moistened seeds. Analysing the effect of the treatments it was found that there was a significant relationship in terms of the effect on seed germination.

It should be mentioned the polarized and constant character of the electromagnetic field made with the help of a Maxwell type coil.

In conclusion, to save the germination time of the okra seeds, a seed treatment can be carried out, before sowing, by exposing them to a magnetic field of 10 Hz or 6.8 Hz.

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EFFECT OF WATER ELEMENT IN HISTORICAL PARKS AND GARDENS IN BUCHAREST

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Abstract

The present study aims to present the investigation results on the importance of water in the landscape and to present a brief history of the use of the water element. The study took into consideration calm waters, falling waters, running waters, water for fountains. There have been included also a landscaping analysis - composition for the seven parks listed in the historical objectives in Bucharest, namely Cişmigiu Garden, Kiseleff Park, Carol I Park, Herastrau Park, Botanical Garden, Ioanid Square, and Agronomy Garden - UASMV. A questionnaire was used to track the importance of water in public spaces, whether visitors are aware of the water element in parks, gardens, and identifying what every visitor feels when he/she is next to the water element (lake, pond, waterfall, well, etc.). Also, the frequencies of visitors to historic parks and gardens in Bucharest was tracked, the questions identified if they knew historical parks and gardens or not, and also the visitor's awareness of the importance of water in Bucharest's historic parks and gardens have also been pursued.

Key words: *Cismigiu Garden, Kiseleff Park, Carol I Park, Herastrau Park, USAMV Park.*

INTRODUCTION

Water has been present in every era and culture in the history of the landscape, from the Egyptian, and Mesopotamian (Babylonian) gardens that used ingenious irrigation mechanisms and systems to the Persian, Indian, Chinese and pre-Islamic ones (Mahmoudi et al., 2016) (Figures 1 and 2).
In the modern time, water pump was introduced so the water gardens became more available, but before that, the gardens designed with water elements were accessible only to the sovereigns of the time (Babnic, 2020).

Figure 2. Islamic garden
(Source: http://www.keywordsuggest.org)

Figure 1. Babylon Hanging Gardens
(Source: http://www.mixdecultura.ro)

Water can be defined as a culture and a way of life (Harris and Dines, 1988; Bayakan-Levent et al., 2002; Iliescu, 2003; Hasanagas, 2010) in addition to its functional qualifications, such as its contribution to the development of agriculture, its use in transport, water has symbolic values for many communities, representing the reflection of the sky, fertility, and abundance in the world, which symbolizes the continuity of life (Booth, 1989; Erdoğan, 2006; Koskina & Hasanagas, 2008). Water is perceived in various context, agricultural (Kherouf & Maoui, 2021), quality (Stavrescu-Bedivan et al., 2021), footprint (Sandu & Virsta, 2021)

The water element was used as an aesthetic factor in landscape design. In addition to its symbolic value, water is a very unique element, providing a different perception of the size and shape of space as it is perceived in reality (Rees & May, 2002). Water has an elemental quality that gives it a great symbolic significance when used as a decorative element. Water together with trees and the vault of the sky reminds us of the wildness of nature (Moughtin & Tiesdell, 1995) and can affect the relationship between the environment and man. The repetitive, flexible and deep nature of water can create different effects on users (Pye, 1995; Machlis & Forney 1996; Erdal, 2003). The waters are suitable in all situations, representing the most interesting objects of a landscape, fixing the attention. From a landscape point of view, the waters flow, stand, fall or gush, adding sounds and certain patterns to the perceived space, by spraying and the specific sounds produced. The repetitive, flexible, and deep nature of water can create different effects on users, depending on the variability of environmental factors (Pye, 1995). In all the districts of Bucharest, green areas have been arranged in order to offer to the inhabitants of the city and to the tourists the possibility of recreation and leisure in the middle of nature, but also on the shores of the lakes that are part of the hydrographic network of Bucharest (Giurescu, 2009).

The present study aims to present the investigation results on the importance of water in the landscape. In the same time, to answer to the necessity to consider the role of the element "water" in the historical arrangements of Bucharest.

MATERIALS AND METHODS

To achieve the aim of the study, three parks (Herastrau, Kiseleff, and Carol I Park) were chosen, three gardens (Cismigiu Garden, Botanical Garden, Agronomy Garden) and one square (Ioanid Square) to determine the weight of the water element and the percentage of water in the researched historical parks and gardens.

The methods used were observation (landscape visual analysis) and recording of existing data in the field. A questionnaire was also used to investigate the following:

• The importance of water in public spaces and parks;

• The sensations that water determines on the visitors from the parks in Bucharest;

• What are the most frequented parks in Bucharest (the frequency of visitors);

• Awareness of visitors on the importance of water.

RESULTS AND DISCUSSIONS

As a result of the research carried out, all the historical parks and gardens in Bucharest in this study use the water element in various forms, namely: static (rivers, lakes, ponds, basins), dynamic (artesian fountains, cascade). The styles of the studied parks and gardens are different: the mixed style (Cismigiu Garden, Carol I Park, Agronomy Garden), mixed, geometric, and English (Herastrau Park), English and French (Botanical Garden), English and French (Ioanid Square).

The classification by size differentiates the following formations of public green spaces, in increasing order of importance: the square, the garden, and the park, the forest park:

- Square - up to a maximum of 3 ha;
- Garden - between 3-20 ha;
- Park - between 20-100 ha;
- Park forests - over 100 ha (Filofteia, 1977).

In Cismigiu Park, the water element is given by the 3 ha Lake, which occupies about 1.5% of the total area of the park. The lake has an aesthetic, and recreational aspect. Other elements representing the water present in Cismigiu Park are the fountain located in the middle of the lake and the waterfall located in the cave. The water element is also encountered in the form of a small pond (Figure 3) (Dimitriu, 2010).

Figure 3. Footbridge in Cismigiu Garden

Kiseleff Park (Figure 4) presents the water element in the following forms: static - a small pond (basin rather) but also dynamic - numerous artesian fountains designed to create aesthetics. There is no lake or river in this park.

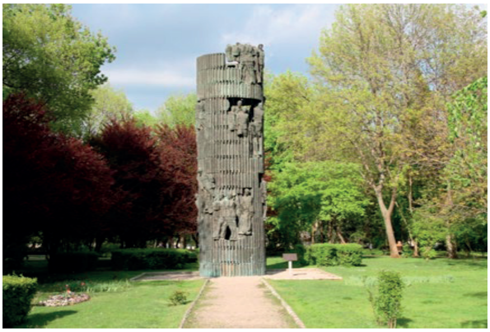

Figure 4. Infantry Monument in Kiseleff Park

In Carol I Park the water element is a basic element being presented in both static and dynamic forms. The lake within the park has a total area of 2 hectares and is intended for recreation. The waters within the Carol I Park account for 2.1% of the total area. In addition to the large lake, inside the park, we also find a small lake and, in its centre, a small island. Also, the water element is represented by the artesian fountains, the most important being the Zodiac fountain. Another fountain appreciated by the constant visitors of Carol Park is George Grigorie Cantacuzino Fountain (Cantacuzino Fountain). Inside the park, we also find a small pond. Various cubicles in the park give people in the park water when they get thirsty. Carol I Park also has a small pond and it is surrounded by rocks giving a soothing picture. Over this pond passes a small paddle which makes accessible the passage from one shore to the other. The small lake on the Carol I Park has a small island. From here visitors can quietly look at the mirror of the water, which gives them a state of quiet (Figure 5).

Figure 5. Footbridge in Carol I Park

Herastrau Park is currently the largest park in Bucharest. Its surface is very large, and water is a central element. The water is represented both in static form (very large lake) and also in dynamic form (artesian fountains). Lake Herăstrău is very large, it is an anthropic lake, which is arranged on the Colentina River. Lake Herastrau is located between Lake Baneasa (upstream) and Lake Floreasca (downstream). Herăstrau Lake is considered to be the largest in Bucharest (Stănescu, 2011) with a total length of the shores of 7,400 m. The surface of the islands on Lake Herastrau is 1,600 m. Initially, the place where Lake Herastrau is located today was a marshy area. Lake Herăstrau, besides its aesthetic value, also has an entertainment function. The visitors can walk on the lake by boat, hydrofoil, and boat (Figure 6). A lock (1933-1936) was built on the left bank of Lake Herastrau, making it possible to ride by boat both on Lake Herastrau, and Lake Floreasca. At the same time, the locker has the role of carrying out the additional drainage of Lake Herastrau in Lake Floreasca due to the risk of high-water level (it can flood the shores of the lake). The artesian fountains are quite numerous in Herastrau Park. Around them, we find benches that visitors can sit on and watch the landscape, the water spilling from these fountains, or read books. The fountains in Herastrau Park have different sizes and shapes (round, rectangular, etc.). The Herastrau Park is also equipped with many cubicles where visitors can get water for drinking. The lake is crossed by backyards. The island of roses is connected by two bridges which make it possible for visitors to visit this island of rare beauty (Iliescu, 2003).

Figure 6. Canoes in lake of Herastrau Park

The Botanical Garden is the oldest historical garden in Bucharest. Here we meet over 10,000 species of plants unique in our country. The water element is also encountered in the Botanical Garden being under two forms: static (lake) and dynamic (waterfall, and artesian fountains). There is an island on the lake and the access to the island is made by bridges connecting the shores of the island lake. The lake and the waterfall gives you a sense of tranquillity when you are near it. The artesian fountains through the splashing water give you the feeling of joy, relaxation, soul fulfilment. The mirror of the lake also creates a relaxing state (Figure 7).

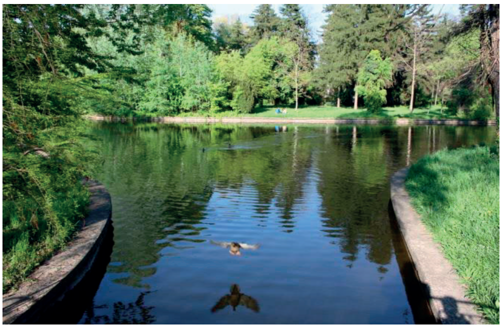

Figure 7. Lake in The Botanical Garden

The Agronomy Garden within USAMV of Bucharest also has a very well-defined water element, both in its static form (lake, pond, and basin within the botanical garden), as well as the numerous artesian fountains (the Faculty of Agriculture and the Faculty of Land Reclamation buildings). The water element is not very extensive in the Agronomy Garden, but it gives you a relaxing state when you are around artesian fountains. The pools in the Botanical Garden are of a round shape and the water is very poorly represented. Initially, one of the pools was designed to grow fish species to delight visitor views. The pond does not have a very large size but its shape and its surrounding by many dendrological species give you the impression of relaxation. If you are near the pond, it is impossible not to stop for even a few seconds to contemplate the water mirror, the picturesque scenery, and the tranquillity that surrounds this wonderful place (Figure 8).

Figure 8. Small pond in the Agronomy Garden

Ioanid Square is the smallest historical park in the present research. The water from Ioanid Square is represented by the static form (a small lake) but also the dynamic form (a small waterfall). The lake and the waterfall are surrounded by both rocks giving the impression of a soothing place. The lake is crossed by a very small decorative wooden bridge, which attracts a lot from the visual point of view. The waterfall overflowing gives the visitor a quiet, well-being, looking-and-seeing continuously as the water runs through the rocks. Relaxation is the word that can best be used in this case (Figure 9).

Figure 9. Statues in the Ioanid Square

In all the parks and gardens studied in the present research, besides the element of water that is encountered in static and dynamic forms, we find a lot of vegetation and greenery (trees, shrubs, lianas, annual flowers, biennials and perennials, etc.) The visitor may be protected from summer heat but also from dust pollution and noise from the city.

Also, in the parks of the present research we found various decorative objects.

The parks under study in the present research are very old and are included in the national patrimony of historical monuments: Cismigiu Garden (1830), Kiseleff Park (1832), Carol I Park (1900 designed, 1906 inaugurated), Herastrau Park (1930-1935 inauguration of marshland and inaugurated in 1939), Botanical Garden (1870), Garden of Agronomy (1870), Ioanid square (1870).

Analysis of questionnaire results

According to the data for the water element questionnaire in the parks, it was found that of the 20 respondents, 64% were female and 36% male (Figure 10).

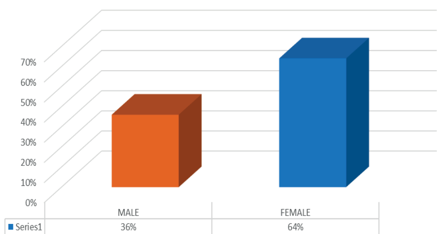

Figure 10. Distribution of gender in the target group

The respondents were aged 74% between 25-50 years, 16% over 50 years, and 10% under 25 years of age (Figure 11).

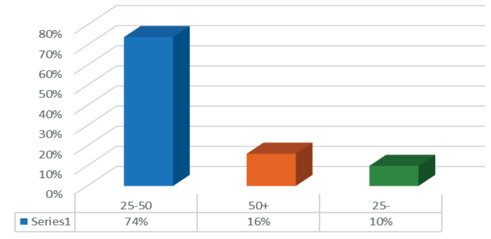

Figure 11. Age of respondents

The level of formal education for those who completed the questionnaire were from secondary schools (high school), 69%, respectively, to higher education, 31%. The data presented in the questionnaire show that most respondents (69%) have average education.

1. To Question 1, "What is the reason why water should be used in parks?", the respondents gave the following answers: they create relaxation, leisure, irrigation, and consumption, vegetation maintenance, and beautification of the park, because it is useful to nature, for recreation, water is considered as an aesthetic element in parks, but also to positively influence the human psyche. Water is also used for irrigation, watering, relaxation, and aesthetics according to other respondents. Water can be used for recreation (water sports). Respondents also mentioned that water can be used to water (irrigate) green areas as a necessity for both humans and plants, but also as a basic compositional factor (Figure 12).

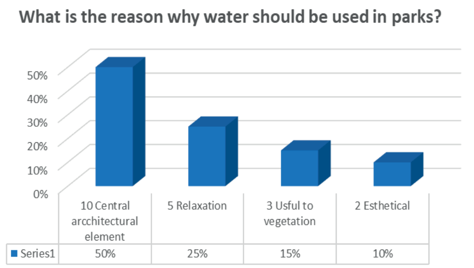

Figure 12. Possible aims for water used in parks

2. On question 2, "Do you think water is an aesthetic centerpiece in parks?", respondents answered as follows: 100% said yes, and 0% said they did not.

Also, 10% of respondents mentioned that water reduces atmospheric drought, 15% has an aesthetic role, 20% creates relaxation, 25%

looks good and 30% responds that it enriches the landscape.

3. For question 3, "Do you think water should only be used for parks irrigation systems?", 95% of respondents mentioned that not, and 5% said yes.

As arguments the answers to this question have been varied as follows: the water gives a relaxing state, it is used for dendro-floral watering, the water maintains the life of the plants, the water induces a cool state during the hot days, ensures the watering of the fauna in the park, etc.

4. At question number 4, "Do you know a historic park in Bucharest that uses water in any form of it? (irrigation, rivers, streams, lakes, waterfalls, artesian fountains, water games). What are these parks?", the respondents answered as follows: 45% mentioned Herastrau and Cismigiu, 30% Cismigiu, 20% Herastrau, 5% do not know.

As the arguments of the question, the interviewees replied as follows:

- Herastrau Park, because it's very nice to bring your girlfriend to a boat that you only find, being forced to have full confidence in your forces.
- The Cismigiu Garden, because the bridge has its purpose in the water, we cross the bridge from one shore to the other, exercising the tiny triumphs of life, which heal our souls, and the sails here pray mirrored in their own depth.

5. In question 5, "Do you think that the recreation / relaxation function of lakes or ponds in parks is important?", respondents answered as follows: 100% - yes and 0% - no.

The answers were argued as follows: boat rides, relaxation, fun, sport fishing if they are also populated with fish, etc. (Figure 13).

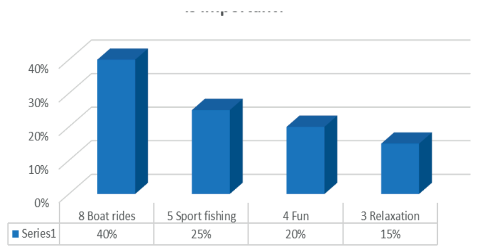

Figure 13. Possible functions of the lakes

6. In question 6, "Do you know parks in Bucharest that have artesian fountains?", respondents answered: yes - 85% and 5% - no, and 10% did not answer.

To the question "Specify which?", 25% of respondents mentioned Herastau Park, and 75% specified other parks such as I.O.R., Cismigiu, Tineretului, etc.

7. In question 7, "Do you know parks in Bucharest that have lakes or ponds?", the respondents mentioned the following: 75% - yes, 15% no, and 10% no answer.

In the question "Specify which?" The respondents gave the following answers: Herastrau Park - 15%, Cismigiu Park - 10%, the remaining 60% - Herastrau, Cismigiu and 5% - I.O.R.

8. To question 8, "What feelings are you induced when looking at a lake or a pond, basin, etc?" Were the following answers: 20% - quiet, 20% - quiet and relaxation, 20% - relaxation, 10 % joy, and 30% other responses like revival, harmony, calm, introspection, meditations, etc

The respondents' arguments to this question were the following:

"When you look at the water of a lake, at that moment you feel more relaxed, more quietly think of different beautiful things, you feel freer. You also feel a revival, a harmony, and a propensity for meditation. Looking in the water of a lake, you actually look deep into your soul, you become gentle better and more reconciled with yourself. Water inspires quiet, a new beginning."

9. In question 9, "What are states of mind are you induced by the strands and/or fountains in the parks?", the respondents mentioned that: the waterfalls and waterfalls in the parks give them a state of relaxation, well-being, quietness, of joy, of joy and happiness, of good mood and creativity, of energy, of coolness, of sleeping, etc. It is noted that most respondents mentioned that they are relaxing when they are near the waterfalls or artesian fountains in the parks (Figure 14).

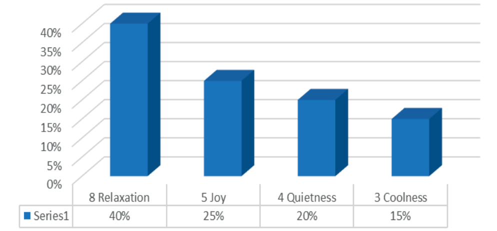

Figure 14. Effects of the fountains in the parks

CONCLUSIONS

The results showed that all the parks, historical gardens and squares studied contain the element of water. And the degree of response between females and males was there is an attraction to water (lakes, ponds, pools, and artesian wells). Water is perceived by the visitors in all designed form.

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GREEN WALL IMPACT ON BENEFICIAL INSECTS IN AN URBAN FRUIT ECOSYSTEM

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Abstract

Green walls are a component of urban green infrastructure and contribute to a range of ecosystem services including habitat provision for urban biodiversity, screening out aerial particulate matter, improving air quality, attenuating noise, and enhancing aesthetics of the cityscape. Insects can be the organic grower's best friend. Whether pollinators or predators, they will help manage pests and keep urban gardens healthy. The organic growers encourage flowers, along with trees, shrubs and water, to provide a valuable and diverse ecosystem.
The green wall was set in the experimental fruit field of the Faculty of Horticulture within USAMV Bucharest, at the border between the vineyard and the organic apple and cherry growing sector. Observations were made on the green wall impact on beneficial insects, as pollinators, hoverflies (Diptera), ladybirds (Coleoptera), parasitic wasps (Hymenoptera), butterflies and moths (Lepidoptera), lacewings (Neuroptera), ground beetles (Coleoptera, Carabidae). The preliminary results indicate that Lonicera japonica, Hedera helix, Mentha, Parthenocissus quinquefolia, Akebia Quinata,Campsis radicans they attract beneficial insects such as pollinators or aphid eaters.

Key words*: Green wall, wild flowers, organic apple, biodiversity, pollinators.*

INTRODUCTION

Urban green infrastructures have the potential to support biodiversity not only within its boundaries, but also nearby due to a landscape-mediated 'spill over' effect of energy, resources, and organisms across habitats. Such effect may be an important process for the persistence of pollinating insects, because allotment gardens often exhibit a rich abundance of flowering plants and thus a prolonged season for nectar supply, allotment gardens can support urban pollinators for long periods of time (Colding et al., 2006; Lin et al., 2015).

Plant-pollinator interactions have valuable impact in agricultural food production and provide indispensable ecosystem functions that support global biodiversity (Ollerton, 2017). It is estimated that 87.5% of flowering plants depend on animal pollinators for reproduction (Ollerton et al., 2011). In agriculture, 87% of the leading global food crops and 35% of global production volumes from crops are dependent upon animal pollination (Klein et al., 2007). Reports of declining pollinators from different parts of the world may constitute an urgent ecological challenge (Potts et al., 2016; Christmann, 2019). Pollination by native honeybees and other pollinating insects is very important for the farmers and the ecosystems. Pollinating insects are crucial for many naturals habitats and the production of a majority of food crops. More than 80% of European crop types, including many fruit and vegetables, depend directly on them (Gardi et al., 2015). However, numbers of pollinators and other beneficial insects have significantly declined across Europe in recent years (Gardi et al., 2003; Gardi, 2004; Gardi, 2010).

Understanding the correlations between the vegetation component of urban ecosystems and pollinating insects helps to understand the needs of current landscape design under new environmental policies, in order to benefit from biodiversity at socio-economic and cultural level within the capital city (Dragoș et al., 2018).

For decades, the conservation of biodiversity has been limited only to protected areas, which currently cover about 15% of the land surface (McDonald, 2008) and where biodiversity is protected from human threats. Biodiversity plays a fundamental role in the functioning of ecosystems and its ability to deliver long-term ecosystem services (Rosenfeld et al., 1998; Konijnendijk et al., 2000; Oberndorfer et al., 2007; Eysenbach, 2008).

Several beneficial insect species play an important role for urban gardens health. The most important group of beneficial insects are pollinators, biological control agents, and soil decomposers. Pollinators are insects which pollinate plants. Insect pollinators include honey bees, beetles, flies, ants, moths, butterflies, bumble bees, solitary bees, and wasps. Butterflies and moths are important pollinators of flowering plants in wild ecosystems and managed systems such as gardens and parks. Biological control of pests is part of an integrated pest management (IPM) strategy. It is the reduction of pest populations by natural enemies and typically involves an active human role. In fact, all insect species are also suppressed by naturally occurring organisms and environmental factors, with no human input. The natural enemies of insect pests, also known as biological control agents, include predators, parasitoids, and pathogens.

Growing seasonal food helps to supply with fresh produce for a healthy nutrition. Vegetables, fruits, and nuts are the life blood of a healthy human life. Beneficial insect species play major roles including as pollinators, decomposers, parasitoids & predators, and food to other taxa that contribute to a thriving garden's health and the environment. They are an integral part of urban ecosystem and landscape. Beneficial species richness and their optimum numbers in a garden can play a very important role in enhancing the crop's production levels. Beneficial insects can help suppress populations of serious pests in gardens, reducing plant damage and reducing the need for synthetic pesticides. This is partly true due to the use of non-selective pesticides, habitat destruction, reduction in native flowering plants (wild flowering trees & shrubs, bunch grasses, and cover crops) which provide nectar and pollen for the pollinators. For a healthy garden, conserving beneficial insect species is very important.

At European level, the cohesion policy for urban and peri-urban horticulture should be in line with the objectives of the European Green Deal, the Farm to Fork strategy and the Biodiversity Protection strategy (Mâcnea C.E. et al., 2021).

Proposing the development of urban gardens, green roofs and hydroponic systems, the importance is stressed of enriching urban biodiversity, better waste management with composting, the collection of rainwater and the improvement of air quality. The aim is to produce, process and consume food locally using available alternative plant protection products, with a low environmental footprint (Mir & March, 2017).

This short article emphasizes the best management practices which can help us to conserve beneficial insects in and around our gardens by implementing simple and easy agricultural practices.

MATERIALS AND METHODS

Green walls aim to identify new eco-innovative technologies useful in plant cultivation in urban horticultural ecosystems, by developing a green wall in a fruit orchard. The green wall was built in the experimental fruit growing field of the Faculty of Horticulture within USAMV of Bucharest, at the border between the vineyard and the organic apple and cherry sectors. The research intends to open a new path in urban horticulture, scientifically substantiating the effects of using such modular, mobile green walls near urban gardens. The behaviour of grass species and other annual/ perennial plants used in the construction of the green wall is monitored.

Green wall in urban environment are a component of urban green infrastructure and contribute to a range of ecosystem services:

- habitat provision for urban biodiversity;
- screening out aerial particulate matter ;
- improving air quality;
- attracting pollinating insects;
- aesthetics of the cityscape.

RESULTS AND DISCUSSIONS

In order to build the green wall, the following steps have been taken:

a. Installation of the experimental system to be used as a green wall in urban ecosystems, which included field soil preparation (Figure 1), stretching out the wire mesh as a support for the plants (Figure 2), installing the drip irrigation (Figure 3), planting the desired plant species (Figure 4).

b. Determining the composition of the floristic mixture, in order to maximize the useful effects of the green wall (Figure 5).

c. Observation on green wall impact on beneficial insects, as pollinators, hoverflies, parasitic wasps, butterflies and moths, lacewings, ground beetles (Figures 6, 7, 8, 9).

Figure 1. Field preparation

Figure 2. Stretching the wire mesh as a support for the plants

Figure 3. Testing the operation of the drip system

Figure 4. Planting plant species to test system functionalit

The studied biological material consists of: shrubs with flowers, climbing plants, grasses and cover crops, that provide nectar and pollen for pollinators.

Several beneficial insect species play an important role for urban garden health.

Observation on green wall impact on beneficial insects, as pollinators, hoverflies (*Diptera*), ladybirds (*Coleoptera)*, parasitic wasps (*Hymenoptera*), butterflies and moths, (*Lepidoptera*), lacewings (*Neuroptera)*, ground beetles (*Coleoptera, Carabidae*).

Natural enemies (parasitoids, predators, and pathogens) of pests balance their numbers so that there is no threat to the garden's productivity. The pollinators (honeybees, bumble bees, butterflies, hoverflies, etc.) play a very important role in pollinating crops and ornamental plants. Similarly, plant and animal decomposers (flies and beetles) play a vital role in recycling the organic matter in supporting the soil health. Indeed, natural enemies can help suppress populations of pest species, reduce crop damage and reduce the need for insecticides. In general, gardens with small sizes, selective & less pesticide use, and more non-crop habitats have the most natural enemies and are able to maintain pests below economically damaging threshold levels. In addition to simply reducing pest damage, native natural enemies can provide benefits such as reduction in the need for pesticides, reduction in the need to release non-native biological control agents, and supporting other facets of wildlife. The conservation of beneficial species is probably the most important and readily available biological control practice available to gardeners

Whether pollinators or predators, these insects\help manage pests and keep the gardens healthy. The organic grower encourages flowers, along with trees, shrubs and water, to provide a valuable and diverse ecosystem.

Figure 5. Determining the composition of the floristic mixture

Figure 6. Bee Hymenoptera - *Apidae, Bombus terrestris*

Figure 7. Butterfly *Papilio machaon*

Figure 8. Brown marmorated stink bug (*Halyomorpha halys*)

Figure 9. Ladybirds - *Coccinellidae* - *Coccinella septempunctata*

CONCLUSIONS

For healthy gardens, it is vital to have beneficial species diversity and their suitable population density for sustainability.

Beneficial species are adapted to the local environment and to the target pest(s), and their conservation is generally easy and cost-effective. With relatively little effort, the activity of the biological control agents can be observed. Lacewings, lady beetles, hoverfly larvae, big-eyed bugs, minute pirate bugs, and parasitized aphid mummies are almost always present in aphid colonies in the gardens. Fungus-infected adult flies are often common following periods of high humidity. The biological control is very important and need to be conserved and considered when making decisions based on the IPM strategies. In many instances the importance of natural enemies has not been adequately studied or does not become apparent until synthetic pesticide use is stopped or reduced. Often the best we can do is to recognize that these factors are present and minimize negative impacts on the beneficial species in gardens. If an insecticide is needed, every effort should be made to use bio pesticides which are selective in nature for gardening. In addition, growing native flowering plants (e.g., sweet alyssum, marigold, basil, sunflower, milkweed, and goldenrod) in and around the gardens for the beneficial species is critical for garden(s) productivity and sustainability.

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STUDY OF THE POSSIBILITIES FOR IMPROVING THE SOWING QUALITIES OF SEEDS AND THE VITALITY OF SEEDLINGS FROM *Cryptomeria japonica* Don. THROUGH PRE-SOWING TREATMENT WITH ULTRASOUND

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Abstract

Recently, in the production of planting material from ornamental species of trees and shrubs from seeds, more and more attention is paid to various physical methods aimed at increasing the germination and viability of seeds in difficult to propagate species. One of these methods is pre-sowing treatment of seeds with ultrasound. The present study was conducted to determine the effect of ultrasound on the germination and viability of seeds of ornamental species of Cryptomeria (Cryptomeria japonica D. Don.). The experiments were set in the laboratory of the Department of Horticulture, Agricultural University Plovdiv. The experiment with Cryptomeria was conducted from the end of February to the beginning of August. Variants with 5, 10, 15 and 20 minutes exposure were studied. Untreated seeds were used for control. Indicators related to the growth and phenological manifestations of plants were studied. It was found that the treatment of seeds with ultrasound affects the sowing qualities of the seeds of the studied specie. Ultrasound treatment has a beneficial effect on germination in Cryptomeria, with the optimal exposure being 20 minutes - so germination increases by 11% compared to control.

Key words*: Cryptomeria, seed propagation, ultrasound treatment, germination, ornamental plant.*

INTRODUCTION

Japanese cedar or cryptomeria (*Cryptomeria japonica* D. Don.), is a coniferous evergreen tree of the *Cupressaceae* family. The plant is indigenous to Japan and southern China and endemic to Japan. It is a widely used timber species in the Far East (Dirr, 1990). *Cryptomeria* is also considered a sacred tree in Japan with great landscape value (Creech, 1984). The plant that the Japanese call Sugi is an allogamous, wind-pollinated conifer species that is frequently used for commercial afforestation in Japan. Approximatelly 45% of all the man-made forests in Japan are composed of this species (Saito O., 2009; Forster E.J. et al., 2021). The species currently is gaining popularity not only in the northeastern United States, but also in the hot and humid southeast. The tree grows very well in rich, deep, acidic, moist soil but will tolerate heavy clay during dry and wet periods (Dirr, 1990; Tripp, 1993). The plant has a pyramidal crown, reaching 15 to 18 m in height, with descending branches. There are many cultivars of Japanese cedar that have a wide range of ornamental characteristics and uses. *Cryptomeria* has no major insect or disease problems (Baker J. and R. Jones, 1987), grows rapidly and makes an excellent evergreen screen (Dirr, 1990; Tripp, 1993). In addition, the emerald green foliage exhibits little dieback or discoloration (Tripp, 1993). This species is recommended as a replacement for Leyland cypress *Cupressocyparis leylandii* [(A.B. Jacks and Dallim.) Dallim. and A.B. Jacks] which has various disease and insect problems (Baker and Jones, 1987). In Bulgaria cryptomeria is used as an ornamental tree due to the beautiful shape of the crown, high cold resistance - withstands up to - 23^0C - and the relative tolerance to diseases and pests (Bogdanov B., 1984). Its extremely limited distribution in our country in public and private parks and gardens is due to the lack of supply of the plant in garden centers and nurseries. The main part of the plants offered in these sites are imported from abroad and the price is quite high. These problems stem from the lack of developed technology for the production of planting material for the conditions of Bulgaria. One of the main points in the technology for the production of planting material is the question of how to propagate. The different ways of propagation should be studied in detail and the one that has the highest efficiency and can be used for mass production of planting material in the conditions of the given area should be singled out. There is a lot of information in the specialized literature about the ways of *Cryptomeria* propagation. Special attention is paid to their advantages and disadvantages. Researchers studying vegetative propagation in *Cryptomeria* are unanimous that the most appropriate method is by stem cuttings. Jull L.G. et al. (1994) demonstrated that *Cryptomeria* stem cuttings can be rooted at all growth stages, but branch order from which cuttings are prepared is critical in achieving high rooting percentages. Although popularity and subsequent demand of *Cryptomeria* have increased, little research has been reported in journals on factors influencing propagation of the species and related cultivars by stem cuttings (Doran W.L., 1957; Waxman S., 1962; Henry P.H., F.A. Blazich, and L.E. Hinesley, 1992). For centuries, Japanese cedar has been propagated in Japan for forestry by seed and stem cuttings (Brix and van den Driessche, 1977; Ohba, 1993; Still S.M. and S. Zanon, 1991), and a body of practical knowledge on propagation and culture exists in the Far East. Some research concerning stem-cutting propagation of the species has been conducted in the United States. Information regarding factors, such as growth stage (timing) and auxin treatment, have been published (Black D.K., 1972; Bogdanov B., 1984); however, much of this information is conflicting and needs to be resolved (Dirr and Heuser, 1987; Lahiri, 1975; Nakayama, 1978; Orndorff, 1974). In addition, tree forms of Japanese cedar exhibit a well-defined branch order (branch position), which may influence rooting. For some conifers, branch order is an important factor affecting adventitious rooting (Black, 1972; Bogdanov, 1984; Miller et al., 1982) and warrants study in Japanese cedar.

Cryptomeria can also be propagated by seeds (Hartmann H.T. et al., 1990). In order to obtain more and better seeds Moriguchi et al. (2004); Moriguchi et al. (2005) and Moriguchi et al. (2007) recommend that in production of seedlings of *Cryptomeria* have to abidance three main factors, namely: first the pollen contamination rates can be >30%; this may be influenced by the surrounding forest plantations; second, the self-fertilization rate in conifer seed orchards has been determined to be generally less than 5% and third, the paternal contributions of constituted clones have been found to differ significantly from the expected equal contributions; this may be influenced by the number of male strobili. Itoo, S. (1984) compared the properties of *Cryptomeria* seeds obtained from a greenhouse (indoors) and outdoors and found that seeds obtained in the open place have better germination. On the other hand, the plants obtained from these seeds vary considerably in the main observed decorative characteristics. According to Mitsch J. (1975) and Russell R.S. (1977), a short period of statification is required for normal seed germination - from 4 to 6 weeks - at low positive temperatures, after which the seeds are ready for sowing. The issues of seed propagation in most cases are related to the study of the possibilities for increasing the germination and viability of the seeds through different ways of pre-sowing treatment. Ultrasound treatment is one of these ways (Awad T. et al., 2012; Chen G. et al., 2012; Miano A.C. et al., 2015) Ultrasound technology has been used to enhance the quality of seeds in many agricultural crops. The effect of ultrasound treatment on seed quality parameters such as germination and vigour has not been sufficiently studied (Yaldagard M., 2008). This physical way of affecting the qualities of the seeds is still poorly studied, and in cryptometry there is no information about it at all. This work investigated whether ultrasound technology affected the germination and vigour of *Cryptomeria* seeds.

MATERIALS AND METHODS

The study was conducted in the period 2020-2021. The experiments were set in the laboratory of the Department of Horticulture, Agricultural University - Plovdiv.

The seeds were collected from well-developed, healthy plants *Cryptomeria japonica* var. *japonica*. Syn: *Cupressus japonica* Thunberg ex Linnaeus f., Suppl. Pl. 421. 1782; *Taxodium japonicum* (Thunberg ex Linnaeus f.) Brongniart (Fu et al., 1999) from TP State

Hunting Farm Krichim. The collection took place in early November 2020. The seeds were separated by hand. Then lightly dried at room temperature. The ultrasonic treatment was carried out in an ultrasonic bath Ultrasonic water bath NAHITA, model 620/1, manufactured by AUXILAB, S.L., Spain with ultrasonic wave frequency 220-240 v - 50 Hz and ultrasonic power 35 W. The experiment was set on February 26, 2021 at the Agricultural University of Plovdiv. *Cryptomeria* seeds were immersed in water and wrapped in gauze and treated with ultrasound for different periods of time. 180 seeds were used, which were divided into 5 groups of 36 each - one non-treated control and 4 groups treated for 5 minutes, 10 minutes, 15 minutes and 20 minutes respectively. Immediately after sonification, the seeds were sown in a peat-pearlite mixture, each in a separate cell on a 180-cell tray. The seeds are then left outdoors and grown under natural conditions. Germination began in mid-April and ended in early May 2021.

The following indicators were studied: Growth rate of the stem; Phenological observations; Seed germination (%); Plant height (cm); Diameter of the stem at the base (mm); Number of internodes (pcs.).

The experiment was reported every week after the emergence of the first plant. The experiment ended 6 months after sowing (in August). All plants of each variant were analyzed. Seed germination was reported three months after sowing.

Figure 1. Seeds of *Cryptomeria japonica* D. Don.

The height of the stem was measured from the soil surface to the top of the plant in cm. The thickness of the stem was recorded at the base, above the root collar using a caliper in mm.

The obtained data were statistically processed by analysis of variance.

RESULTS AND DISCUSSIONS

Table 1 presents the results of studies of the seed quality of *Cryptomeria japonica.* The absolute mass of 1000 air-dried seeds was 1.279 g. This indicator is influenced by the size and fulfillment of the seeds, as well as by the climatic conditions in the growing area. These data are a criterion for the ecological plasticity of a species and its suitability for acclimatization to the conditions of an area. The vitality of the seeds determines their potential ability to germinate. In the seeds used in this experiment, the vitality was 63.94%. Germination is the most important indicator of the suitability of seeds to form normal sprouts under optimal conditions over a period of time. In *Cryptomeria japonica*, seed germination was determined at 7 days - 24.44% (Table 1).

Table 1. *Cryptomeria* seed quality

Absolute mass per 1000 seeds (g)	Vitality, %	Germination, %	Germination energy, %	Embryonic root length (cm)
1.279	63.94	24.44	73.18	1.07

Germination energy indicates the percentage of normally germinated seeds under optimum germination conditions within a period shorter than that for germination. In *Cryptomeria japonica*, the germination energy was determinated for 5 days and was 73.18%, indicating that the seeds germinate jointly and give strong and viable seedlings and, respectively, more viable plants. The average embryonic root length of *Cryptomeria japonica* is 1.07 cm, which is also evidence of seed viability and usability.

The rate of stem growth in seedlings is a criterion for the viability of plants and their successful future development. The growth rate of the *Cryptomeria* seedlings was monitored from the beginning of June to the beginning of August and is presented in Figure 2. From the data becomes clear that the plants from the control species have the lowest growth rate - their initial average height is 2.50 cm and the increase in height in the first week is only 0.16 cm. In the the next three weeks, the stem increase in height, or more precisely the differences in stem height for the seven-day reporting period, is minimal - 0.02 cm in the first week, up to 0.04 cm in the third week. Transplantation was carried out in early July. Very intersting is the fact that after this period of minimal growth, from mid-July, the plants not only do not grow, but also the height of their stems begins to decline intensively - 0.15 cm in the first week of July, 0, 37 cm in the second week of the same month and 0.1 cm the third week of July. The plants then die, most likely because of the contact of the root hairs with air oxygen during transplanting.

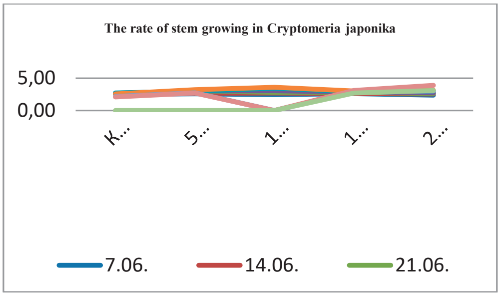

Figure 2. The rate of stem growing of *Cryptomeria japonica*

Seeds treated with different sonication exposure, give significantly taller and more viable plants, with more intense growth rate. The plants from the 5-minute exposure species have the most intensive growth rate at the end of June, with an increase in height for the seven-day period of 0.34 cm. Then follow periods of poor growth and height decrease. This process lasts until the end of July. In the beginning of August the plants die. 10 minutes of ultrasound exposure leads to the death of plants in the third week of July, but until then they are vital and growing at an intensed rate - from 0.1 cm in the third week of June to 0.25 cm in the last week of the same month. The plants with the most intensive growth rate are obtained from the seeds of 15 minutes sonication. In the third week of June - from 16 to 24 June they increase their height by an average of 0.2 cm. These plants are also characterized by an intensive growth rate in the second and fourth week of July - they grow by an average of 0.12 cm and 0.17 cm, respectively. This increase is due to the death of lower plants and an increase in average height. During the remaining weeks of the vegetation period the growth of the plants is 0.02 cm, 0.03 cm and 0.04 cm. in the second and fourth week of June and the first week of July. Here there is also a reducing in the stem height by 0.09 cm, in the third week of July. Plants received 20 minutes sonication are distinguished by the most intensive growth rate and the greatest vitality. Differences in stem height range from 0.08 cm in the third week of June to 0.87 cm in the last week of July.

Table 2. Phenological observations in *Cryptomeria*

Characteristics / Variants	Germination			
	started		masse	
	date	no. days after sowing	date	no. days after sowing
к	19.04	52	26.04	59
5	21.04	54	26.04	59
10	19.04	52	26.04	59

Table 3. Phenological observations in *Cryptomeria*

Characteristics / Variants	First stem branch			
	started		masse	
	date	no. days after sowing	date	no. days after sowing
к	28.06	122	28.06	122
5	28.06	122	28.06	122
10	21.06	115	28.06	122
15	28.06	122	28.06	122
20	05.07	129	05.07	129

Phenological observations on *Cryptomeria* seedlings are presented in Table 2. The data show that pre-sowing sonication did not significantly affect the onset of germination of cryptomeria seeds - all experimental variants except the 5-minute variant germinate 52 days after sowing on 19.04. The same applies to the mass germination of seeds, which occurs 7 days after beginning, on 26.04. or 59 days after sowing, both for treated and untreated seeds. The first stem branching occurs in plants attained from 10 minutes sonication seeds, on 21.06. or 115 days after sowing (Table 3). Next are the plants attained from seeds treated with 5 and 15 minutes sonication, which form the first stem branch 7 days later on 28.06. or 122 days after sowing.

Table 4. Phenological observations in *Cryptomeria*

Characteristics / Variants	Second stem branch			
	started		masse	
	date	no. days after sowing	date	no. days after sowing
к	-	-	-	-
5	-	-	-	-
10	05.07	129	05.07	129
15	-	-	-	-
20	-	-	-	-

Very interesting is the fact that plants growing from untreated seeds form the first stem branch within the same period. At the latest, the first stem branch is formed by the plants growing by 20 minutes seeds sonication - on 5.07., or 129 days after sowing. The second stem branch is formed only by plants from 10 minutes seeds sonication, on 5.07., 129 days after sowing, or 14 days after the appearance of the first stem branch (Table 4). The data illustrating how many of the plants form stem branches are very interesting. The highest percentage of plants forming stem branches are in the variant of 15 minutes sonication - 8.33%. Next are the plants from the variant of 10 minutes sonication - 5.55%. Plants from the variant of 5 and 20 minutes sonication, as well as untreated ones have the same percentage of plants that formed stem branches - 2.77%.

Table 5. Characteristics of the stem in *Cryptomeria japonica*

Characteristics / Variants	Stem high, cm	Number of stem branches, nb	plants with stem branches,%
к	2.72	1	2.77
5	3.23	1	2.77
10	3.60	2	5.55
15	3.10	1	8.33
20	3.90	1	2.77

In Table 5 are presenting the data regarding the height of the stem. The highest average height is reached by plants from seeds of 20 minutes sonication - 3.90 cm. The plants from seeds of 10 minutes sonication are 3.60 cm or 0.3 cm lower. followed by plants from seeds of 5 and 15 minutes, sonication respectively, 3.23 cm and 3.10 cm. The plants from the untreated variant have the lowest stem height - 2.72 cm, or 1.18 cm or 30.25% lower than the variant with the tallest plants.

CONCLUSIONS

1. Plants growing from 20 minutes seeds sonication are characterized by the most intensive and uniform growth rate.
2. Germination of *Cryptomeria* seeds is not affected by pre-sowing sonication. Germination, both initial and mass, is not affected by the duration of sonication.
3. *Cryptomeria* was found to form first and second stem branches after 10 minutes sonication. However, most plants form stem branches after 15 minutes sonication.
4. Pre-sowing 20 minutes sonication resulted in *Cryptomeria* seedlings with an average height of 3.90 cm.
5. The conclusion shows that 10 minutes pre-sowing sonication results in obtaining first and second stem branches; the duration of 15 minutes - results in obtaining the highest plant percentage of stem branches - 8.33%, and 20 minutes sonication resulted in obtaining the highest plants - 3.90 cm. We can conclude that pre-sowing sonication of *Cryptomeria* seeds is recommended in seed propagation of this species. According Aladjadjian A. (2002) the effect of under ultrasonic action is due to the mechanical energy of the ultrasonic wave which transformed into the kinetic energy of the molecules in the seed. This energy is redistributed between the molecules and transformed into chemical energy, increasing the activity of chemicals in the seeds, as a result of which accelerates their growth and development. However, other indicators should be included in further studies in order to specify the exact duration of treatment.

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EVOLUTION OF THE MARINE DUNES OF AGIGEA IN THE CONTEXT OF BIODIVERSITY CONSERVATION

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Abstract

The paper aims to present the evolution of coastal landscape, as well as the defining component of it, sand dunes. The current situation of sand dunes, their impact on ecosystems, the threats and difficulties they face, along with the importance of their conservation were analyzed.
The introduction is based on the statistical data gathered from numerous international reports regarding the environment, specifically the sustainability of coastal and marine zones. The information was processed and compared during the materials and methods phase to the analyzed site, which is unique for Romania, hosting the only natural reserve of marine dunes in the country, the protected area "Marine Dunes of Agigea". The analysis focused on the physical environment, land use, history and heritage, flora and fauna. As for the results and discussions, a synthesis of the analysis and a diagnosis, vision and mission were elaborated, while the conclusion of the paper is summing up a sustainable landscape planning and management strategy.

Key words*: coastal ecosystems, green infrastructure, natural habitat, protected areas, sand dunes.*

INTRODUCTION

Coastal areas are considered to be zones where land and sea influence, meet and interact. The coastal strip varies depending on the nature of the environment, the marine and terrestrial interactions of coastal processes and management needs (European Environment Agency, 1999).
The dunes are an integral part of the coastal environment. Not only do they provide a reserve of sand used by waves during storms, but they form the basis of important ecosystems, supporting valuable plant and animal communities. On sandy shores, in addition to limiting the intrusion of waves to land, coastal dunes act as a barrier to flooding and ensure an important morphological and ecological transition from the marine to the terrestrial environment (Kidd et al., 2001).
Coastal areas occupy less than 15% of the Earth's surface, but they are home to more than 60% of the world's population. Since 1992, at the United Nations Conference on Environment and Development, it has been found that if this trend continues, by 2025 it could be that 75% of the population will live in coastal areas. Most of the potential global coastal ecosystems threatened by unsustainable development (Figure 1) are located in the northern temperate zone and the northern equatorial zones, with Europe having 86% of its coastal areas at either high or moderate risk (Bryant et al., 1995).

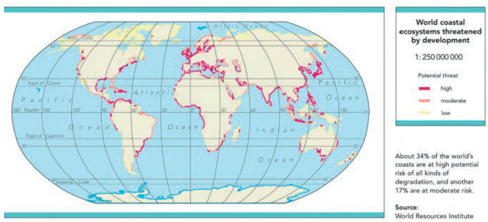

Figure 1. World coastal ecosystems threatened by development. Source: European Environment Agency, Coastal and marine zones, 1999

According to this image and the same source, about 34% of the world's coastal areas have a high-risk potential for all types of degradation, and another 17% of coastal areas have a moderate risk.
Coastal areas are not static. They can change shape quickly, and coastal erosion, due to human activities or natural causes, is a common phenomenon.
In the European Union 25% of the coast is subject to erosion, while 50% is stable, 15% is

degraded, and for the remaining 10% evolution is unknown (Corine, 1998).

In Europe alone, a net loss of 25% of coastal dunes has been reported since 1900, and about 55% of the remaining coastal dune area has lost its natural character (Delbaere, 1998).

The population density and land cover in coastal areas in Europe and Mediterranean Sea are shown below (Figure 2).

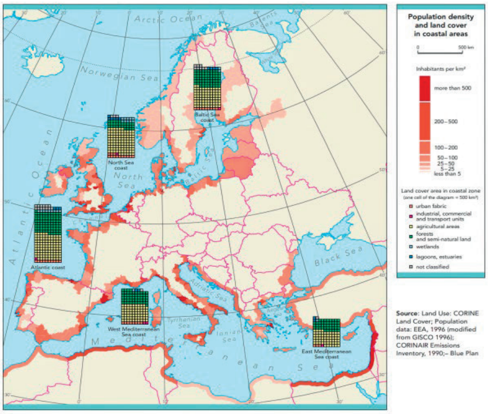

Figure 2. Population density and land cover in coastal areas. Source: European Environment Agency, Coastal and marine zones, 1999

In Israel, for example, about 70% of the population is located in a 1 to 3 km strip along the 190 km Mediterranean coastline.

Beneath (Figure 3) the main factors affecting coastal and marine ecosystems are presented.

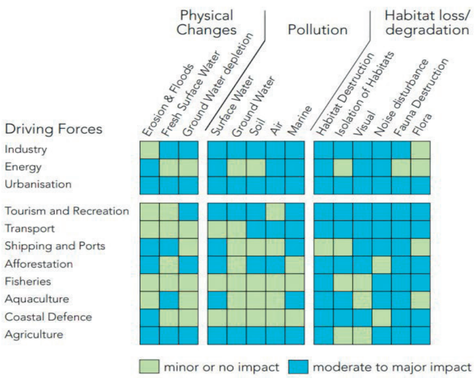

Figure 3. The driving sources and their impacts in coastal areas. Source: European Environment Agency, Coastal and marine zones, 1999

In this context, the paper presents an analysis of the Marine Dunes of Agigea, a protected natural area established in 1939, hosted by the Marine Biological Station "Prof. Dr. Ioan Borcea" which was founded in 1926, in order to put into evidence, the evolution of the site.

The main objective is the conservation of habitats and protected areas, being imperative to protect the natural area of sand dunes. Another goal of the project is to incorporate the history of the site into a sustainable solution that maximizes the existing potential.

The protected natural area "Marine Dunes of Agigea" is the only marine dune reservation in Romania. It is part of the Natura 2000 network of protected areas as a site of community importance with the code ROSCI0073.

The protected site is part of category IV of the International Union for Conservation of Nature, meaning protected areas that are subject to active management interventions for the protection and conservation of natural habitats, flora and fauna characteristics.

MATERIALS AND METHODS

The analysis is focused on assessing with the intervention site and understanding it, so that the solution responds in a clear and concise way to the current situation.

The study was based on several methods of theoretical research, such as consultation, information, documentation and synthesis of bibliography and specialized materials, but also practical research through field visits, field photographs and on-site observations.

The aim of the paper is to identify and solve the needs and issues that the studied area faces. The proposed solution seeks to transform the station into an attractive spot, dedicated to both locals and tourists, especially during the summer, while maintaining the priority research function.

The physical environment analysis (Figure 4), was necessary for the study of winds and currents in the process of formation and stabilization of dunes.

As dunes form as a consequence of the deposition of sand by the waves, its transport carried by the wind inland and stabilized by vegetation, therefore the direction and speed of the wind play a key role, and the dunes recede and grow as they change (Mascarenhas, 1998).

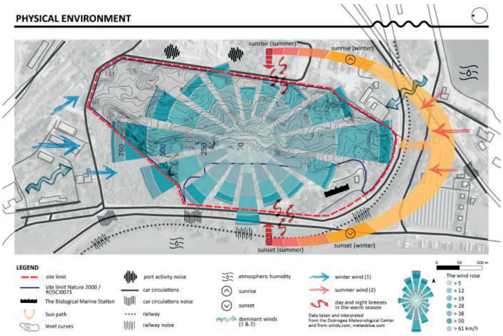

Figure 4. The physical environment analysis

As for the Marine Dunes of Agigea, with the construction of the Port of Constanța South - Agigea, between 1967-1984, the sand dunes were separated from the sea by a concrete road, a railway and a road bridge leading to the ferry terminal. Currently, the site is at a distance of about 200 meters from the sea shore, which over time has led to a series of changes in ecological, microclimatic and edaphic conditions, followed by significant changes in the composition and structure of the vegetation (Management Plan ROSCI0073, 2011).

The historical analysis (Figure 5) reveals that in 1926 was established the Marine Zoological Station "King Ferdinand I" by a royal decree. The site was spread over an area of 9 ha with 1 km of sea, in the immediate vicinity of the coast, an area made up of "land with the sands (dunes) on which during the war some barracks were built by the Germans, now almost complete ruin" (Borcea, 1929).

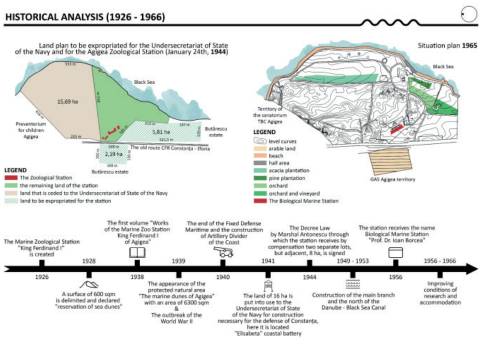

Figure 5. The historical analysis, 1926-1966

At the initiative of Prof. Dr. Ioan Borcea, director of the Zoological Station, a protection zone with an area of 6300 m² has been delimited since 1928 and is declared a "marine dune reservation", which becomes a Nature Monument by Decision of the Scientific Bureau of the Commission "Flora of Romania", published in the Journal of the Council of Ministers no. 142 in 1939.

In the same year, 1939, the protected natural area "Marine Dunes of Agigea" appears, but as a result of the outbreak of the Second World War, Marshal Ion Antonescu decides to fortify the coastal defensive line and part of the site is put into use by the Secretariat of State of the Navy that places here the coastal battery "Elisabeta".

On January 24th 1944, the law decrees signed by Marshal Antonescu, the "State Leader" of Romania, were published in the Official Gazette of Romania, which decides the legal frameworks of an event fulfilled from 1940-1941, when the almost 16 hectares were used for constructions necessary for the defense of Constanța. It is specified that the Zoological Station receives by compensation two separate, but adjacent, plots of land of 8 hectares from the close estate named "Butărescu", while the Ministry of Defense bears the costs of compensation.

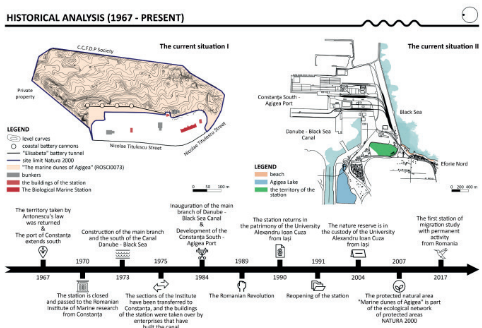

Figure 6. The historical analysis, 1966-present

Research increased in the following decades (Figure 6), but in 1970 the station ceased teaching and went on to research, library and museum under the auspices of the Institute of Marine Research until 1990. During this time, the dune reserve suffered severe damage caused by the construction near of some objectives such as: the Constanța South - Agigea Port, the Danube - Black Sea Canal, the road that separates it from the Black Sea coast, but also by increasing the noise level, due to grazing, the invasion of steppe and ruderal

species, unlimited access of vehicles on their way to new construction areas (Management Plan ROSCI0073, 2011).
Since June 28th 1990, the reservation has been under the tutelage of the Marine Biological Station "Prof. Dr. Ioan Borcea", administered by the "Alexandru Ioan Cuza" University of Iași. From March 2004, the university exercised the custody attributions of the protected natural area (Management Plan ROSCI0073, 2011).

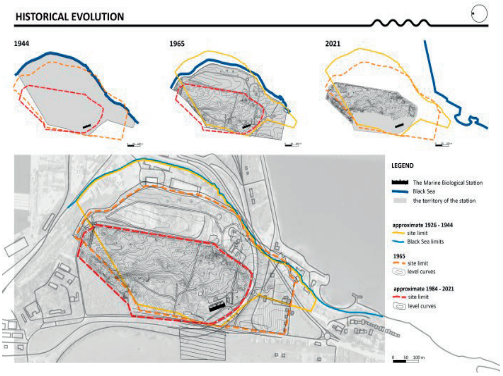

Figure 7. The historical evolution analysis

The historical evolution analysis (Figure 7) shows the administrative boundaries of three different eras of the station. It is noticeable the way in which the site has undergone territorial changes, shrinking significantly and moving considerably away from the Black Sea with the industrial and urban development.
Regarding the flora of the site, in the reservetion, there are two types of priority natural habitats according to the Natura 2000 classification. They were identified based on the 10 characteristic plant associations (Figure 8).

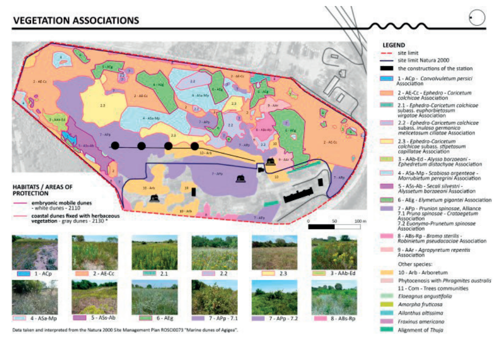

Figure 8. Plant associations

The embryonic mobile dunes (white dunes) and the coastal dunes fixed with herbaceous vegetation (gray dunes) are considered to be of great conservative importance because they shelter communities of rare or very rare plants at the European Community level and national level (Management Plan ROSCI0073, 2011).
Of the associations, only 6 are specific to coastal sands, the others being woody plants and ruderal associations. To the 10 plant associations is added an atypical phytocoenosis with *Phragmites australis* located in a depressed area in the northwest corner of the reserve (Management Plan ROSCI0073, 2011).
The mixed reservation, botanical and zoological, currently houses over 120 species of vascular plants. It is famous especially due to the large local populations of some rare species in Romania, such as: *Alyssum borzaeanum*, *Ephedra distachya*, *Convolvulus persicus*. Other floristic rarities present in the reservation area are: *Silene thymifolia*, *Leymus racemosus* subsp. *sabulosus*, *Dianthus leptopetalus*, *Astragalus varius*, *Salvia aethiopis*, *Seseli campestre*, *Echinops ritro* subsp. *ruthenicus*, etc. (Făgăraș et al., 2008).
Of the 241 taxa registered in the floristic inventory of the protected natural area "Marine Dunes of Agigea" we analyzed in a table 152 species (Figure 9, Figure 10, Figure 11), including planted specimens, located in the administrative space, outside the dune reservation.

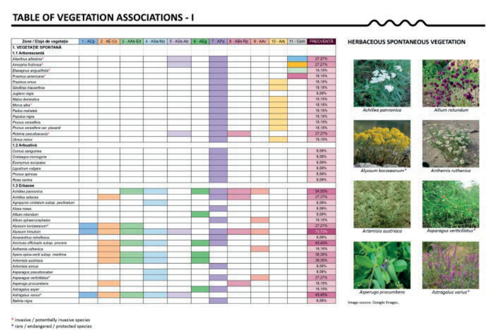

Figure 9. Table I - Plant associations

To the 10 associations were added other 2 components, the arboretum and the wood communities. In the table, we assigned to each column the color from the plant association (Figure 8) in order to see if the species are found in several associations.

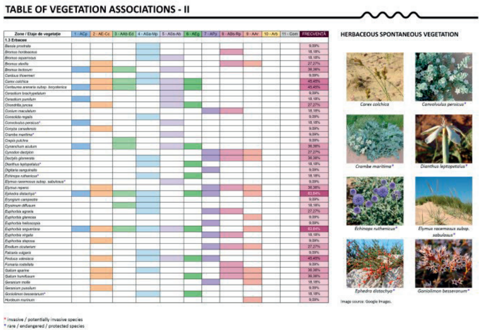

Figure 10. Table II - Plant associations

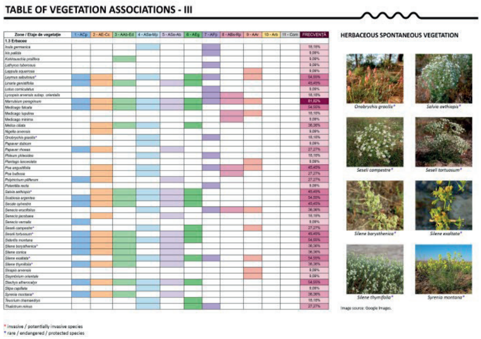

Figure 11. Table III - Plant associations

Moreover, we were able to determine the frequency of the species depending on the number of plant associations in which they are found (Figure 12).

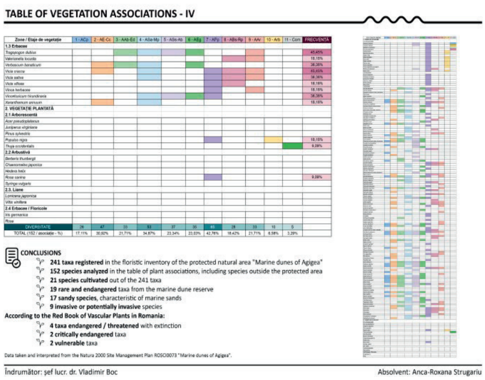

Figure 12. Table IV - Plant associations

As for the fauna of the site, according to the faunal inventory, 442 registered species were reported, of which 353 species are invertebrates and 89 species are vertebrates.

RESULTS AND DISCUSSIONS

The synthesis of the analysis (Figure 13) resulted in the presence of the research point with a vast environmental diversity, but not sufficiently exploited. The studied site is located on the outskirts of Agigea, in an industrial area and not frequented by tourists due to the vicinity of the Constanța South - Agigea Port. In fact, accessibility in the area is reduced and direct access to the Marine Biological Station "Prof. Dr. Ioan Borcea" is difficult. The station is not valued, and its history is not known to the general public.

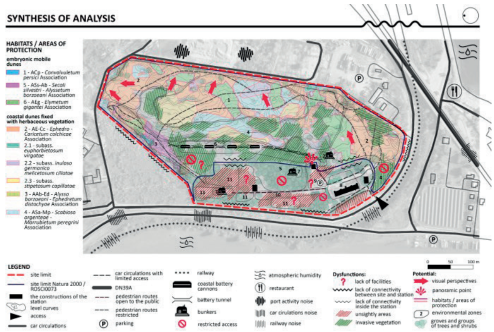

Figure 13. The synthesis of analysis

The spaces at the site level are not arranged and not coherent from a functional organizational point of view. At the ecological level, there are many invasive and potentially invasive specimens that are spreading in conservation areas. From a functional point of view, many areas within the station are tough to access or even have restricted access. There is a physical barrier between the site and the station, represented by the concrete fence that surrounds the station.

The lack of connectivity between the site and the station, as well as the lack of connectivity inside the station, along with the lack of facilities, unsightly areas and the expansion of invasive vegetation, are some of the dysfunctions reported.

However, the potential of the site is high, with many visual perspectives to the port, industrial landscape, which can be interpreted as a type of tourism. Also, Villa Turn, the building made by Marshal Antonescu during the Second World War is a panoramic point with direct views on

habitats and protection areas, respectively dunes, but also on plant associations.
As a diagnosis (Figure 14), the station is a unique natural setting, not integrated in the territorial context. It represents a research point that offers favorable conditions to studies, with floristic and faunal diversity. From the functional point of view, it is noticeable the reduced accessibility and lack of diversity of facilities and functions. Aesthetically, multiple environments are present, being predominant those with natural character. Economically, there is a high potential for tourism due to the location between Constanța and Eforie Nord.

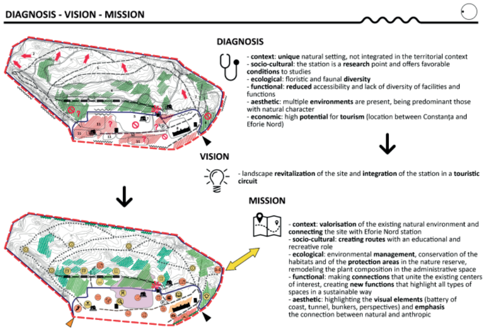

Figure 14. Diagnosis - Vision - Mission

The vision involves the landscape revitalization of the site and the integration of the station in a touristic circuit. The mission is to enhance the existing natural environment and to connect the site with Eforie Nord. Another objective is to create routes with an educational and recreational role. Ecologically, an environment management is recommended, for the conservation of the habitats and for the protection areas in the nature reserve, remodelling the vegetation structure/planting design in the administrative space. Functionally, the project aims to make connections between the existing centres of interest, creating new functions that highlight all types of spaces in a sustainable way. Aesthetically, is it important to call attention to the visual elements (battery of coast, tunnel, bunkers, perspectives).
The macro strategy (Figure 15) illustrates the improvement of connectivity by proposing a bicycle track from the train station, Agigea Ecluză, to the station. This route can also be travelled on a pedestrian alley. On the route there is proposed a street tree alignment, but also various stopping points and bike rental spaces. This route is also addressed to the locals, therefore solving the urban fragmentation of the railway line, which seems to separate the "village hearth" from the new subdivisions from Lake Agigea, near the opening of the Danube - Black Sea Canal in the sea, creating two different entities.

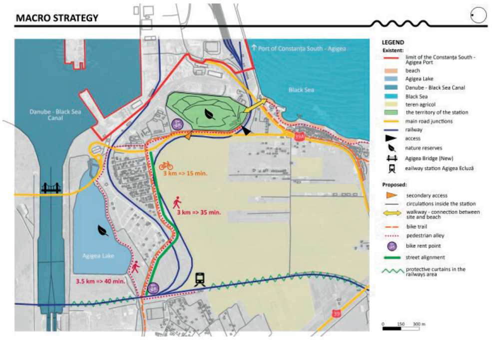

Figure 15. Macro strategy

The strategy at the level of the research station (Figure 16), aims to reorganize the functions, to correlate them with each other.

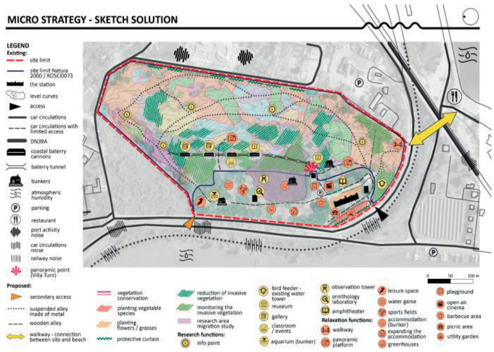

Figure 16. Micro strategy

A secondary access is proposed in the northwest corner of the site, where there are currently several concrete spaces, which will be used and revitalized. In the southern part of the site, a walkway is proposed, a connection between the station and the beach, where there are also two restaurants, and this intervention could facilitate pedestrian access between the two spaces.
The walkway will be located above the railway and below the national road DN39A, the road bridge leading to the ferry terminal.

The general solution (Figure 17) presents a composition designed to interconnect existing points of interest with the proposed functions and interventions, as well as with primary and secondary access.

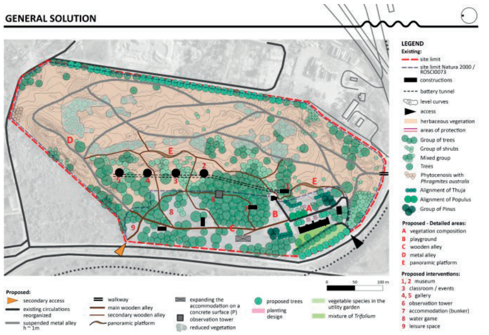

Figure 17. General solution

The vegetal composition is preserved in general, with the mention that the invasive or potentially invasive trees and shrubs on the territory of the dunes in the reserve are reduced, and the surrounding woody vegetation is monitored.

The proposed interventions are meant to highlight the visual elements, but also to emphasize the connection between natural and anthropic. Another proposal is the revitalization of the coastal battery "Elisabeta" by reorganizing the tunnel and its cannons. This space will consist of a museum, a lecture or event room, as well as a gallery, hosting various exhibitions.

In the reservation is encouraged the expansion of existing sandy plant associations in order to conserve the existing habitats and protected areas.

In front of the main building, a planting design concept is proposed (Figure 18).

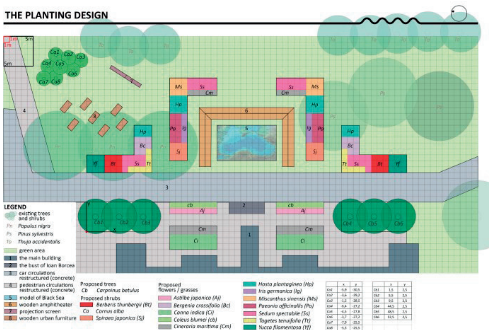

Figure 18. The planting design

In this area, there is the model of the Black Sea, used for teaching purposes. Consequently, we suggest creating an amphitheater around it, including an outdoor cinema area with urban furniture.

It is also shown the seasonal variation (Figure 19) of the decorative features of the proposed plants. They were chosen taking into account the flowering period, the period of decoration, habitus, color, perfume.

The playground (Figure 20) is proposed in front of the Villa, in a space where research does not prevail. It is made entirely of wood, to make a connection of children with nature.

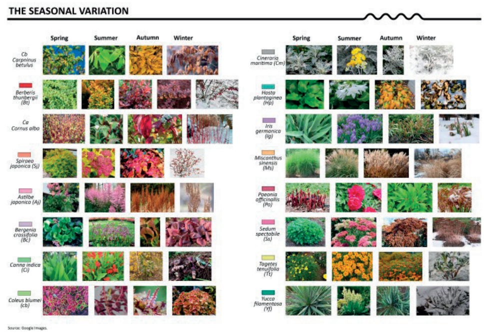

Figure 19. The seasonal variation

Figure 20. Playground

We proposed two panoramic platforms (Figure 21) of organic shapes, made of wood, which will be viewpoints. These platforms are connected to the wooden and metal alleys. The spaces are characterized by the research function, along with the role of walking, contemplation, and relaxation.

Figure 21. Panoramic platforms

The connection between the administrative space and the natural reserve is made through wooden alleys (Figure 22), slightly raised above the ground level, but also through the already existing concrete spaces, restructured and reintegrated within the solution.

Figure 22. Wooden alleys

Within the protected natural area "Marine Dunes of Agigea", interventions are minimal in order to not endanger habitats and protection areas, with more emphasis on their conservation and the application of environmental management measures. The access to the reservation is made on a suspended alley (Figure 23), at about 1 meter high, made exclusively of metal grid, so that the wind can continue to circulate in normal parameters, without any opaque surface that would complicate this essential aspect. We mention the fact that we started from the initial structure of the existing paths, but we created a firmer and more sinuous route. The alley has information points, located at the intersection of associations or where flora or fauna rarities are present.

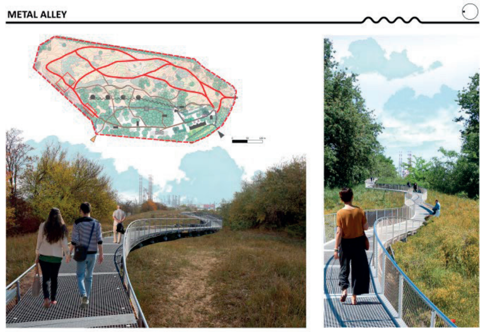

Figure 23. The metal alley in the reservation

CONCLUSIONS

As a conclusion and recommendation, we created a simulation, a staging of works (Figure 24), in case of starting the project in 2022.

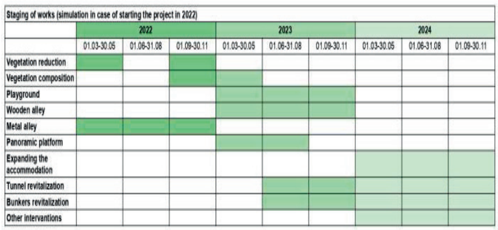

Figure 24. Staging of works

Regarding the protected natural area "Marine dunes of Agigea", it is recommended to monitor the dune habitats in order to be signaled in advance of any major change in the structure and composition of flora and fauna.

From an ecological point of view, it is recommended to eliminate or reduce, followed by monitoring, invasive and potentially invasive species from the marine dune reserve. Removing them could facilitate the expansion of protected areas and significantly improve their conservation value, as these species contribute greatly to the sanding and humidification of the sands, the stabilization of the dunes and their ruderalisation. Another recommendation is to replace the concrete wall with a type of fence that allows better airflow (Management Plan ROSCI0073, 2011).

The implementation of conservation management measures should lead to an improvement in the current conservation status of dune habitats and representative species, by eliminating threats and creating conditions for the expansion of sand dunes, specific to marine dunes, for which the nature reserve has been established and declared a Natura 2000 site (Management Plan ROSCI0073, 2011).

As a spatial structure, marking and respecting the visiting routes proposed in the solution could limit the access of a large number of people, but also avoid their trampling of dunes, specific vegetation, floristic rarities, and disturbance of wildlife species in the nature reserve.

Last but not least, the connection with the neighborhoods is a priority, as the station is already located on the outskirts of Agigea. The integration of the station in the urban regulation plans would reduce its isolation and would facilitate its development as a touristic and recreational point.

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MISCELLANEOUS

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URBAN GREEN AREAS USING SUSTAINABLE AQUAPONICS

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Abstract

Aquaponic technologies and systems are considered growing industries in many countries, with great environmental and socio-economic benefits. Aquaponics uses a system that combines two technologies such as aquaculture specialized in fish farming, and hydroponics, which studies the cultivation of plants. Climate change and the impact of global warming on the ecosystem, declining aquatic animal stocks and the response to growing demand are turning points in our century and in the era of sustainability. Through a phytotron type system, different solutions can be implemented to collect performance indices, impose plant watering criteria (follow the parameters of the aquaponic system such as temperature, water level, humidity and others) using cloud data storage support other facilities offered by the Internet of Things (IoT) concept. Therefore, this study focuses on the sustainable use of aquaponics as a platform for all-in-one solutions covering technical, managerial, socio-economic, institutional and environmental measures in the implementation requirements taking into account the possibility of aquaponics systems to have on the side of electricity a certain energy autonomy if these aspects are considered from the implementation of the aquaponic system.

Key words*: aquaponics, durability, energy storage, sustainability, urban agriculture.*

INTRODUCTION

The main advantage of aquaponic systems is the possibility of installation in various environments, requiring a relatively small investment to create such a system. It offers remarkable landscapes and brings a beneficial change to the environment and contributes massively in reducing the CO_2 footprint. Also, the materials used in the development of these aquaponic systems do not involve an expensive recycling technology, noting that in a sustainable way they are considered to have met most of the criteria (Simke, 2020). Figure 1 ilustrates an aquaponics system where the plants are placed in floating plates without soil in the fish ponds.

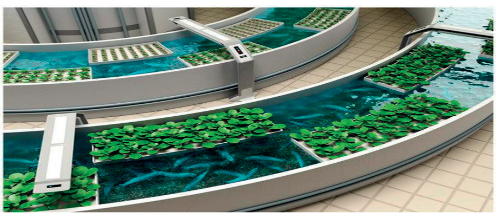

Figure 1. Aquaponics systems with water from fish tanks: the plants are placed in floating plates without soil (Simke, 2020)

Several sustainable development goals (SDGs) were highlighted for the study of aquaponics, mentioning 5 principles from the sustainable development goals set by the United Nations in 2015:
- SDG2: Zero hunger;
- SDG7: Clean energy for everyone;
- SDG8: Both job satisfaction and economic growth;
- SDG12: Responsibility to create, responsibility to use;
- SDG14: Protect the richness of the sea, life underwater.

Aquaponic systems have a smaller effect on the ecosystem because it has the capacity for self-regulation, also there is no need to use pesticides, herbicides, or fertilizers. According to Simke (2020), 70% of water consumed on a global scale in feed production could be saved by up to 30-70% if efficient irrigation systems were used and up to 90% if systems used aquaponics. The above shows the potential of the respective aquaponic systems and their high adaptability to the environment. According to Naylor et al. (2020), the aquatic field, respectively freshwater fish have contributed a lot to food security compared to any other aquaponic production in the last 200 years.

MATERIALS AND METHODS

We studied studies published in the 2010-2021 period, to extract the main ideas on sustainability, design, energy production using compressed air and works about aquaponic systems. Following the consultation of the specialized published studies on the fields of interest, we could ascertain a special compatibility between the electricity production systems with the help of compressed air and the aquaponic systems. In order to outline an overall idea and to create a connection between several fields that apparently have no connection, solutions were sought for the energy independence of aquaponic systems, the sustainability part was consulted, respectively the optimization of energy consumption. Looking for different solutions, different concepts were encountered, approaching the concept of storing renewable energy in potential energy in the form of compressed air which subsequently maintained the movement of a device for producing electricity adding extra strength due to the floating/ buoyancy principle explained in the following sections.

RESULTS AND DISCUSSIONS

Research on aquaponic systems has begun to increase from 2010 to the present, so the more researched, the newer connections can be created in various fields of scientific research. In the following were taken into account factors related to the life cycle, important parameters in a system as well as the possibility of capitalizing on losses in a system for producing electricity using compressed air.

Aquaponics/Aquaculture Life Cycle Sustainability

In order to implement an aquaponic system from the point of view of the life cycle, it must follow the paths of a project, namely the process of conceptualization, initiation, planning, implementation, monitoring and closing the steps or closing the project where we put the conclusions and determine if the project closed as expected or not. Thus, all the steps mentioned above lay the foundations of the life cycle of the project.

If we want to ensure the success of the project started and increase the chances of control and the central management system that is based on a technical expertise such as aquaculture and aquaponics, we must consider to define well and in a timely manner the main factors. and their proper selection. According to them, the importance of the management plan and the ranking of the implementation steps becomes obvious. Within an aquaponic system, the harmonization and distinction of the different types of life cycle must be delimited.

The life cycle of a technical project can be classified into three main categories:

1. Predictive or fully based on the plan;
2. Incremental or process-based iterative;
3. Agile or adaptive change-oriented.

Mostly, the types of life cycles are differentiated by the reference stages or the sequential implementation of the phases (for example: phases carried out by overlapping, in fraction or in parallel, etc.), the level of definition of the scope and the implementation of the scope (e.g. project level, phase level, reference level, iteration level, etc.), project types and complexity and level of stakeholder involvement. The first step is the design and sustainable modelling of an aquaponics project and is done with the realization of an adequate analysis of the life cycle. To explore this proposal, project life cycle types are taken from the PMBOK Project Management Institute, detailed in Table 1.

Table 1. Summary of distinctive factors for project life cycle types (Rowley, 2013; PMI, 2017)

	Predictive	Iterative	Adaptive
Conceptual Chaude	Plan-Driven	Process-Driven	Change-Driven
Phases implementation	Sequential, overlapping	Sequential overlapping	Sequential overlapping, parallel
Scope definition	At the beginning of project	At the beginning of each phase	At the beginning of phase or iteration
Scope description	Covers all project phases	Only for each phase	Only for each phase or iteration
Detailed Planning	At the beginning of project OR rolling wave	Only for each phase	Only for each phase or iteration
Application purpose	Well-defined projects or products	Large and complex projects	Product is not well understood, rapidly changing environments
Stakeholders' involvement	Beginning, when scope changes, and project end	Periodic	Continuous

According to the Project Management Institute report, adaptive or agile life cycles are mainly applied in most information technology IT projects.

Pillars of durability and efficiency of aquaponics

According to FAO (2020) the consumption demand for food products resulting from aquaculture results in an average annual consumption of 20.5 kg per capita with a slight wet growth fish products being estimated at $ 401 billion.

China remained the main producer in 2018, with a total production of 35% as can be seen in Figure 2.

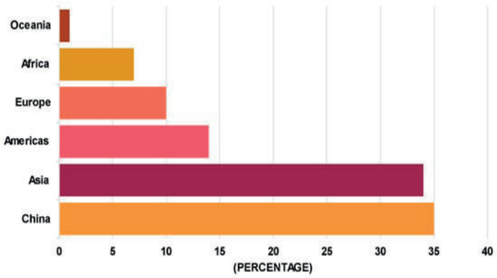

Figure 2. World aquaculture production (Simke, 2020)

Aquaponics require:

- Small investment at startup;
- Low operating cost, including minimal supervision;
- Low maintenance and management costs.

Overall, due to the high demand for trained human resources in various fields, capital investments and treatment systems are considered expensive.

The comparative analysis of the filter performance was done with an average time with certain types of fish by measuring the effluent criteria based on water quality in litres per hour.

For this process, these parameters can be assessed using an environmental quality objective (EQO) approach, technology-based approach or a combination of these approaches for different types of filters, comparing performance according to the required parameters (Table 2) (Ragas et al., 2005; Jegatheesan et al., 2007; Masabni & Sink, 2021).

Table 2. Parameters of aquaponics to produce aquatic products

Parameter	Unit
Dissolved oxygen (DO)	
Ammonia (NH_3)	
Nitrite (NO_2^-)	
Nitrate (NO_3^-)	mg/L
Phosphate ($P0_4^{3-}$)	
Ferric chloride ($FeCl_3$) (for flocculation)	
Water Hardness	
Turbidity (NTU)	
Conductivity	(µS/cm)
pH	
Water temperature	°C/F

IoT and AI technologies present in Aquaponic systems

In general, Internet of Things (IoT) technologies and artificial intelligence have come in the context of facilitating human interaction, providing useful information and mechanisms to increase the comfort of the human factor, especially in urban areas, contributing to the idea of smart cities. But this aspect has expanded in other areas as well, becoming a tendency to look for the respective comfort to be able to easily follow the parameters of the processes. Among the proposed solutions for managing water consumption in agriculture is aquaponics. Aquaponic brings together plant growth and cultivation technologies as well as fish farming technologies by creating a closed-loop ecological system that relies largely on aerobic bacteria that facilitate the transformation of ammonia, taken from food scraps and fish droppings, into substances plant nutrients such as nitrates that are taken up by plants leaving filtered water that returns back into the fish pond. Thus we can speak of a mixed environmental monitoring solution consisting of two processes such as aquaponics for fish farming and hydroponics for plant cultivation. According to FAO 2014, aquaponic systems can produce over 560 fish species, resulting in the ability to adaptation and a diversified variety for both fish and vegetable crops (Carlos et al., 2018). In addition to the aspects mentioned above, we can also list the suitability of these systems for the reconversion of buildings in urban areas due to the high degree of flexibility and the possibility of growing plants without soil bringing very inseminated water savings (according to sources they can reduce water consumption by 80%, respectively 99% compared to conventional methods), compared to conventional systems here watering is carried out underground so it greatly reduces the occurrence of plant diseases, resulting in a healthy diet without chemical treatments or fertilizers. It is possible to better control the growth of plants, due to the control of temperature, water pH and nutrients. Compared to conventional cultivation where if you do not use herbicides you need to intervene to weed, this system is much easier because the plant growth area is pure with no germination seeds or if they appear they are insignificant, they can be removed without too much intervention time, somewhere an average time of 5-10 minutes a day and this to feed fish and check the proper operation of equipment. In addition, aquaponics has applicability in the education system for either pupils or students in university centre who can use different parameters of IoT systems to study different regulation techniques for different species of fish and plants in aquaculture, so as to integrate the student in a deep environment and to offer him a pleasant, concrete experience and applied notions in the field of technologies. Maintaining proper operation, water level, nutrient level measurement, system pH control, is easy to manage in small installations, but for a large structure it is necessary to incorporate an IoT-based system. There are various researches in the field of IoT-based aquaponics such as Yanes et al. (2020) which propose the use of aquaponics as an alternative based on the two technologies mentioned above, namely aquaculture and hydroponics, thus using this combination increases the efficiency of water use, conditioning the user to use pesticides or of chemical fertilizers due to the presence of fish, making this concept healthy, green, and sustainable. For Lee et al. (2020), an IoT-based cloud monitoring system is proposed for an aquaponic system used to measure various parameters such as: water level, water temperature, dissolved oxygen and pH value. Three infrared distance sensors at different heights located in the aquarium are used to monitor the activity of the group of fish, the data is uploaded on ThingSpeak™, a cloud platform that allows the data interpretation, uploaded via wi-fi. For Mahkeswaran & Keong Ng (2020), an aquaponic system is proposed for the home environment, thus suggesting that if each family could produce its own fish and plants, the need for food in cities and in all countries will be reduced, especially in Singapore. The classic method requires large areas of land and numerous human resources, and food security would be at a standstill, so the proposed aquaponic systems are smart and sustainable, include a wide range of sensors, actuators, as well as a microcontroller with internet connection for monitoring and control of all parameters. Uses sensors for measuring water level and temperature, sensor for determining pH, electrical conductivity, air temperature, and humidity, dissolved oxygen, light sensor adapted for the Arduino Mega 2560 microcontroller. For the mobile application it was created with Blynk, being an IoT platform for hardware control and data analysis. According to Ibtissame et al. (2020), aquaponic systems have many benefits in academic research, for example; students having the opportunity to learn tools such as mathematics, chemistry, biology and engineering, in addition, studying the interactivity between fish, plants and bacteria in a living ecosystem reporting water quality tests (measuring and tracking) fish growth rates and plants, as well as the aquaponic system are used to demonstrate various principles.

Urban Agriculture (UA)

Due to the cities development and the global population growth, the agricultural policy to cope with this trend is thus adapting to the consumer, causing major changes to food production systems due to research and innovation in the field, to meet growing demands. Over the past three decades, agricultural growth and innovation combined with the advancement of information technology have brought to the fore modern and promising cultivation techniques that are valuable in terms of the sustainability and economic viability of controlled agriculture (C.E.A.). According to Ellis (2012) agriculture in the past has been associated with urban centers to a greater extent than today.

Although modern and traditional agriculture have been separated into upper layers, they remain somewhat attached to each other at the roots. By the end of the 2000s, large cities had reached the point where most people did not even need to associate food with natural resources (Abel, 2010). The concepts of urban agriculture (AU) and the associated benefits have enjoyed considerable attention and popularity lately, namely 8 years, growing to provide satisfaction to urban dwellers who focus on continuous development. A variety of systems can be included in the concept of urban agriculture (AU) in different architectures and residential areas, increasing the tourist potential of the metropolis (Figure 4), from common, personal gardens, for social and self-sufficiency purposes, to complex systems. with the potential to produce food indoors by artificial lighting or in climate-controlled areas started by small plant-producing businesses.

In most cases, urban agriculture (AU) is mostly practiced indoors, or adapted to new names, namely, vertical agriculture (VF) (Despommier, 2010), integrated agriculture inside buildings (Caplow, 2009) and agriculture (Thomaier et al., 2015). Thus, this type of food production has managed to penetrate most cities in the world, attracting special public interest.

Because the market is regulated according to demand and supply and in this field it can be seen that the need of consumers is constantly changing, so many are oriented towards the freshest and best quality food that does not compromise nature.

Several reports have indicated that several projects are involved in bringing agricultural products to cities (Mok et al., 2014; Taylor & Lovell, 2012). It is appropriate to consider the definition of the concept of urban agriculture that conforms (Benis & Ferrão, 2017; Pölling et al., 2016; Cahya, 2016, Ahlström & Zahra, 2011). Balas et al. (2020) stated taht AU is an industry that produces, processes and markets food and fuel, largely in response to the daily needs of consumers in a city or area.

Some of the proposed solutions for a closed field production system in the AU are plant factories, vertical agriculture (VA) and roof greenhouses. Figure 3 shows a conceptual demonstration of the concept of urban agriculture that can be implemented in areas with a dense population.

Conceptual demonstration of urban agriculture in high-density urban areas in transformation and conceptual design of a smart urban farm inside a center.

Figure 3. Conceptual demonstration of urban agriculture in high-density areas of changing cities (weburbanist.com)

Figure 4. Conceptual design of a smart urban farm inside a centre (weburbanist.com)

Aquaponic systems and the possibility to become autonomous

An aquaponic system consumes electricity for:
- water supply/recirculation;
- water oxygenation (bubbling air into water);
- plant lighting;
- command and control system.

In order to reduce the costs of water oxygenation and especially to find an efficient method of storing green energy, in the case of aquaponic systems the most convenient solution is to store them in potential energy using compressed air.

Thus having a potential energy in the form of compressed air, which is used to oxygenate the water in the aquaponic system, a system can be implemented to recover this potential energy with the help of an electricity generation system using the principle of buoyancy (buoyancy, buoyancy - powered generator BPG).

Hybrid CAES/GDP conceptual system

The CAES (Compressed air energy storage)/ BPG (buoyancy-powered generator) consists of two essential components cylindrical vessels that are positioned on a vertical mechanical mechanism and implement the principle of buoyancy and an installation that converts electricity produced from renewable sources into potential kinetic energy, such as a compressed air generator coupled to a source storage. Compressed air is used by the buoyancy mechanism to generate electricity and recover the energy consumed for oxygenating the water shown in Figure 5. The general concept of the generator in its simplest form in which light cylindrical vessels were used to capture air is illustrated in Figure 6.

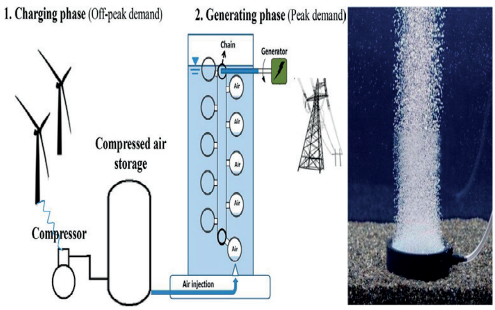

Figure 5. The basic components used are the development of the CAES/ BPG system for energy storage, applicable to water oxygenation (Hossein et al., 2019)

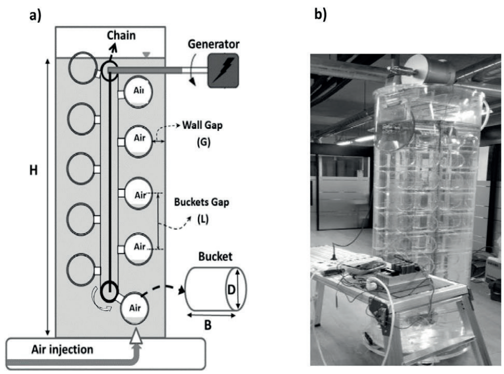

Figure 6. Systematic illustration of the power generator on the buoyancy principle and (b) a capture of the experimental installation by Infinity Sav Team (2019)

In order to be able to put into practice the system of electricity production with the help of floating, a system called BPG was created consisting of:

- A basin with 2 horizontal axes, one positioned at the bottom of the basin and the other positioned at the top of the basin;
- Four gears equal in diameter positioned on the 2 horizontal axes of the basin;
- A gear with a smaller diameter positioned on the upper horizontal axis that ensures the transmission of rotation to the generator;
- Two chains with conveyor rollers that vertically connect the gears on the 2 horizontal axes;
- Rigid cylindrical vessels of equal size, arranged at equal distances on the chain with conveyor rollers that move the axes, respectively through the axis located at the top of the basin engages through a chain the generator with permanent magnets, thus producing electricity.

Thus, when the compressed air is injected into the cylindrical vessels, air intake losses occur due to the impossibility of the cylindrical vessels being captured, respectively by their movement, but coupled to an aquaponic system these losses can be considered an advantage contributing to the oxygenation of the water to be used in the aquaponic system, this in the idea of coupling such a BPG system with an aquaponic system.

The operation of this CAES/BPG system is due to both the floating phenomenon and the respective design and construction of the device to be able to use this advantage, so the air has a density about a thousand times lower than the density of water, adding as a plus the state of air aggregation through this concept the storage of renewable energies becomes much more environmentally friendly, all components can be recycled without too high costs. As can be seen in Figure 5, the cylindrical vessels are coupled by means of the chain with conveyor rollers forming on either side of the axes two almost symmetrical parts the difference being only the orientation of the orifices of the cylindrical vessels so in one part these openings are oriented down by capturing the injected air and at the moment of rotation, passing the other side, the orifice of the vessel will remain upwards so the air will leave the cylindrical vessel allowing water to enter.

Because the movement of the bucket through the water it is gentle, the force transfer is performed by an assembly consisting of a chain gear and a gear with a well-established diameter ratio to ensure optimal kinetic energy transfer (Alexuta, 2021). The speed of the cylindrical vessels can be adjusted by the flow produced by the air source, respectively the regulation of the flow introduced by the air injection installation. Increasing the outlet torque can be increased by adding more containers on the vertical chains and increasing the depth of the pool vertically. Other methods of increasing the output force refer to increasing the volume inside each cylindrical vessel or raising the flow released by the mechanism for injecting air into the cups. There are some parametric criticisms that affect the proper functioning of the system such as chain rotation speed, container movement speed, container size and shape, container number, tensile and frictional force due to container and chain movement, exhaust air to fill the container, the space ratio and the distance ratio, noted in Figure 6 with G signifying the gap between the cylinder wall and the moving container, and L signifying the gap between the means of the moving containers. For an understanding of how the electricity generator works with buoyancy (Hossein et al., 2019).

CONCLUSIONS

Due to the possibilities offered by an aquaponic system for growing both plants and fish, considerable work has been developed stating various ideas such as food security, short chain food supply, sustainability, control of renewable resources and much more. In addition to the optimal production of aquatic products, the accommodation of aquaponics and aquaculture continues in the global food system for quantification, evaluation and market development towards sustainable development. By implementing a system of electricity production with the help of Archimedes' law, the autonomy of the system can be increased during the grey periods (night for solar energy, and when there is no wind for wind energy) of renewable sources of electricity production. The CAES (Compressed air energy storage)/BPG (buoyancy-powered generator) system applied under aquaponic systems significantly reduces the energy consumed, offering a viable option to become autonomous, depending largely on the green energy sources used, solar or wind. The potential of the idea of coupling the mechanical systems of electricity production in an aquaponic system, respectively the capitalization of losses for the benefit of the aquaponic system would increase the potential of aquaponic systems but this can be highlighted after testing.

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RESULTS OBTAINED BY INVESTIGATING PUMPKIN (*Cucurbita maxima* L.) USING FT-IR SPECTROSCOPY

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Abstract

Cucurbita maxima is a plant with a flexible and hanging stem that belongs to the Cucurbitaceae family. The fruit of this species (pumpkin) is characterized by a strong orange peel with distinctive grooves. The Cucurbita maxima L. has a considerable economic importance, beyond this variety huge quantities of seeds and shells can be obtained. Although the shells do not have a final application, they contain large amounts of tocopherols and five times more carotenoids than seeds.
Pumpkins are commonly grown for human consumption, for decoration, and also for livestock feed. Because, the pumpkin is cultivated in many countries having very important medical properties are necessary more studies regarding his bioactive compounds and nutritional values. Vibrational spectral techniques, FT-IR, offer several advantages in the context of current research and using this techniques we can identify molecular components in the samples studied. IR spectroscopy is based on the absorption of radiation in the 400-4000 cm^{-1} range which excites molecular vibrations. Our research had the objective to analyze a vibrational spectra of C. maxima pumpkin variety seeds and pulp, using a method which is based on microscopic infrared study (FT-IR).

***Key words**: pumpkin seed and pulp, FT-Raman (Fourier-transform infrared spectroscopy).*

INTRODUCTION

Cucurbita maxima is an annual herbaceous plant cultivated for its fruit, flowers and seeds. It is an annual plant with a flexible and hanging stem. It has cordiform (heart-shaped) leaves, pentalobulate, large in size with well-marked veins. The flowers are yellow and fleshy, the fruit has a great variation: it can be ovoid or spherical, green or deep orange.
The fruits of this domestic species, pumpkins, (Fig,1), contains inside numerous oval, convex and smooth seeds 2-3 cm long, which in turn contain a white core. There are summer varieties with light-colored fruit and softer seeds, and the autumn varieties that are drier and sweeter, used in confectionery.
The pulp of the fruit is yellow-orange, dense and sweet in taste (Lopez-Anido 2021). Pumpkin is one of the lowest calorie vegetables, instead it is a rich source of antioxidant dietary fiber and vitamins. It is also a rich source of many polyphenolic flavonides, such as alpha and beta carotene, lutein. Carotenes are converted into vitamin A in the body according to needs. Pumpkin also has a high content of fiber, fatty acids and phytosterols (Xiong, 2002). Pumpkin pulp contains many nutritional components, including polysaccharides, carotenoids, minerals, amino acids and active proteins (Zhou et al., 2007). The pulp is a rich source of vitamins: A, B, C, E (Men et al., 2020). Pumpkin seeds content an important source of energy because the values reported to the dry matter is 30-70% protein and 40-50% fat (Winkler et al., 2002; Tepper et al., 2000). In addition, pumpkin seeds are a good source of vitamins and minerals importants for human health: Ca, K, P, Mg and Zn (Fan and Li, 2005).
Pumpkin is a plant that is cultivated and widely found in Romania. There is an increasing interest for many varieties uses of pumpkin because of their high nutritional value, digestive effects and good sensory characteristics (Noelia et al., 2011; Konopacka et al., 2010). The pulp of pumpkin is usually used in soups, pies, and

bakeries while the seeds are used as a snack and pharmaceutical. Therefore, pumpkin has high potential application in the food, cosmetic and pharmaceutical industries. Due to the good acceptance and industrialization of the derived products from pumpkins, more scientific information about the chemical and physicochemical properties of this fruit is important (Quintana et al., 2018).

Figure 1. Pumpkin (*Cucurbita maxima*)

Carotenoids, a complex family of isoprenoids, are relevant in plant foods and agriculture, as nutrients, antioxidants, pigments, and ripening indicators (Sharma et al., 2013; Fiedor and Burda, 2014). They are the main reason for orangered yellow colors of plant tissues, they act as antioxidants, and confer benefits for human health, including provitamin A activity in some cases (Rodriguez-Amaya, 2015).
Fourier transform infrared spectroscopy (FTIR) is a potential alternative for simplifying the analysis of food constituents (Andronie et al., 2021; Keseru et al., 2016; Andronie et al., 2019). Several FTIR-based methods have been developed to discriminate and determine various properties of food matrices (Bassbasi et al., 2014; Biancolillo et al., 2020; Belton et al., 1995; Mellado-Mojica et al., 2016; Anjos et al., 2015, Andronie et al., 2016; Bebu et al., 2020) showcasing an important reduction of chemicals and time consumption, as the main advantage compared to conventional analytical techniques. Moreover, the use of attenuated total reflectance (ATR) devices simplifies the application of FTIR for qualitative and quantitative analysis of materials, including food matrices.
The capability of this technique for the determination of carotenoids in food matrices has been demonstrated in tomatoes, with good performance in lycopene quantification (De Nardo et al., 2009). However, there are no many previous studies of either FTIR-ATR or other MIR-based techniques for the evaluation of carotenoids in pumpkin or squash pulp, two of the richest sources of these compounds among fresh produce.
The pulps of pumpkins having a high percentage of carbohydrate and the seeds are characterized by a high amount of oil and protein.
The Fourier-transform infrared spectra in combination with chemometrics and fatty acid composition was used to analyze pumpkin seed oil blended into extra virgin olive oil (Rohman et al., 2013).
The purpose of this study was to analize the minerals and determination of total polyphenols in pumpkin powder and also to study functional groups of the pumpkin seed using FT-IR spectroscopy.

MATERIALS AND METHODS

Experimental for determination of total polyphenols in pumpkin

The pumpkin powder was obtained by drying the pumpkin pulp at room temperature for 7 days.
The principle of the method for the determination of total polyphenols in ingredients of plant origin such as pumpkin powder is that in a basic medium and in the presence of phenols, the mixture of phosphotungstic and phosphomolybdic acids is reduced to blue oxides of tungsten and molybdenum with maximum absorption of 765 nm.
In order to obtain pumpkin pulp extract, 40 ml of methanol (extraction solution) was added to 5 grams of pumpkin powder, mixed with the laboratory blender, and then centrifuged for 5 minutes at 10,000 rpm. 5 ml of distilled water, 1.5 ml of sodium carbonate solution (10%), 0.5 ml of sample extract and 0.5 ml of Folin-Ciocalteu solution were added into a 15 ml vial. The samples were left for 45 minutes in the dark at room temperature and then measure at a wavelength of 765 nm against a blank solution (ethanol). The measurements are compared with a gallic acid calibration curve (25, 50, 100, 250 ppm) and the results are expressed in gallic acid equivalent.

For the determination of total polyphenols we used a Lambda 25 Ultraviolet-Visible Molecular Absorption Spectrophotometer, Perking Elmer.

Experimental for determination of minerals

The preparation of the samples consisted of weighing 0.2 grams of powder obtained from pumpkin seeds and placed in a Berzelius beaker. A volume of 9 ml of 65% HNO_3 was added to the weighed sample and placed on a sand bath for mineralization. The sample was boiled in the sand bath for about an hour, after which the glass was removed from the sand bath and allowed to cool. After cooling, 2 ml of 30% hydrogen peroxide (H_2O_2) were added, each glass containing the mineralized sample for complete oxidation of the organic matter and left on the sand bath for about 20 minutes. The resulting solution was filtered into 25 ml volumetric flasks, flushed with ultrapure water and transferred to plastic bottles. The samples were then analyzed with an inductively coupled plasma-optical emission spectrometer.

The Perkin Elmer inductive coupled plasma mass spectrometer, ELAN DRC II type, with quadrupole and equipped with a reaction cell for the elimination of interferences, with a mass range of 3-240 m/z, resolution below 1 amu (IU-36, Serial Q1970307H), was used to determine metals.. The device is calibrated using standard calibration solutions prepared from stock solution (Multi-element Calibration Standard 3, PerkinElmer).

Experimental for FT-IR spectroscopy

The seeds samples of pumpkin were crushed using a commercial blender obtaining a powder that was used on the same day.

Measurements were carried out on the infrared scale of 350-4000 cm^{-1} and a spectral resolution was set at 4 cm^{-1} using a Jasco FT-IR-4100 spectrophotometer (Oklahoma City, OK United States) using KBr pellet technique.

The sample was prepared using calcinated potassium bromide as a matrix material and was mixed at a proportion of 3 mg of the sample (powder of seeds) to 200 mg KBr. Then the mixture was condensed in 15 mm dies at a pressure equal to 10 t till 2 min (Crișan et al., 2019). All spectra were acquired over 256 scans. The spectral data were analysed using Origin 6.0 software (Figure 2).

Statistical analysis

The IBM SPSS v.19.0 for windows, was used for statistical analysis. Basic statistics, was implemented in order to emphasize the arithmetic mean (X) ± standard deviations (SD) of the content of the total polyphenols and minerals in the pumpkin powed. The mean concentration of minerals was compared using One-Sample T-test with the standard values reported by USDA (The U.S. Department of Agriculture) relative to different categories of pumpkin seeds: dried pumpkin seeds respectively pumpkin seeds, roasted without salt. Differences of the means were considered to be significant when p-value < 0.05 (Paunovic et al., 2019).

RESULTS AND DISCUSSIONS

Polyphenols results

The result obtained after the determination of the total polyphenols in the pumpkin powder of pulp is presented in table 1 and it can be seen that it does not exceed the maximum allowed limits, being 480 mg/kg.

Table 1. Total polyphenols

Sample (grams of pumpkin powder)	\Extraction volume (ml)	\Result mg /kg GAE equivalent
5 ± 0.11	40	480 ± 0.02

Note: *The mean difference at significant p< 0.05.

Mineral results

Following the determination of the minerals in the pumpkin powder of pulp, it was observed that the analyzed samples did not contain heavy metals (cadmium, cobalt, nickel = <LDS).

The unit of measurement of the samples is mg/kg and the results obtained are presented in tables 2. The values presented in tables 2 reveal that the pumpkin core has a high content of calcium (Ca)-1048.1 and potassium (K)-1045.3 mg/kg. Also important is the concentration of magnesium (Mg)-718.2 mg/kg. The content of the other minerals can be seen in the tables presented.

Statistical analysis for the minerals content

ANOVA analysis was followed by Tukey's test in order to perform multiple comparisons regarding the minerals content in the pumpkin powder and the Turkey test revealed significant differences between the mean amount of minerals contained.

Table 2. The results obtained for minerals

Ca (mg/kg) 1048.1±0.8	**K (mg/kg)** 1045.3±0.82	**Mg (mg/kg)** 718.2±0.78	**Fe (mg/kg)** 96.8±0.34
Na (mg/kg) 88.9±0.37	**Al (mg/kg)** 17±0.3	**Cu (mg/kg)** 15±0.8	**Zn (mg/kg)** 9.3±0.36
Sr (mg/kg) 4±0.3	**Pb (mg/kg)** 0.6±0.1	**Co (mg/kg)** <LOD	**Ni (mg/kg)** <LOD
Cr (mg/kg) <LOD	**Cd (mg/kg)** <LOD	**Mn (mg/kg)** <LOD	**Ba (mg/kg)** <LOD

*LOD= below the detection limit of the device

The average amount of Ca, Fe contained in the pumpkin powder of pulp is statistically significantly higher than the reference values specified by USDA for dried pumpkin seeds respectively for pumpkin seeds, roasted without salt.
The average amount of K, Zn recorded in the pumpkin powder of pulp is statistically significantly lower than the reference values specified by USDA for dried seed or roasted seeds.
The average amount of Na determined in the pumpkin powder is statistically significantly higher than in the case of dried pumpkin seeds respectively statistically significantly lower than in the case of roasted pumpkin seeds without salt.

FT-IR analysis

We used FT-IR spectroscopy to evaluating the functional groups as part of a compound in chemical analysis. In the food industries, this technique it was also used as a monitoring quality control because of its rapid screening and quantification of chemical components in samples.
Most chemical bond components have vibrational movements in the medium infrared spectrum (4000-350 cm^{-1}), such as lipids, proteins, carbohydrates and nucleic acid molecules.
The FT-IR spectrum was used to identify the functional groups of the active components present in the sample, based on the peak's values in the region of IR radiation. The presence of carotenoids, amides of proteins was reported, according to FT-IR analysis.
As can be seen in Figure 2, the FTIR spectra of pumpkins *(Cucurbita maxima* L.) of seeds were evaluated in frequency of 4000-500 cm^{-1}.

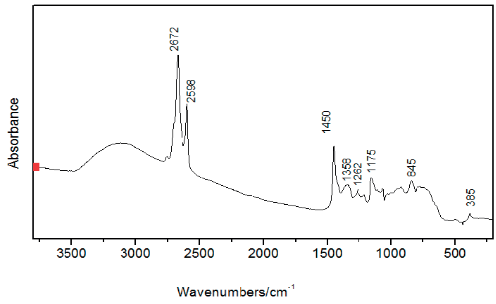

Figure 2. FT-IR spectra of pumpkin (*Cucurbita maxima*) seeds

Generally, a frequency at 3450-3250 cm^{-1} is hydroxyl groups (O-H) from carbohydrate or other compounds such as carboxyl acid and ketone. The absorption around 3120-3030 cm^{-1} is associated with (N-H) groups from amide. The bands at 1105-1010 cm^{-1} is associated with lipids (Indrianingsih et al. 2019). The range of frequency at 2950-2850 cm^{-1} is related to CH absorptions.
The region of approximately 1450 cm^{-1} is associated with vibrations of antisymmetric deformation of CH_3 groups (change in HCH angles) and CH_2 groups (scissor vibrations) (Berezin et al., 2005; Schluckeer et al., 2003).

CONCLUSIONS

Based on the obtained results, it can be concluded that the FT-IR spectroscopy is a reliable instrumental technique for the determination of mean components in seeds of plants.
The results of this study will be used for the preparation of new products and the extraction of microcomponents with technological functionality.

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ORGANIC SOURDOUGH MINI BAGUETTE FORTIFIED WITH JERUSALEM ARTICHOKE FLOUR, FOR DIABETICS

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Abstract

Fermentation of the dough with sourdough cause the improvement of the sensory and nutritional qualities of bakery products. Also, sourdough bakery products (in a longer fermentation process) are digested more slowly in the human body, which causes a lower glycemic impact on the human body. This paper presents the research results undertaken to obtain the organic sourdough mini baguette fortified with Jerusalem artichoke flour, for diabetics. This product has superior sensory qualities, high nutritional value, has antioxidant capacity and low glycemic index, being in the diet of diabetics. Thus, the mini baguette fortified with jerusalem artichoke flour, is distinguished by the content in mineral elements (K: 245.17-260.85 mg/100 g; Ca: 136.21-145.21 mg/100 g; Mg: 90.02-98.45 mg/100 g; Fe: 1.88-1.97 mg/ 100 g; Zn: 1.47-1.56 mg/100 g), total fiber (8.94-9.75 mg/100 g), total polyphenols (115.75-118.98 mg GAE/100 g). Also, this bakery product has antioxidant capacity (125.43-128.65 mg Trolox/100 g) and has low content in available carbohydrates (39.24-38.15%). The use of sourdough in the organic mini baguette composition ensures its freshness and microbiological stability for a period of at least 4 days. The use of sourdough as a natural fermentation agent and Jerusalem artichoke flour as a fortifying agent ensures a mini-baguette with superior sensory qualities, high nutritional value and antioxidant capacity, which can be used in the prevention and diet therapy of diabetes.

Key words*: diabetics, Jerusalem artichoke flour, mini baguette, organic, sourdough.*

INTRODUCTION

Sourdough is the result of the fermentation of flour from cereals and pseudocereals or legumes, among others, through the action of microorganisms present in the preparation (Weckx et al., 2019). Some sourdoughs may also contain added microorganisms. Sourdoughs are stable ecosystems composed of lactic acid bacteria (LAB) and yeasts used in the production of bakery products (Zlateva et al., 2019). Traditionally, sourdough was used as a leavening agent, but today it is more and more used to improve the organoleptic characteristics of bakery products and to reduce the need for additives in their composition (Gänzle et al., 2016).
The sourdough fermentation process is based on the unique symbiosis between yeasts and lactic bacteria. The use of sourdough in bread composition cause the improvement of the aroma, the texture and the increase of the shelf life of the bread.
Bioaccessibility and bioavailability of nutrients are essential for ensuring optimal nutrition of the fermentation medium, but also for the quality of the final product and its beneficial effects on consumers health (Păcularu-Burada et al., 2020; Siepmann et al., 2018). Thus, the use of sourdough in the fermentation process of the dough to obtain bread can lower its glycemic index, improve the dietary fiber complex, release bioactive peptides and increase the absorption of minerals and bioactive compounds (vitamins, phenolic compounds) in the human body (Păcularu-Burada et al., 2020; Siepmann et al., 2018). At the same time, the microbial metabolism of the lactobacilli present in the dough cause the formation of compounds with nutritional value, such as peptides and amino acid derivatives (amino-butyric acid) as well as prebiotic exopolysaccharides (Păcularu-Burada et al., 2020; Chiș et al., 2019; Rashmi et al., 2020). Catana et al. (2018) obtained an assortment of minibaguette fortified with dehydrated fruits of

Aronia melanocarpa, using sourdough as a fermentation agent. This mini-baguette stands out for its sensory qualities, the content in mineral elements (Potassium: 10547 mg/kg; Calcium: 1598.57 mg/kg; Magnesium: 1095.44 mg/kg; Iron: 19.80 mg/kg), total polyphenols (197.91 mg GAE/100 g) and antioxidant capacity (2.64 mg Trolox Equivalents/g).
Also, Burnete et al. (2020) made an assortment of hypoglycemic bread, in which a type of natural yeast fortified with polyphenols and inulin was used as a fermentation agent. This bread is intended for diabetics, having a low carbohydrate content, high nutritional value and antioxidant potential (117.45-125.15 mg TE/100 g).
This paper presents the research results undertaken to obtain the organic sourdough mini baguette fortified with Jerusalem artichoke flour, for diabetics.

MATERIALS AND METHODS

Materials

To obtain the "Mini baguette fortified with Jerusalem artichoke flour" the following materials were used: organic sourdough, whole wheat flour, barley flour, Jerusalem artichoke flour, apple waste flour (*Jonathan variety*), seeds (sunflower, pumpkin, flax, white sesame, black sesame) milk, olive oil and sea salt.

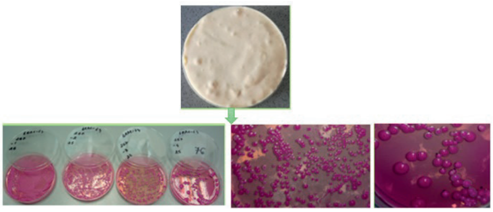

Figure 1. "Mini baguette fortified with Jerusalem artichoke flour"

For the control sample (C: "White mini baguette with natural sourdough") the following materials were used: organic sourdough, white wheat flour, white sesame and black sesame seeds, milk, olive oil and sea salt.
Organic sourdough was obtained and tested at the Human Nutrition Laboratory in IBA-Bucharest. Jerusalem artichoke flour was obtained within the Vegetable-Fruit Processing Pilot Experimental Station, from IBA Bucharest.

Mini baguette-making

To obtain the product "Mini baguette fortified with Jerusalem artichoke flour" three experimental variants (CDV1, CDV2 and CDV3) and the control sample were made
(C: "White mini baguette with natural sourdough"). The variable factors were the levels of fortification with Jerusalem artichoke flour and apple waste flour (3%, 4% and 5%, relative to the amount of whole wheat flour in the product composition). The experimental variant CDV3 presents the minimum level of fortification (3%).
Figures 2 and 3 show the products "Mini baguette fortified with Jerusalem artichoke flour" (CDV3) and the control sample.

Figure 2. Product "Mini baguette fortified with Jerusalem artichoke flour" (CDV3) and control sample

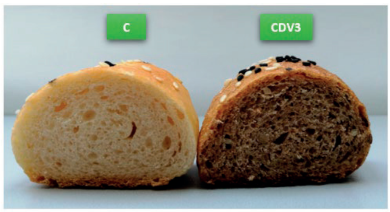

Figure 3. Product "Mini baguette fortified with Jerusalem artichoke flour" (CDV3) and control sample (in section)

To make the products, the biphasic process was applied, which ensures a high bioavailability of minerals and bioactive compounds and a better digestibility of proteins.

Methods

Statistical Analysis

The organic sourdough mini baguette fortified with Jerusalem artichoke flour, for diabetics and Control sample were analyzed in triplicate. Mean and standard deviation are reported for each analytical parameter studied.

Sensory analysis
The sensory analysis was carried out using the descriptive method and by "Comparison method with unitary score scales" method (Burnete et al., 2020).
Instrumental color analysis was performed with a CM-5 colorimeter (Konica Minolta, Japan) equipped with SpectraMagic NX software.
Instrumental texture analysis was performed with Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA) equipped with Bluehill 3.13 software.

Physico-chemical analysis
The physical-chemical analysis was carried out using the following methods: ACC 44-15A (moisture content), AOAC 979.09 (protein content), AOAC 963.15 (fat content), AOAC 923.03 (ash content) and AOAC 991.43 (total dietary fiber). The mineral elements were determined by atomic absorption spectrophotometry, after calcination of the samples. Energy value (kcal/100 g and kJ/100 g) were calculated according to the Commission Regulation no. 1169/2011 (European Commission, 2011).

Total polyphenol content
Total polyphenol content was performed by Folin-Ciocalteau spectrophotometric method, according to Horszwald and Andlauer (2011), with some modifications (Burnete et al., 2020).

Antioxidant capacity
Antioxidant capacity was performed by DPPH (1,1diphenyl-2-picryl hydrazyl) method, according to Horszwald and Andlauer (2011), with some modifications (Burnete et al., 2020).

Microbiological analysis
Microbiological analysis was performed according to SR ISO 21527-1:2009 (Yeasts and molds) and SR EN ISO 21528-1:2017 method (*Enterobacteriaceae*).

RESULTS AND DISCUSSIONS

Sensory analysis
Following the sensory analysis, it was found that the product "White mini baguette with natural sourdough" has a pleasant, specific taste and aroma, an elastic, dense core, with uniform pores and a volume of 247 cm^3/100 g. The product "Mini baguette fortified with Jerusalem artichoke flour" made in the experimental versions CDV1 and CDV2, has an elastic, dense core, with uniform pores and a pleasant, specific taste and aroma, but has a low volume (CDV1 - V = 216 cm^3/100 g; CDV2 - V = 180 cm^3 /100 g) and after 2 days from the date of manufacture they have a hard texture. In contrast, the product made according to the experimental variant CDV3, presents appropriate sensory qualities and has a volume close to that of the Control sample (V = 243 cm^3/100 g). If the percentage of Jerusalem artichoke flour and apple waste flour increase, we noticed as a result a decrease in product volume and an increase in firmness (product texture). Thus, the product made according to the experimental variant CDV1, in which the level of fortification with Jerusalem artichoke flour and apple waste flour is 5%, shows, after one day from the date of manufacture, the highest value of firmness (12.82 N) and gumminess (4.03). Elasticity and cohesiveness recorded close values in the case of the Control sample and the three experimental variants. The products were packed in polypropylene foil.

Table 1. The textural properties of the product "Mini baguette fortified with Jerusalem artichoke flour", and the control sample C

Product	Period (days)	Firmness (N)	Elasticity	Cohesiveness	Gumminess (N)
C	1	6.03 ± 0.05	0.99 ± 0.02	0.43 ± 0.03	2.58 ± 0.28
CDV1	1	12.82 ± 1.64	0.97 ± 0.00	0.32 ± 0.03	4.03 ± 0.78
CDV2	1	8.47 ± 1.47	0.97 ± 0.00	0.37 ± 0.03	3.03 ± 0.73
CDV3	1	6.10 ± 0.28	0.97 ± 0.00	0.40 ± 0.02	2.36 ± 0.23

The experimental data on the main instrumentally determined texture parameters (firmness, elasticity, cohesiveness, gumminess) are presented in Table 1, and compression curves are presented in Figures 4-6.

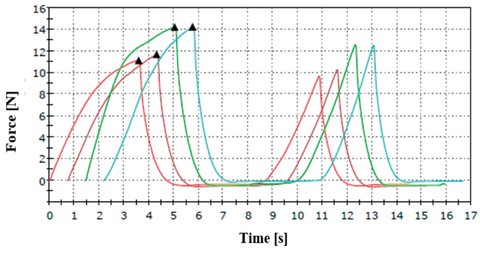

Figure 4. Compression curves for the product "Mini baguette fortified with Jerusalem artichoke flour" (CDV1)

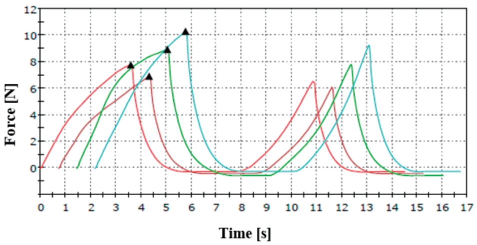

Figure 5. Compression curves for the product "Mini baguette fortified with Jerusalem artichoke flour" (CDV2)

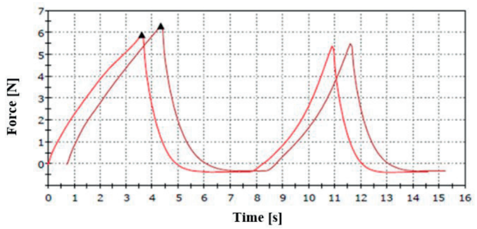

Figure 6. Compression curves for the product "Mini baguette fortified with Jerusalem artichoke flour" (CDV3)

Following the sensory analysis by a panel of 10 tasters, applying the "Comparison method with unitary score scales", the products received the following scores and qualifications: control sample C - 19.92 points ("very good" qualification), CDV1 - 17.76 points ("very good" qualification), CDV2 - 17.92 points ("good" qualification) and CDV3 - 19.76 points ("very good" qualification) (Figure 7).

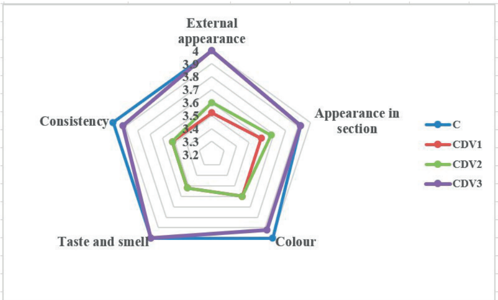

Figure 7. Sensory evaluation of the product "Mini baguette fortified with Jerusalem artichoke flour" (CDV1, CDV2 and CDV3) and of the control sample (C)

Following the instrumental analysis of the color the results showed that the product "Mini baguette fortified with Jerusalem artichoke flour" (CDV1, CDV2 and CDV3) recorded lower luminance values, compared to the control sample (C). The use of whole wheat flour in the composition of the product cause the darkening of its color, the minimum luminance value being registered in the case of the experimental variant CDV1, L* = 66.30 (Figure 8).

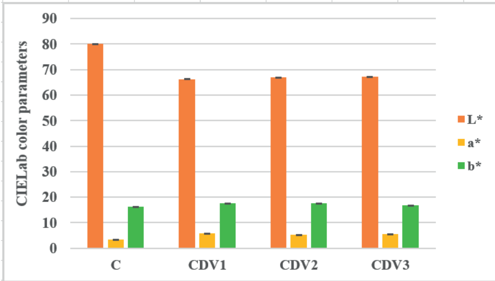

Figure 8. Color parameters of the product "Mini baguette fortified with Jerusalem artichoke flour" (CDV1, CDV2 and CDV3) and of the control sample (C)

Also, the product "Mini baguette fortified with Jerusalem artichoke flour" (CDV1, CDV2 and CDV3) has higher values of the color parameters a* and b*, compared to the control sample (C).

Corroborating the results of the sensory analysis with those of the instrumental analysis of the texture, in order to make the product "Mini baguette fortified with Jerusalem artichoke flour", the experimental variant CDV3 was chosen as the optimal variant.

Physico-chemical analysis

Physico-chemical analysis of the product "Mini baguette fortified with Jerusalem artichoke flour" and of the control sample C is presented in Table 2.

Table 2. Physico-chemical composition of the product "Mini baguette fortified with Jerusalem artichoke flour" and of the control sample C

Component	C	CDV1	CDV2	CDV3
Acidity (degrees)	3.8±0.01	4.38±0.01	4.30±0.01	4.20±0.01
Moisture (%)	32.88±0.82	33.16±0.83	33.54±0.84	33.05±0.83
Ash (%)	0.84±0.009	1.40±0.015	1.49±0.016	1.58±0.017
Protein (%)	9.65±0.08	11.60±0.10	11.78±0.10	12.15±0.11
Fat (%)	1.88±0.02	4.67±0.05	4.55±0.05	4.42±0.05
Carbohydrates (%)	54.75±0.04	49.17±0.02	48.64±0.02	48.80±0.02
Available carbohydrates (%)	53.94±0.03	44.18±0.01	43.93±0.01	44.37±0.01
Total dietary fiber (%)	0.81±0.01	4.99±0.09	4.71±0.09	4.43±0.08
Energy value (kcal/100g)	273	275	273	275
Energy value (kJ/100g)	1157	1161	1153	11560

Following the physico-chemical analysis, it was found that the product "Mini baguette fortified with Jerusalem artichoke flour" has a low carbohydrate content and stands out for its protein, ash and total fiber content, compared to the control sample (C). These chemical characteristics of the product cause a quick and lasting satiety when consumed and a reduced glycemic impact, being beneficial in the diet of diabetics and obese people.

The product "Mini baguette fortified with Jerusalem artichoke flour" has protein, ash and fiber content, comparable to those reported by Odunlade et al. (2017) in the case of white bread, fortified (fortification level 1-3%) with leafy vegetable powders (protein: 9.50 to 13.93%; fiber: 1.81-4.00%), ash: 1.05 to 2.38%) .

The "Mini baguette fortified with Jerusalem artichoke flour" stands out for its content in mineral elements" (K, Ca, Mg, Zn, Cu and Fe). Their content in minerals is presented in Figures 9 and 10.

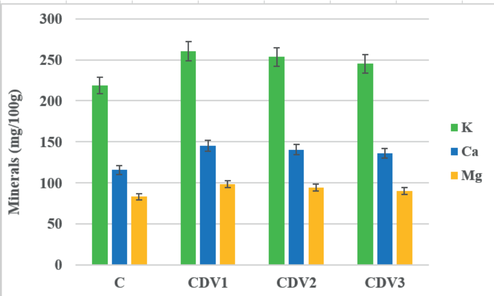

Figure 9. Mineral content (K, Ca and Mg) of the product "Mini baguette fortified with Jerusalem artichoke flour" and of the control sample C

The product made according to the experimental variant CDV1, has the highest content in potassium, calcium and magnesium (K: 260.85 mg/100 g; Ca: 145.21 mg/100 g; Mg: 98.45 mg/100 g). Bread and bakery products are an important source of macro and micro elements (Odunlade et al., 2017; Szymczycha-Madeja, 2017). In the human body, minerals have structural, catalytic and regulatory functions. Thus, they activate enzymes and regulate fluid pH for metabolic reactions and cellular osmotic exchanges (Gharibzahed et al., 2017).

The product "Mini baguette fortified with Jerusalem artichoke flour" has a lower calcium and magnesium content than bread fortified with leafy vegetable powders (Ca: 250-330.5 mg/100 g; Mg: 157.5-179 mg/100 g). Instead, this product has a significantly higher mineral content compared to that reported by Torrinha et al. (2019) in the case of some types of bread, made from wheat flour, in Europe (K: 74-205 mg/100 g; Ca: 19.2-45.9 mg/100 g; Mg: 20-32.4 mg/100 g).

The product "Mini baguette fortified with Jerusalem artichoke flour" made according to the experimental version CDV1, has the highest content in iron, zinc and copper (Fe: 1.97 mg/100 g; Zn: 1.57 mg/100 g; Cu: 1.01 mg/100 g).

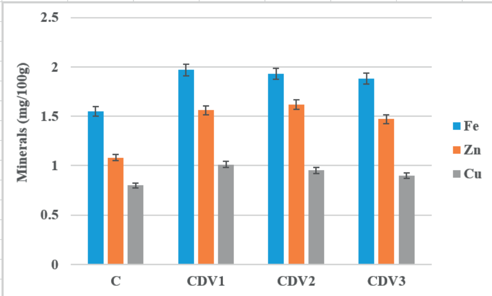

Figure 10. Mineral content (Fe, Zn and Cu) of the product "Mini baguette fortified with Jerusalem artichoke flour" and of the control sample C

The product "Mini baguette fortified with Jerusalem artichoke flour" has the iron and zinc content higher or comparable to that reported by Torrinha et al. (2019) in the case of some types of bread, made from wheat flour, in Europe (Fe: 1-2.2 mg/100 g; Zn: 0.27-1.59 mg/100 g). The Fe content of the product "Mini baguette fortified with Jerusalem artichoke flour" is higher compared to that reported by Zlateva et al. (2019), in the case of bread fortified with Spirulina powder (fortification level 2%): 1.59 mg/100 g .

Total polyphenol content

Due to the valuable ingredients used in the composition of the "Mini baguette fortified with Jerusalem artichoke flour", the product stands out for its content in total polyphenols (Figure 11).

The product made according to the experimental variant CDV1, has the highest content in total polyphenols (129.56 mg GAE/100 g), about 1.8 times higher than the Control sample (C). The total polyphenol content of the product "Mini baguette fortified with Jerusalem artichoke flour" (CDV1, CDV2 and CDV3) is lower than that reported by Catană et al. (2018), in the case of the "Minibaguette with Aronia" product (197.91 mg GAE/100 g), but higher than that reported by Saharan and Jood (2021) in the case of bread fortified with Spirulina: 85 mg GAE/ 100 g (fortification level 2%) and 119 mg GAE/100 g (fortification level 4%).

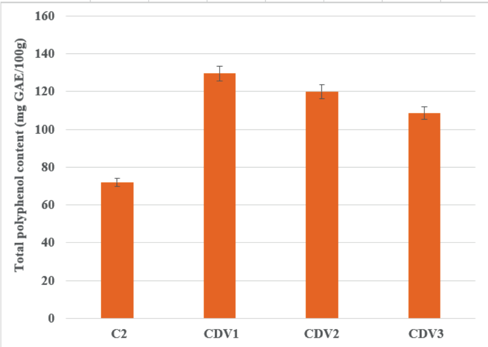

Figure 11.Total polyphenol content of the product "Mini baguette fortified with Jerusalem artichoke flour" and of the control sample C

Bread and bakery products (buns, baguettes, mini baguettes, croissants, etc.) are important foods in the human diet. Therefore, their enrichment in polyphenols is of real interest. Polyphenols have direct antioxidant and anti-inflammatory effects in the human orgasm, contributing to the prevention of conditions caused by oxidative stress. At the same time, recent scientific evidence mentions that polyphenols and their metabolites inhibit the growth of pathogenic bacteria in the colon (Aravind et al., 2021).

Antioxidant capacity

Due to its complex biochemical composition, the product "Mini baguette fortified with Jerusalem Jerusalem artichoke flour" (CDV1, CDV2 and CDV3) has antioxidant capacity. (Figure 12).

The highest value was recorded in the case of the experimental variant CDV3: 135.67 mg Trolox/100 g. The product made according to this experimental variant have a higher antioxidant capacity compared to that reported by Burnete et al. (2020), in the case of the product "Hypoglycemic bread with antioxidant potential" (125.15 mg Trolox /100 g).

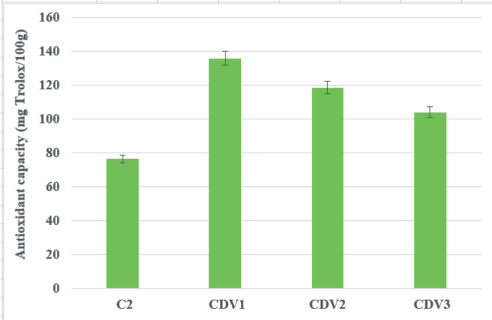

Figure 12. Antioxidant capacity of the product "Mini baguette fortified with Jerusalem artichoke flour" and of the control sample C

The antioxidant capacity of the product "Mini baguette fortified with Jerusalem Jerusalem artichoke flour" (CDV1, CDV2 and CDV3) is lower than that reported by Catană et al. (2018), in the case of the product "Minibagette with Aronia" (264 mg Trolox/100 g).

Microbiological analysis

The microbiological analysis of the product "Mini baguette fortified with Jerusalem artichoke flour" and the Control sample (C) is presented in Table 3.

The microbiological analysis revealed that the product complies with the provisions in force even 7 days after the date of manufacture. The microbiological stability of the product is due to polyphenols that have an antioxidant and antibacterial effect (Bouarab-Chibane et al., 2019) and the lactic acid bacteria from organic sourdough, which has antimicrobial and antifungal properties (Bartkiene et al., 2019).

Table 3. Microbiological analysis of the product "Mini baguette fortified with Jerusalem artichoke flour" and of the control sample C

Product	Microbiological indicator					
	Yeasts and molds (CFU/g)			*Enterobacteriaceae* (CFU/g)		
	24h	5 days	7 days	24h	5 days	7 days
C	< 10	-	-	< 10	-	-
CDV1	< 10	< 10	< 10	< 10	< 10	< 10
CDV2	< 10	< 10	< 10	< 10	< 10	< 10
CDV3	< 10	< 10	< 10	< 10	< 10	< 10

Corroborating the results of the microbiological analysis with the results of the sensory analysis and the results of the instrumental analysis of the texture, the shelf life of the product "Mini baguette fortified with Jerusalem artichoke flour" was set at 4 days.

CONCLUSIONS

The product "Mini baguette fortified with Jerusalem artichoke flour" made with organic sourdough presents superior sensory characteristics, being evaluated by a panel of 10 tasters with a score of 19.76 points ("very good" qualification).

The product "Mini baguette fortified with Jerusalem artichoke flour" has a low content in available carbohydrates (44.37%) and stands out for its content in proteins, total fibers, mineral elements (K, Ca, Mg, Fe, Cu, Zn), total polyphenols and antioxidant capacity, being beneficial in the diet of diabetics, obese people, as well as people with conditions caused by oxidative stress.

Thanks to polyphenols and lactic acid bacteria, the product "Mini baguette fortified with Jerusalem artichoke flour" shows microbiological stability, having a shelf life of 4 days.

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FORTIFICATION OF BISCUITS WITH CARROT POMACE POWDER IN ORDER TO INCREASE THE NUTRITIONAL VALUE AND ANTIOXIDANT CAPACITY

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Abstract

Carrot pomace powder is stands out by its content in protein, minerals, β-carotene, vitamin C, B vitamins (vitamin B3, vitamin B6), vitamin E and polyphenols. The aim of this study was to fortify the biscuits with carrot pomace powder, in order to increase their nutritional value and antioxidant capacity. Fortified biscuits have superior sensory qualities and have a complex biochemical composition, being noticed by their protein content (11.22%), total fiber (10.90%), potassium (435.55 mg/100 g), calcium (185.47mg/100 g), magnesium (127.68 mg/100 g), iron (2.26 mg/100 g), zinc (1.72 mg/100 g), total polyphenols (260.75 mg GAE/100 g), β-carotene (1.30 mg/100 g). At the same time, the fortified biscuits with carrot pomace powder have antioxidant capacity: 338.23 mg Trolox/100 g. Due to the addition in the composition of biscuits of dietary fiber with antioxidants, both the increase in nutritional value and the increase in their minimum durability are obtained. The minimum shelf life of fortified biscuits is 25 days. Using differential scanning calorimetry, it was found that fortified biscuits have a lower enthalpy value compared to control biscuits. Thus, it can be concluded that carrot pomace powder delays the starch downgrade (which is equivalent to an increase in shelf life). The fortification of carrot pomace powder biscuits ensures the improvement of their sensory, nutritional quality and microbiological stability.

Key words*: carrot, pomace, powder, biscuits quality, antioxidant capacity.*

INTRODUCTION

Fruit and vegetables are responsible for approximately 22% of food loss and waste along the supply chain. By-products from fruits and vegetables can be capitalized by obtaining flours with a high mineral content, in fibers and bioactive compounds, mainly related to fibers, thus bringing value to the food industry, by creating food products with high nutritional value and antioxidant potential, having beneficial health effects (Santos et al., 2022).
Carrot roots are traditionally used for the preparation of salads and soups, and at the industrial level, to obtain products with high nutritional value, such as juice, carrot concentrate, dry powder, canned carrots (Sharma et al., 2012; Barzee et al., 2019). The yield of carrot juice is quite low, therefore up to 50% of the raw material remains in the form of pomace (Surbhi et al., 2018).
Catana et al. (2019) obtained from carrot waste resulting from the processing of carrots in the form of juice, a functional ingredient like powder that stands out for its mineral content (K: 668.55-704.73 mg/100 g; Ca: 76.85-86.39 mg/100 g; Mg: 27.86-32.56 mg/100 g; Fe: 2.49-2.97 mg/100 g), ash (6.84-7.28%), protein (6.55-6.89%), total sugar (13.65-16.85%), total fiber (47.80-51.67%), vitamin C (13.85-15.63 mg/100 g), β-Carotene (10.70-13.65 mg/100 g), total polyphenols (265.14-295.85 mg/100 g) and have an antioxidant capacity (95.54-103.23 mg Trolox Equivalents/100 g). Sindhu et al. (2016) made an assortment of biscuits with the addition of defatted soy flour and carrot pomace powder and reported that the addition of these functional ingredients caused a significant increase in fiber and β-carotene content of the product.
The biscuits in which 17% defatted soybean flour and 12% carrot pomace powder were

used, have superior sensory qualities and high nutritional value. Also, Aglawe and Bobade (2018) made sweet fried cookies, using in its composition, carrot pomace powder (fortification level 5, 10 and 15%) and defatted soybean flour. Increasing the level of fortification with carrot pomace powder increases the content in protein, ash and total fiber. The sweet fried cookies product made with a 10% fortification level presented superior sensory qualities. Kausar et al. (2018) made a cake fortified with carrot pomace powder (fortification levels (4, 8, 12 and 16%). Fortification of the cake with carrot pomace powder resulted in an increase in ash content (0.42-1.16%), fat (21.43 %-22.64%) and crude fiber (0.2-2.35%). The fortification level of 12% with carrot pomace powder resulted in a cake with superior sensory qualities and high nutritional value.
This paper presents the research undertaken for the fortification of the biscuits with carrot pomace powder, in order to increase their nutritional value and antioxidant capacity.

MATERIALS AND METHODS

Materials

In order to get the product "Biscuits fortified with carrot pomace powder" the following materials were used: whole wheat flour, sugar from coconut flowers, virgin coconut oil (cold pressed), oat flakes, dried fruit, eggs, yogurt, walnut kernels, flax seeds, lemon juice, sodium bicarbonate, natural vanilla and sea salt. The level of fortification with carrot pomace powder was 25% (the reference was made to the amount of whole wheat flour used in the composition of the product).
"Control Biscuits" (C) were not fortified with carrot pomace powder.
Carrot pomace powder was obtained within the Vegetable-Fruit Processing Pilot Experimental Station, from IBA Bucharest (Figure 1).

Figure 1. Carrot pomace powder

The products "Biscuits fortified with carrot pomace powder" and "Control Biscuits" were made in the Cereal and Flour Processing Pilot Experimental Station.

Biscuits-making

The products "Control Biscuits" (C) and "Biscuits fortified with carrot pomace powder" were shaped and their baking was done in a "MONDIAL FORNI-ITALIA" oven for about 27 minutes, at a temperature of 180ºC.
Figures 2 and 3 show these products of "Control Biscuits" (C) and "Biscuits fortified with carrot pomace powder"

Figure 2. “Control biscuits” (C)

Figure 3. Product “Biscuits fortified with carrot pomace powder”

Methods

Statistical Analysis

The biscuits fortified with carrot pomace powder and Control biscuits were analyzed in triplicate. Mean and standard deviation are reported for each analytical parameter studied.

Sensory analysis

The sensory analysis was carried out using the descriptive method and the "Method of comparison with unit score scales" (Burnete et al., 2020).
Instrumental color analysis was performed with a CM-5 colorimeter (Konica Minolta, Japan) equipped with SpectraMagic NX software.
Instrumental texture analysis was performed with Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA) equipped with Bluehill 3.13 software.

Physico-chemical analysis
The physico-chemical analysis was carried out using the following methods: ACC 44-15A (moisture content), AOAC 979.09 (protein content), AOAC 963.15 (fat content), AOAC 923.03 (ash content) and AOAC 991.43 (total dietary fiber). The mineral elements were determined by atomic absorption spectrophotometry, after calcination of the samples. β-carotene content was determined by high-performance liquid chromatography Diode-Array Detector (Catană et al., 2020). Energy value (kcal/100 g and kJ/100 g) were calculated according to the Commission Regulation no. 1169/2011 (European Commission, 2011).

Thermal properties
Thermal properties of biscuits were investigated by differential scanning calorimetry (DSC), using DSC 8000 equipment.
The working method included 2 steps: the equilibration step for 1 min at 20°C and the heating step up to 120°C with a heating rate of 10°C/min. Using the thermal analysis program Pyrex Manager, the parameters were calculated: peak temperature (TP) and enthalpy value (ΔH).

Total polyphenol content
Total polyphenol content was performed by Folin-Ciocalteau spectrophotometric method, according to Horszwald and Andlauer (2011), with some modifications (Burnete et al., 2020).

Antioxidant capacity
Antioxidant capacity was performed by DPPH (1,1diphenyl-2-picryl hydrazyl) method, according to Horszwald and Andlauer (2011), with some modifications (Burnete et al., 2020).

Microbiological analysis
Microbiological analysis was performed according to SR ISO 21527-1:2009 (Yeasts and molds) and SR EN ISO 21528-1:2017 method (*Enterobacteriaceae*).

RESULTS AND DISCUSSIONS

Sensory analysis
Following the sensory analysis, it was found that "Biscuits fortified with carrot pomace powder" have a sweet and pleasant, characteristic taste, with natural vanilla aroma, they are well baked and tender. Following the sensory analysis by a panel of 10 tasters, applying the "Comparison method with unitary score scales", the products received the following scores and the qualification "very good": control sample C-20 points, and the product "Biscuits fortified with carrot pomace powder" (Sample-FB), 19.92 points (Figure 4).

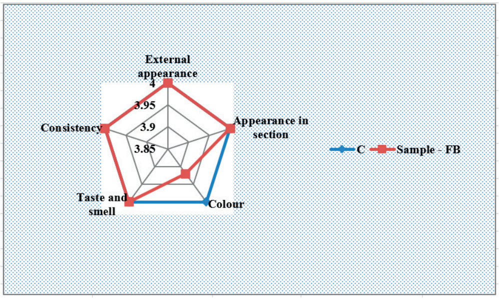

Figure 4. Sensory evaluation of the product "Biscuits fortified with carrot pomace powder" and of the control sample (C)

Textural properties of the products "Biscuits fortified with carrot pomace powder" and "Control biscuits" (C) are presented in Table 1.

Table 1. The textural properties of the product "Biscuits fortified with carrot pomace powder" and the control sample C

Product	Period (days)	Hardness (N)	Brittleness (MPa)
Control (C)	1	2.93 ± 0.10	14.47 ± 1.25
	7	3.99 ± 0.54	16.08 ± 1.41
	14	6.03 ± 1.11	17.95 ± 1.55
	36	7.32 ± 0.46	24.75 ± 1.84
	42	8.19 ± 2.43	28.95 ± 2.12
"Biscuits fortified with carrot pomace powder" (Sample-FB)	1	4.20 ± 0.18	8.08 ± 0.10
	7	6.46 ± 1.15	13.17 ± 0.89
	14	6.71 ± 1.35	16.91 ± 1.48
	36	9.59 ± 0.27	22.38 ± 1.75
	42	11.75± 0,27	23.17 ± 1.78

According to the experimental data we achieved, it causes an increase in hardness and a decrease in brittleness, compared to the control sample (C). Thus, after 42 days from the date of manufacture, the product "Biscuits fortified with carrot pomace powder" recorded values of 11.75 N for hardness and 23.17 MPa for brittleness.
The product "Biscuits fortified with carrot pomace powder" registered a significantly lower value of hardness one day after the date of manufacture (4.20 N), compared to the products "Cookies fortified with raspberry

pomace flour", "Cookies fortified with red currant pomace flour", "Cookies fortified with strawberry pomace flour", which recorded hardness values in the range of 11.88-36.09 N (Tarasevičienė et al., 2021).

Following the instrumental analysis of the color, we found that the fortification of the biscuits with carrot pomace powder causes a darkening of their color, compared to the Control sample (C) (Figure 5). Thus, the product "Biscuits fortified with carrot pomace powder" registered a luminance value (L* = 49.85), lower than that of the Control sample (C). Also, this product recorded the lowest values for the parameters a* (red-green color coordinate) and b* (yellow-blue color coordinate): a* = 9.88 and b*= 18.20.

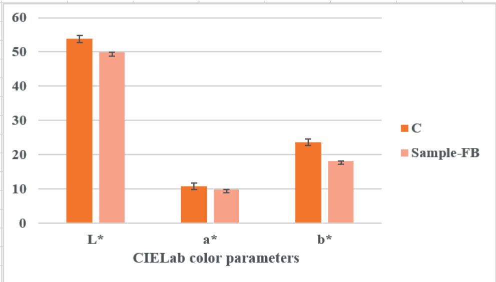

Figure 5. Color parameters of the product “Biscuits fortified with carrot pomace powder” and of the control sample (C)

Physico-chemical analysis

The physico-chemical analysis of the product "Biscuits fortified with carrot pomace powder" is presented in Table 2. Following the physico-chemical analysis, it was found that the fortification of biscuits with carrot pomace powder causes an increase in the content of ash (from 1.86% to 2.25%), fat (from 21.53% to 24.49%), and β-carotene (from 0.02% to 1.30%), in total dietary fiber (from 6.53 to 10.90%) and a decrease in available carbohydrates (from 43.20% to 35.59%).

The product "Biscuits fortified with carrot pomace powder" has a higher protein and fiber content than that reported by *Parveen* et al. (2017) in the case of biscuits fortified with carrot pomace powder (5, 7.5 and 10%) and beetroot pomace powder (1%): protein 7.38-7.95%; crude fiber 3.87-4.17%. Also, at the same time, the biscuits fortified with carrot pomace powder, made in this experimental study, have a significantly lower carbohydrate content (46.49%), compared to that reported by Parveen et al. (2017) in the case of biscuits fortified with carrot and beetroot pomace powder (carbohydrates: 62.09-64.73%).

Table 2. Physico-chemical composition of the product “Biscuits fortified with carrot pomace powder” and of the control sample (C)

Component	**C**	**Sample-FB**
Moisture (%)	15.45±0.39	15.64±0.39
Ash (%)	1.86±0.02	2.25±0.02
Protein (%)	11.43±0.10	11.22±0.10
Fat (%)	21.53±0.23	24.49±0.23
Carbohydrates (%)	49.73±0.02	46.49±0.02
Available carbohydrates (%)	43.20±0.01	35.59±0.007
β-carotene (mg/100g)	0.02±0.0003	1.30±0.02
Total dietary fiber (%)	6.53±0.12	10.90±0.20
Energy value (kcal/100g)	425	429
Energy value (kJ/100g)	1778	1788

The high β-carotene content of the product "Biscuits fortified with carrot pomace powder" (1.30 mg/100 g) should be noted. β-carotene (also called pro vitamin A) is a powerful antioxidant that provides protection against numerous conditions, including cancer, arteriosclerosis, cardiovascular disease and ulcers (Knockaert et al., 2012; Syamila et al., 2019). That is why the fortification of food products in general and bakery products in particular with β-carotene from natural sources is of real interest.

Also, the fortification of the biscuits with carrot pomace powder caused an increase in their content in mineral elements. Thus, the product "Biscuits fortified with carrot pomace powder" stands out for its content in mineral elements (Figures 6 and 7): K-445.75 mg/100 g; Ca: 185.47 mg/100 g; Mg: 127.68 mg/100 g; Fe: 2.26 mg/100 g; Zn: 1.72 mg/100 g; Cu: 0.85 mg/100 g.

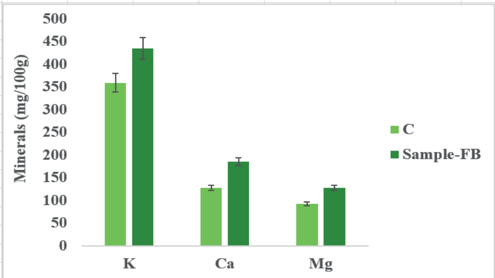

Figure 6. Mineral content (K, Ca and Mg) of the product “Biscuits fortified with carrot pomace powder” and of the control sample (C)

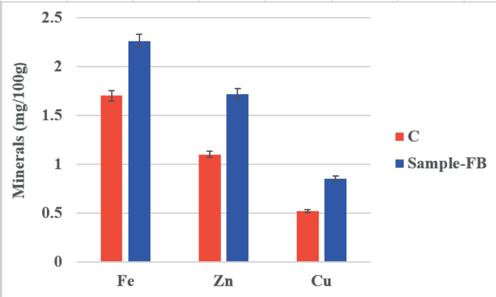

Figure 7. Mineral content (Fe, Zn and Cu) of the product "Biscuits fortified with carrot pomace powder" and of the control sample (C)

The potassium content of the product "Biscuits fortified with carrot pomace powder" is lower than that reported by Catană et al. (2018) in the case of the product "Biscuits with Aronia and cinnamon" (K: 592.16 mg/100 g), and the calcium and magnesium content is higher compared to that reported by these authors (Ca: 158.43 mg/100 g; Mg: 102.90 mg/100 g). At the same time, the iron and zinc content of the "Biscuits with Aronia and cinnamon" product is about 2.5 times and 4.7 times higher, respectively, compared to that of the biscuits fortified with carrot pomace powder, carried out in the framework of the research undertaken in this experimental study.

Thermal properties

The thermal properties of the products "Biscuits Martor" (C) and "Biscuits fortified with carrot pomace powder" were investigated using the differential scanning calorimeter, DSC 8000, 25 days after the date of manufacture. Thus, the peak temperature (TP) and the enthalpy value (ΔH), in the case of the 2 products, are presented in Table 3.

Table 3. Thermal properties of the product "Biscuits fortified with carrot pomace powder" and of the control sample (C)

Product	T_p, °C	ΔH, J/g
Control (C)	56,2	0,78
"Biscuits fortified with carrot pomace powder" (Sample-FB)	55,8	0,60

According to the experimental data we achieved, the fortification of biscuits with pomace powder causes a decrease in the peak temperature and the enthalpy value, compared to biscuits Control (C). Thus, it can be concluded that the fortification of carrot pomace powder delays the degradation of starch, which is equivalent to an increase in the shelf life.

Total polyphenol content

Due to carrot pomace powder fortification and the other ingredients that have a complex biochemical composition (oat flakes, dried fruit, walnut kernels, flax seeds, lemon juice), the product "Biscuits fortified with carrot pomace powder" has a high content of total polyphenols 260.75 mg GAE/100g, about 53% higher than the Control sample (C). In the same time, the total polyphenol content of this product is higher, about 1.4 times higher than that reported by Zlatanović et al. (2019), in the case of cookies fortified with apple pomace flour (fortification level 25%). The product "Biscuits fortified with carrot pomace powder" has a content in total polyphenols, comparable to that reported by Catană et al. (2018), for the "Biscuits with Aronia and ginger" (263.22 mg GAE/100 g).
Biscuits are a product consumed by all categories of consumers, and their fortification in order to increase the nutritional value and bioactive compounds (polyphenols, β-carotene, etc.) is of real interest. Polyphenols represent an important class of phytochemical compounds with multiple effects on human health.
Thus, based on the research undertaken, there are preclinical and clinical evidences, which highlights the fact that diets rich in polyphenols, in the long term, prevent the occurrence of various chronic diseases, such as neurodegenerative diseases, cardiovascular diseases, cancer, diabetes, inflammatory disorders and infectious diseases (Rudrapal et al., 2022).

Antioxidant capacity

Due to the content in bioactive compounds (polyphenols, β-carotene, etc.), the product "Biscuits fortified with carrot pomace powder" has antioxidant capacity: 338.23 mg Trolox/100 g (about 1.4 times more than the control sample).
The product "Biscuits fortified with carrot pomace powder" has an antioxidant capacity comparable to that reported by Catană et al.

(2018), in the case of "Biscuits with Aronia and ginger" (342 mg Trolox/100 g).

Microbiological analysis

The microbiological analysis of the product "Biscuits fortified with carrot pomace powder" and the Control sample (C) is presented in table 4.

Table 4. Microbiological analysis of the product “Biscuits fortified with carrot pomace powder” and of the control sample C

Microbiological indicator	Product			
	Control (C)		“Biscuits fortified with carrot pomace powder” (Sample-FB)	
Yeasts and molds (CFU/g)	24h	< 10	24h	< 10
	10 days	< 10	10 days	< 10
	20 days	< 10	20 days	< 10
	30 days	< 10	30 days	< 10
	40 days	< 10	40 days	< 10
	50 days	-	50 days	< 10
Enterobacteriaceae (CFU/g)	24h	< 10	24h	< 10
	10 days	< 10	10 days	< 10
	20 days	< 10	20 days	< 10
	30 days	< 10	30 days	< 10
	40 days	< 10	40 days	< 10
	50 days	-	50 days	< 10

Following the microbiological analysis of the "Biscuits fortified with carrot pomace powder" product, it was found that it complies with the provisions in force even 50 days after the date of manufacture. The microbiological stability of the product is due to antibacterial activity of carrot pomace powder. Bello et al. (2019) demonstrated that aqueous extract of peel carrot (concentrations 25-200 mg/mL) present bacteristatic action against *S. aureus* and *E. coli*.

Corroborating the results of the microbiological analysis with the sensory results and the results of the instrumental analysis of the texture, the shelf life of the product "Biscuits fortified with carrot pomace powder" was set at 25 days.

CONCLUSIONS

Taking in account the circular economy and the increasing value of added food products, their fortification with carrot pomace powder is of real interest.

Fortifying the biscuits with carrot pomace powder ensures a product with superior sensory qualities, high nutritional value and antioxidant capacity. The product "Biscuits fortified with carrot pomace powder" was appreciated by a panel of 10 tasters, receiving a score of 19.92 points and a "very good" rating.

The product "Biscuits fortified with carrot pomace powder" has a low content in available carbohydrates (46.49%), but a high content in proteins, total fibers, mineral elements (K, Ca, Mg, Fe, Cu Zn), total polyphenols. Also, due to its complex biochemical composition, this product has antioxidant capacity.

The product "Biscuits fortified with carrot pomace powder" according to the provisions of Regulation (EC) NO. 1924/2006 of the European Parliament and of the Council, it is high in fiber because it has a fiber content of more than 6g/100g.

Due to the antibacterial activity of carrot pomace powder, the product "Biscuits fortified with carrot pomace powder" has microbiological stability, with a shelf life of 25 days.

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LIPIDS EXTRACTION METHODS APPLIED ON *Nannochloropsis* sp. BIOMASS - A REVIEW

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Abstract

Nannochloropsis sp. is a microalga of particular interest for the production of lipids containing omega 3 - fatty acids, specifically eicosapentaenoic acid (EPA), a fatty acid mostly found in the flesh of cold-water fish and crustaceans with high importance in human health. Because of the rigid cell wall structure of Nannochloropsis sp., the extract of EPA requires specific methods. There are certain critical points regarding lipidic extraction: the application in food and feed, and the fractionation methods that provide a high recovery rate of EPA. Therefore, green extraction methods have recently gained more and more interest, having minimal environmental and health impacts, as they use less or no organic solvents, being sustainable productive, and efficient. The methods used for lipid extraction should ensure that during the process, the lipid extraction is obtained without influencing the fatty acid composition. The purpose of this review is to summarize the existing research parameters regarding different green extraction methods applied for obtaining lipid fractions, emphasis on supercritical fluid extraction, ultrasound-assisted extraction, and accelerated solvent extraction methods.

Key words: *eicosapentaenoic acid, fractionation methods, microalgae.*

INTRODUCTION

As it is presented in many studies, in the global context the world population is increasing. Along with this, there will be an increase in the need for food and implicitly resources of any kind. Also, these resources in turn are renewable and non-renewable, both being exhaustible at a certain point.

In this context, we are forced to find or explore alternative sustainable edible sources that can satisfy human and animal nutritional needs and reduce competition in the use of traditional ones.

One such source rich in micronutrients are microalgae (Montoya-Arroyo et al., 2022).

Microalgae represent a group of autotrophic microorganisms living in aquatic and terrestrial ecosystems and producing organic substances by photosynthesis.

Today the main areas of use of the microalgae are biomass production (as a biological additive) and the cultivation for isolation of their biologically active substances (Vyacheslav Dolganyuk et al., 2020).

Microalgae are considered an alternative to unconventional sources of biologically active compounds and food supplements for animal and human nutrition (Lorenzo Zanella, Fabio Vianello, 2020). As a source of proteins, polysaccharides, lipids, polyunsaturated fatty acids, vitamins, pigments, phycobiliproteins, enzymes, etc., plays an antioxidant, antibacterial, antiviral, antitumor, regenerative, antihypertensive, neuroprotective, and immune-stimulant role. Considered one of the greatest primary producers of any aquatic habitat, they have high growth rates requiring only water, nutrients, and carbon dioxide (Salbitani et al., 2021).

Therefore, there is a demand for these compounds in domains such as medicine, the chemical industry, fish farming, the energy industry, and agriculture in the production of feed and functional foods (Vyacheslav Dolganyuk et al., 2020).

The consumption of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), like eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) is associated with the health benefits, healthy, balanced diet and well-being (Douglas R. Tocher et al., 2019).

At present, the only commercial source of EPA is marine fish oil, a rather unsatisfactory source because of problems of contamination, taste, odour and stability. In addition, the presence of considerable amounts of other PUFAs in the fish oil complicates the EPA purification process, resulting in high retail prices of the pure product These factors have led to investigation of alternative EPA sources.

One such industrially promising species is *Nannochloropsis* sp. with elevated photosynthetic efficiency and lipid productivity.

Due to its high lipid content (37-60%), high yields of the omega-3 (ω-3), in particular, eicosapentaenoic acid (EPA) makes them become a candidate for commercial applications for human consumption (Salbitani et al., 2021).

An important issue in lipid extraction to consider is the selection of the right extraction method. In fact, extracting oil from biomass is necessary to choose a fully compatible solvent or a mixture of solvents (ratio) that will not alter its bioactivity.

The robust and complex cell wall structure of microalgae leads to the fact that the lipids are trapped in the cytoplasm by cell walls and cell membranes, so the lipids from the cells cannot be completely extracted, this aspect affecting the lipids' yield.

Therefore, there are several gaps in choosing the extraction method for a specific compound from different complex matrices. The robust structure of *Nannochloropsis* sp., requires an integrated approach to lipid fraction extraction methods. Among other things, this will take into account several aspects related to the cell structure, the nature of the solvents used, the extraction yield and last but not least the extraction costs, all of which are raised to a scalable level.

In this context, the paper presents an analysis of the literature regarding the impact of the extraction methods associated to extraction solvents, preceded by cell disruption operations on lipid extraction from *Nannochloropsis* species.

MATERIALS AND METHODS

There are several types of extraction procedures both time and solvents consuming, such as conventional (Soxhlet, Bligh-Dyer and Folch). Also, conventional extraction techniques involve the use of organic solvents considered as not safe for humans and the environment (methanol, chloroform, acetone, etc.).

Therefore, the solvent extraction methods can be combined with the ultrasonic crushing method, microwave, autoclave, bead milling methods and other crushing methods to improve the rate of lipid extraction (Corrêa et al., 2021; Ren et al., 2021; Alhattab et al., 2019; D'Hondt et al., 2017; Naghdi et al., 2016). The solvent extraction methods have the advantages of high lipid yield, high lipid quality, and easy realization of large-scale production. However, usually in the extraction methods are used organic solvents which are toxic and volatile with a negative impact on all ecosystems (Naghdi et al., 2016). In recent years there is a demand to reduce the amount of solvent used in microalgae lipids extraction and also for greener, safer, and more natural products that do not require the involvement of toxic solvents thus minimizing the environmental impact (Ren et al., 2021; Imbimbo et al., 2020; Naghdi et al., 2016).

The solvents must be chosen depending on the compound polarity. The combination of polar and non-polar solvents leads to an increase in lipid extraction/recovery. The polar solvents have the ability to release the lipids from their protein-lipid complexes which facilitate their dissolving in the non-polar solvent (Abimbola et al., 2021; Naghdi et al., 2016). The lipid extraction/recovery is higher when these methods are applied on wet biomass, so the polar solvent can penetrate the water layer and make the lipids available for non-polar solvent solvation. The best solvent-free techniques are the ones which can be performed on a diverse variety of algae with low energy consumption and minimum initial set-up costs for infrastructure (Eikani et al., 2018; Naghdi et al., 2016).

Despite being well known and often used, conventional methods use toxic solvents and are not in accordance with environmental and human health concerns (Corrêa et al., 2021). Bligh-Dyer and Folch methods are classical methods widely used which can be performed directly on microalgae because they extract lipid from the microalgal cell without the additional requirement of cell rupturing (Matos et al., 2019; Nagappan et al., 2019, Ranjith et al., 2015). The chloroform used in classic methods has been shown that is carcinogenic and ozone-depleting (Byrne et al., 2016; Chua et al., 2017).

Generally recognized as safety solvents like hexane, buthanol, ethanol, ethyl acetate (EtOAc), 2-methyl tetrahydrofuran (MeTHF), can be used alone or in combination for lipid extraction from microalgae by dissolving hydrophobic cell membrane components. Solvent mixtures like hexane-ethanol (4:1), and hexane-isopropanol have been demonstrated to be more efficient then using solvents alone, for lipid extraction. The alcohols from the mixture break the electrostatic forces and a hydrogen bond between membrane lipid and associated protein, so non-polar component of the solvent mixture can enter into the cell, followed by neutral lipid extraction (Nagappan et al., 2019; Ahmad et al., 2018).

According to Abimbola et al., 2021, ethanol efficiently extracts lipids from algae without the need for the cell disruption step. In their studies found that hexane extraction recovers oil from: *N. salina* and *N. oculata*, with about 60% Fatty acid methyl esters (FAME) content. Ethanol extraction method recovers more lipids at higher Total solids content (TS). The solvent biomass ratio was of 25. Ethanol extraction giving higher yields with 23% more recovery at 20 and 10 wt % TS while about 14% more recovery was obtained at 15 wt % TS.

Dimethyl ether (DME), a green solvent, features a high affinity for both water and organic compounds with an ability to penetrate the cell walls and successfully extract lipids from microalgae without the requirement of drying the biomass (Wang et al., 2021; Nagappan et al., 2019).

With Soxhlet extraction using n-hexane as solvent was obtained a 45% (dw) yield of lipid from dried *Schizochytrium limacinum* and with ethanol, extract 48% (dw) lipid from dried *Synechocystis* PCC 6803. With supercritical extraction using CO_2 and ethanol was obtained a 34% (dw) yield of lipid from dried *S. limacinum* powder. 18.1% (dw) lipid yield from dried *Chlorella* spp. powder was obtained using a mixed extraction solvent of methanol: ethyl acetate at a volume ratio of 2:1.

Liquid dimethyl ether (DME) can be used also, for the extraction of lipids from microalgae. Liquid DME is partially miscible with water and has a high affinity for organic compounds. Thus, DME is suitable for extraction of lipids from wet biomass samples with simultaneous dewatering with a 25 mL DME for 8 mL of microalgae (Wang et al., 2021)

Extraction of lipid from microalgae can be performed also with water which it is food grade chemical, and resistant to auto-oxidation. The property of a low boiling point allows easy solvent recovery after initial extraction, thereby reducing the microalgal lipid extraction cost (Nagappan et al., 2019).

Bernaerts et al., 2020, used as a method of vacuum filtration and rotary evaporation using hexane: isopropanol (3:2 v/v) for lipid extraction from *Nannochloropsis* sp.

Herrero et al., 2004, obtained the higher oil yield from dried extract of *Spirulina* sp. using water and ethanol as solvents, carried out by Accelerated Solvent Extraction system (ASE 200), the extracted amount increases using higher extraction times and/or higher extraction temperatures (15 minutes at 170ºC).

Angles et al, (2017) found that the best solvents for lipid extraction were Methyl-tert-butyl ether (MTBE) and cyclopentylmethyl ether (CPME). They represent alternatives to chlorinated solvents or alkanes and they are followed by MeTHF and EtOAc, which are green solvents.

Iovine et al. (2019), used as solvents for accelerated solvent extraction by Dionex ASE 200, hexane and a mixture of chloroform, methanol/water (C/M/W) for accelerated solvent extraction at 50°C, two cycles of 10 minutes. Using hexane, the fatty acids obtained were 2.83 times higher than fatty acids obtained without pre-treatment (mechanical pre-treatment using the Planetary ball).

Sánchez-Camargo et al., 2018 used high-pressure homogenization (HPH) to break down the strong cell wall and supercritical fluid extraction (SFE) with pure CO_2 was applied as a first step to extract valuable compounds (such as non-polar lipids and pigments). Extraction of the remaining residue for the recovery of bioactive compounds employing pressurized liquid extraction (PLE) with green solvents such as water and ethanol. Optimum extract was achieved with pure ethanol at 170°C for 20 min.

Blanco-Llamero et al. (2021) used Pressurized Liquid Extraction (PLE), carried out with ASE 350 DIONEX extractor using 20-25 mL from the following solvents: ethanol, 2-MeTHF, and different mixtures of hexane: ethanol (3:4), 2-MeTHF: ethanol (1:3), MTBE: ethanol (1:3), 2-MeTHF: isopropyl alcohol (1:3), and 2-MeTHF: isobutanol (1:3), heated to 90, 120, and 150°C and static extraction time was 15 min for each experiment. The mixtures of isopropanol, isobutanol, and MTBE produced the highest SFA content, ethanol, mixtures of 2-MeTHF: ethanol, 2-MeTHF: isobutanol, and mixtures of hexane: ethanol were the ones with higher PUFA content. The authors, also optimized the methods using a 1:3 ratio of 2-MeTHF and ethanol, with good results for the extraction of polar lipids with omega-3 PUFAs from algal biomass. 2-methyl-THF can be used successfully use as hexane substitute in solvent mixtures with alcohols when extracting by Pressurized Liquid Extraction polar compounds from microalgae (*N. gaditana*, *I. galbana*, *T. chuii*) being in agreement with green chemistry.

Derwenskus et al. (2019), also use pressurized liquid extraction (PLE), performed using accelerated solvent extractor (ASE 350). In the study they used 5 g of dry (30% w/w) and wet algae biomass (*Phaeodactylum tricornutum UTEX 640* and *Chlorella vulgaris*), with biomass/solvent/water ratio (g/ml/ml) of 1/14/0 for dry biomass and 1/12/3 for wet biomass. Extraction temperatures were chosen between 50 and 150°C. Static extraction time was set to 20 min with a rinse volume of 60% and a nitrogen purge time of 300 s. The solvents used, where the following: ethanol, ethyl acetate and hexane. The more suitable extraction for triacyl glycerides (TAG) from wet biomass of *C. vulgaris*, was achieved with medium-polar solvents like ethyl acetate. Fatty acid yields of above 75% w/w were achieved for wet biomass of both microalgae in a single extraction step at temperatures of up to 150 °C.

Park et. Al., 2020, use for dry extraction 1 g of *N. oceanica* and hexane (96%), mixture of hexane and methanol (99.6%) (7:3, v/v), and mixture of chloroform (99.0%) and methanol (7:3, v/v). The total volume of each solvent was 40 mL. For wet extraction, 1 g of dry microalgae was at first mixed with 4 g of distilled water having a concentration of 200 g/L (80% water content). Amount of 5 g of wet microalgae was mixed with the solvents mention for dry extraction and also the total volume of each solvent was 40 mL. The samples were stirred at 1,000 rpm for 6 h at room temperature, and then distilled water was added for separation of the organic solvent layer, and after that 4.000 rpm centrifugation for 5 min. The best results of microalgae oil were obtained with hexane-methanol extraction.

CELL DISRUPTION PROCEDURES AND ASSOCIETED RESULTS

According to Laura Soto-Sierra et al. (2018), for releasing an intracellular compound, a disruption treatment is necessary. Preferably, one that selectively releases the compound using the least possible energy (Figure 1).

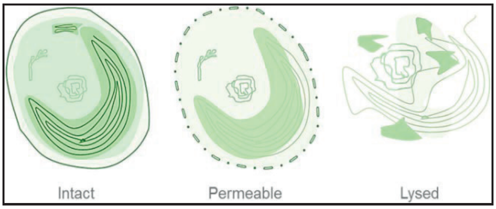

Figure 1. Levels of cell disruption. From non-disrupted (left) to complete cell disruption-lysis (right) (LauraSoto-Sierra et al., 2018)

Choosing a cell disruption method (Figure 2) may depend on the cell wall structure of the sample, compound location, size, solubility, and applied energy. Based on the disruption force, the methods can be classified as physical (drying, sonication, and pulsed electric field), mechanical (bead milling, homogenization) and chemical/biological (pH shift, enzymes, microwave, etc.).

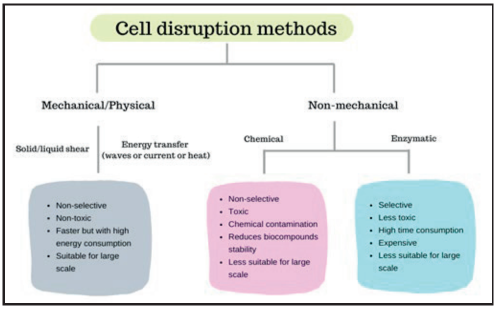

Figure 2. Cell disruption methods
(www. https://encyclopedia.pub/, 2022)

Bead beating includes mechanical stirring and grinding that cause disruption to the cell (ShunyuYao et al., 2018). Bead mill or bead beating are shaking vessels filled with quartz or metal, in which microalgae biomass are disrupted by agitation, friction, collision and grinding, mechanical stirring. Therefore, cells are damaged by direct impact with the beads (steel, zirconium, glass or ceramic) (LauraSoto-Sierra et al., 2018) at high speed. Also, the size of beads and the bead filling ratio are important. For microalgae the optimal beads diameter is 0.5 mm (Ashok Ganesan et al., 2022).
The disadvantage of this method is referring to the difficulty to scale-up (Fabiana Passos, 2015).

Sonication (Figure 3) is a physical treatment based on bubble cavitation by ultrasound waves that promote a non-specific cell-surface barrier disruption (Jose A.Gerde et al, 2012). The ultrasonication method for cell disruption is based on liquid-shear forces caused by emission of high frequency wave sounds.

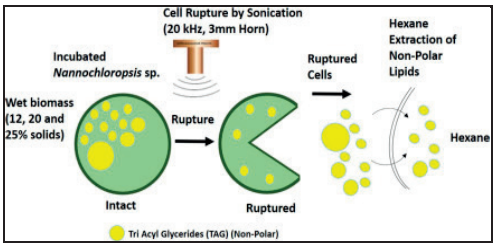

Figure 3. Cell disruption by sonication
(ShunyuYao, et al., 2018)

In liquid, these sound waves create gas bubbles or cavities that achieve a critical size and releasing large amounts of energy; during the extraction local temperature increases and forms hydroxyl radicals that damage the cell wall, and also may produce lipid hydroperoxides (Ying Liu, 2022). Sonication is able to disrupt (Figure 4) the cells at relatively low temperatures avoiding thermal protein denaturation. Using this method is low toxicity, and time saving and can be scaled-up and operated continuously.
Another possibility for cell disruption efficiency is cell weakening by incubation, preceding ultrasound extraction procedure (ShunyuYao et al., 2018). This consists on the dilution of microalgae biomass, obtaining a slurry, and incubated at 40°C on a plate, continuously stirring, during 24 hours.
As procedure, the extraction method is directly corelated to the microscopic evaluation of cell morphology.

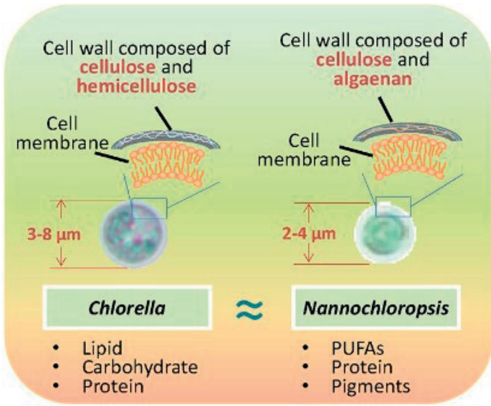

Figure 4. Cell rupture by ultrasonic treatment in *Nannochloropsis* and *Chlorella* species

It was observed that ultrasound could change the external structure of cell surface. The high local temperature and pressure caused by collapsing bubbles could lead to the breaking of *Nannochloropsis* cells into small fragments, and resulted in release of oil into the liquid (Figure 5).
Nevertheless, the extraction procedure involving the organic solvent penetrates the cell membrane and dissolves the lipids as well as the lipoproteins of chloroplast membranes. It has been found that cell disruption efficiency is strongly corelated to chlorophyll and carotenoids content (Aris Hosikian, 2010).

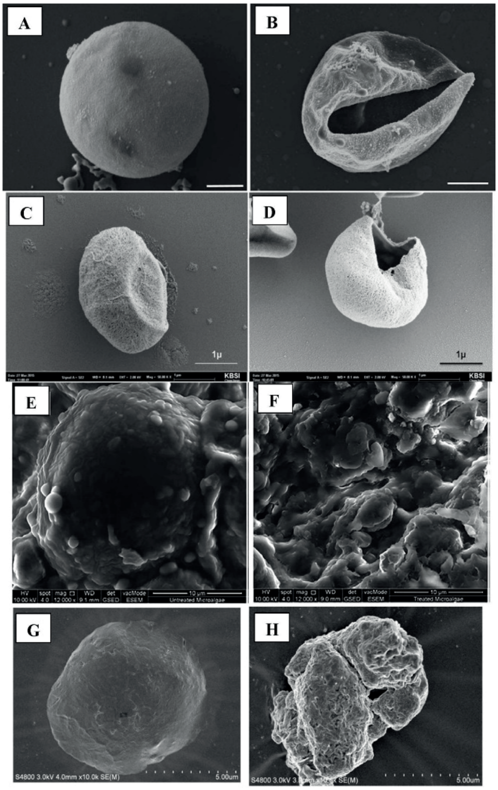

Figure 5. Deformation in cell surface induced by ultrasound (Ying Liu et al., 2022)

This aspect can be an important one in reducing the actual work time and costs, but also the selection of the type of extraction before GC-MS analysis

CONCLUSIONS

Taken together, our data showed that manipulation of photosynthetic pigments, can be an item related to the extraction procedure.
There are several generally recognized as safe solvents and green solvents that are used in the extraction methods for feed and food, but may be used in other components for reducing the environmental impact.
However, the extraction procedure is more efficient when is preceded by cell disruption technique or cell weakening procedure. is not very effective for some microalgae species and it is commonly combined with chemical treatments for efficiency improvement and to reduce energy demand.

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PROCESSING METHODS USED FOR ORGANIC VEGETABLE CHIPS - REVIEW

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Abstract

Due to their compounds and their beneficial properties for the body, vegetables are important ingredients in a balanced daily diet. By definition, vegetables are edible parts of the plant that can include: stems, roots, tubers, bulbs, leaves, flowers and beams. It is recommended to eat fresh vegetables, but when this is not possible, they can be preserved in various forms. One of the oldest techniques for preserving vegetables is drying, which involves reducing the water content of vegetables by exposing them to the sun or artificial heat sources. Dried vegetables are produced by different processes. In general, dried vegetables follow the same steps: selection of vegetables according to their shape and quality, peeling, slicing, preservation, dehydration (natural or artificial), sweating or salting, visual inspection and packaging. Drying is beneficial because it extends the shelf life, reduces postharvest waste, and massively helps reduce storage and transportation costs. This article reviews various methods of pre-processing of organic vegetables (cutting forms, different forms of bleaching, etc.) and different drying methods (classic by varying the temperature, vacuum, etc.).

Key words: *organic, vegetables, drying, chips.*

INTRODUCTION

Vegetables are annual or perennial crops with an important role in human nutrition due to nutrient content (vitamins, minerals, fibres, phenolic compounds, etc.). Their consumption can have beneficial effects in the fight against obesity, cardiovascular diseases, diabetes and even different types of cancer (Bahceci et al., 2005; Cruz et al., 2006).

Worldwide, there are approximately 10,000 plants used as vegetables that can be classified in their turn, depending on the edible part, as: green plants (classified as: leafy vegetables with stems and fruits and vegetables with flowers) and roots (classified in their turn in legumes, roots, bulbs and tubers) (Erickson, 2015; Farkas & Hubbard, 2000; Garrote et al., 2004).

Vegetable can be eaten whole or partially, raw or cooked (Aguero et al., 2008). Although the best way to consume most vegetables is in fresh form, vegetable chips are becoming an increasingly popular snack. On the market there are chips made from carrots, parsnips, beets or sweet potatoes, prepared by the traditional method - frying in a hot oil bath (Figure 1).

Figure 1. Vegetable chips from the market (www.nutracheck.co.uk, www.trafochips.nl, www.terrachips.eu)

Besides the classic method of frying in a hot oil bath, drying is one of the oldest techniques for preserving vegetables (Hamid & Mohamed Nour, 2018). This involves reducing the water content of vegetables by exposing them to the sun or to artificial heat sources, or by alternative methods such as freeze drying. Drying is beneficial because it extends the shelf life, reducing microbe development, reduces postharvest waste, and massively helps reduce storage and transportation costs (Lenart, 1996). Dehydrated vegetables are consumed all over

the world and are generally made from potatoes, carrots, celery, parsnips, beetroot, pumpkin, beans or tomatoes. Because some vegetables cannot be dehydrated directly, they are processed as a paste with the addition of salt and spices, after which they are formed and dehydrated (Huang et al., 2011).
Generally, dried vegetables follow the same steps: selection of vegetables according to their shape and quality, peeling, slicing, preservation, drying (natural or artificial), sweating or salting, visual inspection and packaging (Figure 2).

PROCESSING TECHNOLOGIES

In order to obtain chips from vegetables, several stages must be completed (Figure 2): vegetable selection, peeling and slicing, pre-processing, drying, etc. (FAO, 2020; Matz, 1984).

Pre-processing

An important stage to obtain chips is the blanching. Blanching is used to maintain the color, freshness and nutritional quality, stabilize the texture and inactivate the enzymes responsible for producing the unpleasant smell. The process involves thermal inactivation of unwanted benzenes (peroxidase and lipoxygenase), decreasing the rate of enzymatic deterioration of vegetables (Aguero et al., 2008; Bahceci et al., 2005; Cruz et al., 2006; Garrote et al., 2004; Morales et al., 2002; Nissreen and Helen, 2006; Saldivar et al., 2010; Soysal and Soylemez, 2005).
In the study carried out in 2010, Saldivar et al. found that soybean pods retained soluble sugars when steam-blanched for 10 minutes. When blanched samples were quickly frozen, they showed minimal microstructural damage due to the formation of smaller ice crystals compared to unblanched vegetables (Wang et al., 2007). Furthermore, blanching contributes to a better preservation of pigments and can also limit the degradation of chlorophylls and carotenoids (Lisiewska et al., 2004).
Although blanching seems like a wonderful pretreatment, it also causes unwanted changes to the food. Thus, being a thermal process carried out at high temperatures, changes may occur regarding the thermal processing or the loss of soluble nutrients - sugars, vitamins, minerals, etc. (Olivera et al., 2008, Piotr and Waldemar, 2007, Rungapamestry et al., 2007, Song et al., 2003, Volden et al., 2009).
The lowered moisture content provides the chips with good preservation of concentrates for a long time. Various methods can be used, such as sublimation, vacuum, convection or infrared, as such or combined.

Heat drying process

The classic drying method frequently used, and also the simplest, is drying vegetables by electric or solar convection (Mujumdar, 2006). The method involves subjecting the samples to currents of hot air until the amount of water in it is reduced to a minimum. The method comes with many disadvantages, including browning and the decrease in the amount of nutrients in the vegetables due to high temperature air currents.

Infrared radiation (IR)

IR is a form of electromagnetic waves that comes from a heat source which requires no medium for its emission, and is located at the outer range of the visible red light. The infrared radiation wavelength varies from 0.75 to 1000 μm. Infrared radiation can be divided into three different categories, namely near-infrared (NIR 0.75-2 μm), mid-infrared (MIR 2-4 μm) and far-infrared (FIR 4-1000 μm) (Jain & Pathare, 2004). IR drying method uses energy in the form of infrared rays to penetrate food products to a small depth, then turning into heat. Compared to thermal radiation technology, infrared rays have the advantage of lower energy consumption, with increased efficiency. The quality of the final product is also higher, due to the shorter treatment time. The technology also has the advantage of equipment simplicity, adaptability and easy combination with other heating/treatment methods.
In 2021, Ochirov et al. experimented with this technique on carrots. Experimental studies were carried out on an experimental infrared drying plant, in an oscillating mode "heating - cooling" which allows intensifying the drying process and shortening its duration. Recording results on the change in weight and size of carrot slices along the length was carried out

every ten minutes. The resulting carrot chips have a high nutritional value, no significant changes in color, odor and taste after prolonged storage in plastic wrap.

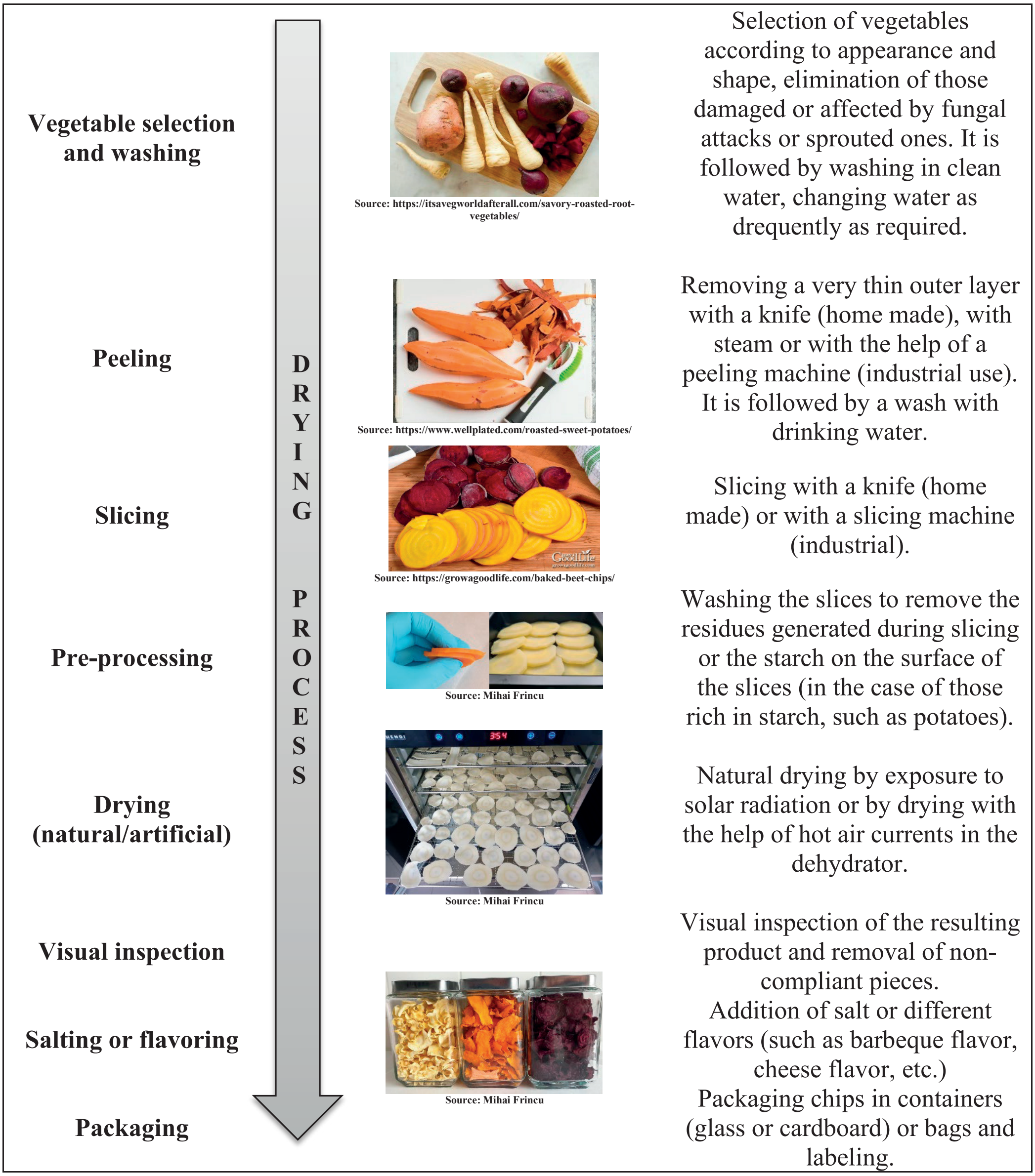

Figure 2. Process of obtaining chips from vegetable (Matz, S. A., 1985; FAO, 2010; Lenart, A., 1996)

Cold drying processes

During lyophilization, the sliced vegetables are first frozen at a very low temperature, after which they are introduced into the device's chamber. At low temperature, under the action of vacuum, the water is transferred from the product to the machine's condenser by sublimation. After all moisture sublimes, a very fine porous structure remains that is easily rehydrated (Fan et al., 2018; Bhatta et al., 2020; Liu et al., 2021).

In 2019, a comparative study between three drying methods (hot-air, combined hot-air-microwave, and vacuum-freeze) was conducted by Jia et al. The effect of drying methods on sensorial, textural, nutritional, and other quality characteristics of persimmon chips was followed. Their results showed that freeze-dried chips and combined hot-air-microwave technique had the best nutritional and quality scores compared to hot-air dried samples. Therefore, higher amounts of β-carotene, ascorbic acid, total phenol and sugar content were determined in freeze-dried chips. All this led to chips with greater flavor and taste and overall acceptability of freeze-dried chips.

Deep frying

In food production, deep frying is one of the most used techniques. It lends itself to a wide variety of products: from chicken products, fish products, pastries, vegetable chips to French fries. The method involves cooking food at a temperature higher than the boiling point of water in vegetable oil or animal fat (Moreira 2007; Erickson, 2015; Farkas and Hubbard, 2000). The process is based on the simultaneous heat and mass transfers from the oil to the product, a complex process due to two mass transfer operations that take place between the product and the oil when it is fried and vice versa. An example is of products containing starch (potatoes) where starch and water pass from the product into oil, oil which later takes their place in the product (Ziaiifar, 2009; Ziaiifar et al., 2008; Oke et al., 2017).

INFLUENCE OF PROCESSING ON VEGETABLE QUALITY

Vegetable chips

As specified in the introduction there are various vegetables used for chips production, but the most common are: potato, carrots, beetroot, parsnips and sweet potato.

Potatoes chips

Potato (*Solanum tuberosum* L.), with a production of 370 million tons in 2021, is one of the major crops grown worldwide. Potato is produced all over the world, in a huge variety of soils, the first 10 countries in terms of production being: China, India, Ukraine, Russian Federation, United States of America, Germany, Bangladesh, France, Poland, Netherlands (FAO, 2021).

Chips made from potatoes are generally obtained by frying in oil at high temperatures. This process generates large amounts of toxic compounds. In 2015, Mariotti et al. studied different forms of blanching aimed at mitigating the furan and acrylamide formation in potato chips without increasing their oil uptake. Potato slices were blanched between 5 and 15 min, varying the temperature from 50°C to 80°C in order to simultaneously leach out ascorbic acid and reducing sugars, the most important precursors of furan and acrylamide generation in thermally treated starchy foods. After the blanching, potato slices were fried at 170°C up to 98% DM. The optimal variant was the one in which the potato slices were blanched for 17 min at 64°C, conditions in which a significant reduction of furan (91%), acrylamide (54%) and oil content (19%) was observed.

Carrot chips

Carrots is also part of the root crops grown worldwide. Annual production exceeds 44 million tons, and the countries with the highest production are: China, Uzbekistan, USA, Russian Federation, Ukraine, UK and Germany (www.Atlasbig.com). Carrots are used for human consumption as well as animal feed; they are cooked alone as chips or with other vegetables in the preparation of soups, stews, curries, and pies; fresh grated roots are used in salads; tender roots are pickled. Also, Carrots possess many medicinal properties and are used in Ayurvedic medicine. They are a rich source of b-carotene and contain appreciable amounts of thiamine and riboflavin (Ratnadass et al., 1990).

In 2018, Peng et al. studied freezing and its effect as a pre-treatment for carrot chips. They treated carrots in 5 ways: control (4°C for 12 h), 3 variants with freezing at −18, −40 and −80°C for 12 h and a 5th placed in a polystyrene container, was treated with liquid nitrogen for 5 minutes, followed by transfer to the freezer and stored at −80°C for 12 h. After pretreatment, the carrot pieces were dried by Instant controlled pressure drop (DIC). Freezing as a pretreatment at −18 and −40°C led to carrot chips with superior porous structure, relatively low hardness and expected high volume expansion after DIC treatment.

Beetroot chips

In the specialized literature, there is no evidence about the origin of the beet, but it is believed to have originated in the Mediterranean regions and near Asia. With an annual production of approximately 280 tons/year, beet is a widespread plant that is cultivated throughout the year around the globe. The main countries that cultivate it are: Russian federation, France, Germany, USA, Turkey, Poland and China (FAO 2021, www.atlasbig.com).

Various studies have been done on the production process of beetroot chips. Thus, Juvvi et al. studied in 2016 the possibility of obtaining beetroot chips with a lower oil content in the laboratory, using a vacuum fryer. They studied 20 different combinations of temperature, absolute pressure and frying time. They obtained the best results when deep frying beetroot at a temperature between 101 and 110°C, pressure between 2.9 and 4.4 kPa and 6 minutes of cooking time, with oil content ≤ 15.7 (comparing with 28.41% in traditionally fried beetroot chips) and an overall acceptability ≥ 7.5 compared to 6 for traditionally fried beetroot chips.

Raupp et al. (2011) studied the effect of the drying process on the antioxidant potential and the content of phenolic compounds in beets. To obtain chips, the beet slices were dried in a dehydrator. The optimal drying variant, with a higher content of beneficial compounds, was drying at a high temperature and a shorter time (100 + 90°C/5.6 hours; 90°C/6 hours).

In 2017, Nistor et al. compared 3 combined techniques for obtaining beetroot chips: free convection (at 50, 60, and 70°C), forced convection at 40°C and 315 W microwave power. The aim was to investigate the effect of the drying techniques on the quantity of betalains, polyphenol and microstructure changes (SEM). A strong thermal shock, provided by convection at 60°C followed by microwave wattage 315 W/9 min, leads to a better preservation of bioactive compounds content (0.631 ± 0.0042 mg/g of betacyanin and 0.795 ± 0.0019 mg/g betaxanthin) when compared to convection at 50, 60 and 70˚C. They concluded that combined drying methods led to a significant preservation of the phytochemical content as compared to the traditional methods.

In the next year (2018) Hamid et al. studied the effect of different drying methods on the quality of beetroot chips. They were using three drying methods (sun, oven and freeze-drying), and the chemical composition, minerals, nitrates, betalains, total phenolic, total flavonoid and color were measured for fresh slices and dried chips. At all 3 drying options, the results showed that the chemical composition, total energy, minerals and nitrate of the dried slices were increased compared to that of fresh slices of beetroot. Sun and oven drying of the slices reduced total betalains and betacyanin, instead the content of betaxanthin was increased. Regarding the content of total polyphenols and antioxidant activity, they increased after sun and oven drying but total flavonoids were decreased. In terms of color of the chips powder measurements, the color of the powder obtained from freeze-dried slices was stable compared to other drying methods, the maximum lightness reduction was observed in powder of sun-dried beetroot slices.

Parsnips chips

The parsnip, a plant closely related to carrot and parsley, is part of the root vegetables and is historically dated from the time of the Romans. It has an annual production of approximately 40 million tons/year (FAO, 2021). Although parsnips are a biennial root vegetable, they are generally grown as an annual. The plant has a cream colored tuberous root used mostly boiled, fried, pureed roasted or steamed.

In 2021, Ledbetter et al. studied some novel pre-frying treatment applied to potato, beetroot and parsnip to inhibit the formation of acrylamide, 5-hydroxymethylfurfural (HMF), glyoxal (GO) and methylglyoxal (MGO). Therefore, the slices of vegetables (2 mm for potatoes and 3 mm for parsnip and beetroot) were treated as follows: cold soak (soaking in 2 L of cold tap water for 2 min), hot soak (soaking in 2 L of tap water at 70˚C for 2 min), cold soak followed by hot soak (soaking in 2 L of cold tap water for 2 min, followed by soaking in 2 L of tap water at 70˚C for 2 min) and soaking in 2 L of a 0.01M $CaCl_2$ solution for 2 min followed by blanching at 70˚C in 0.1M citric acid for 2 min.

Soaking in additive solutions was proven to be effective in lowering acrylamide in all tested crisps. However, it significantly increased HMF levels in beetroot and parsnip crisps.

Sweet potato chips

Sweet potato is an important and leading vegetable crop of tropical and subtropical countries. It is considered a native of tropical America. It has an annual production of almost 90 million tones/year, China being the largest producer of sweet potato. Other sweet potato-producing countries are Malawi, United Republic of Tanzania, Nigeria, Angola, Ethiopia, United States of America (FAO, 2021).

In 2019, Sugumaran et al. compared the physicochemical and sensory analysis of sweet potatoes based on different processing methods: deep-fry, freeze-dry, sun dry, air-fry and oven bake methods. The nutritional values of the freeze-dried sweet potatoes had the highest values of ash (1.77 g/100 g), crude protein (5.65 g/100 g) and crude fiber content (3.56 g/100 g) and the lowest amount of fat content (1.51 g/100 g) compared to other samples. They also had good results with oven-dried sweet potato chips, which have moderate amounts of ash, crude fiber and crude protein. Besides, it consists lower fat content compared to deep-fried samples and similar sensory attributes score of range 5 to 6 as deep-fried samples.

CONCLUSIONS

There is a growing demand for organic vegetables around the world now more than ever. With the increase in the number of the population, problems arise in obtaining quality organic vegetable products. A solution could be organic vegetable chips, which are increasingly appreciated by consumers due to their nutritional values and long shelf life. There are several techniques for obtaining chips, each with its advantages and disadvantages. In terms of favourite organic vegetables, sweet potato, carrot, parsnip and beetroot seem to be the favourites of consumers, but also of researchers who use them in their studies.

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TAILORED HI-GROW TABLETOP SYSTEM FOR ADVANCED STRAWBERRY CULTIVATION

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Abstract

There is a continuous interest in developing experimental technologies that can be further transferred to small producers or house fields with limited soil surface area. The aim of this study was to implement a hi-grow tabletop technology for strawberry in the USAMV of Bucharest experimental open field. This growing technology will allow experimentation with different fertilization treatments and strawberry cultivars as well as an evaluation of abiotic and biotic stress on plant growth. 'Albion' variety used as the day-neutral strawberry cultivar bore fruit from June to the end of October. In the first year the plants developed well, despite various stress factors (heat, pests, diseases). This study will continue with the implementation of a shading system to protect the plants from direct solar radiation, reduce the temperature, and prevent the sunburn of the fruits.

Key words: *Fragaria x ananassa Duch., growing technology, hi-grow tabletop system, 'Albion' cultivar.*

INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.) is a globally grown plant (Oğuz et al., 2022). It is even considered the most widely cultivated crop, covering almost all continents (Hanif & Budiyati, 2011; Oğuz et al., 2022).

Strawberries are appreciated for their biochemical composition, taste and flavour and they are some of the most popular summer fruits in European countries (Kahu et al., 2010). Strawberry production has a clear upward trend; according to data from the FAO in 2020, strawberry production reached almost 8.9 million tons and the world area harvested reached more than 380 thousand tons. According to UN Department of Economic and Social Affairs, Population Division (2022), the global population will reach 8 billion on 15 November 2022 and it is forecast to exceed 9.5 billion in 2050. Correlated with population growth, space and resources are becoming more and more limited, therefore the need to produce more strawberry crops at lower costs, by using new production techniques, is increasing (Bălan et al., 2015; Defterli et al., 2015; Lelieveld, 2015; Rusu et al., 2015; Schmidt et al., 2017; Vizitiu et al., 2017; Oğuz et al., 2022). Despite the population growth, labour force has become harder to find and more expensive, which leads to development of optimized plant cultivation technologies to ensure the highest productivity on the smallest possible area (Koufiotis et al., 2016; Asănică et al., 2017).

There are many technologies that can be used for strawberry cultivation, with the most used being classic raised-beds mulched using plastic or straws (Lille et al., 2003; Uselis et al., 2008), for both conventional and organic crops. Organically cultivated strawberries showed better fruit quality and improved stress tolerance compared to conventionally grown ones (Tõnutare et al., 2009; Reganold et al., 2010).

High tunnels are frequently encountered, being a standard for countries like Spain, the United

Kingdom, Mexico (Lantz et al., 2021). The high tunnel system is one of the choices of small farmers to practice season extension. It extended the strawberry fruit production by up to 5 weeks in USA, using increased temperatures in winter and early spring (Gu et al., 2017). The use of low tunnels cannot be disregarded and may have some advantages such as extending the harvest period (Lewers et al., 2017) with increasing fruit yield and attempts are being made to study their effect on early season production (Laugale et al., 2017; Fernandez et al., 2021).

Hydroponics, vertical farming, and horizontal systems are technologies that have many advantages, but the initial cost of investment is quite high. These modern technologies have been the subject of many studies (Hanif & Budiyati, 2011; Ramírez-Gómeza et al., 2012; Borrero, 2021; Oğuz et al., 2022).

Cultivar choice is the most important factor for conventional/organic strawberry production (Kahu et al., 2010). In Romania several popular local and foreign varieties are widely used in strawberry growing technology, such as: 'Coral', 'Premial', 'Real', 'Magic' 'Onebor', 'Clery', 'Alba' (originated from Italy), 'Benton', and 'Mira' (originated from Canada). Newer varieties, *e.g.*, 'Albion', 'San Andreas', and 'Benicia' (USA), have been taken into study (Tudor et al., 2014) in a plasticulture system considering the pedo-climatic conditions of Bucharest.

'Albion' is a day-neutral (everbearing) cultivar (Shi et al., 2021). It was released in 2006 by University of California and its parents are the cultivar 'Diamante' and the advanced selection Cal 94.16-1, varieties crossed in 1997 (Teklić et al., 2010).

Compared with short day cultivars, day-neutral strawberry varieties present better performance in areas with highland climate (Polat et al., 2016), because perpetual flowering genotypes are not affected by photoperiod and produce flowers and fruits regularly, allowing year-round harvesting in closed systems and from spring to late autumn in conventional systems (Hossain et al., 2019).

Given the multitude of technologies for strawberry growing systems, nutrition requirements will be determined according to variety necessities.

Strawberry plants require a high amount of nitrogen (N) in order to be able to sustain a good production. The total amounts of N, phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) accumulated by plants can gradually increase as growth stages progress.

However, due to environmental pressures related to excess fertilizer use, a more optimal fertilization strategy is required (Yoon et al., 2014). There are different types of fertilization techniques, including foliar fertilisation (Valentinuzzi et al., 2018), fertigation (Yoon et al., 2014) or classic fertilization using organic fertilizers, *e.g.*, compost, manure (Gaskell et al., 2009).

The aim of this study was to implement a tailored hi-grow tabletop technology for strawberry in the USAMV of Bucharest experimental open field. This growing technology will allow experimentation with different fertilization treatments and strawberry cultivars as well as an evaluation of abiotic and biotic stress on plant growth.

IMPLEMENTING THE GROWING TECHNOLOGY

The experimental field within the Faculty of Horticulture, USAMV of Bucharest - 44.4708° N and 26.0662° E (Asănică et al., 2017), was established in spring 2022, with a single cultivar ('Albion') in 2 rows. The stages of hi-grow tabletop technology for strawberries are presented below.

Tabletop strawberry growing system

Prior to the construction of the support for the tabletop system, the ground was leveled in order to have a uniform distribution of the pillar height (Figure 1).

The iron pillars supporting the tabletop system had a height of 2 m and a diameter of 50 mm. They were coated with zinc to provide them a better durability. The pillars were fixed in the ground at about 50 cm in order to support the consoles for the irrigation drains. The metallic consoles with a rectangular profile had 70 cm length and were coated with zinc. The irrigation drains were made of anti-corrosive sheet metal, painted white, with a thickness of 0.6 mm (Figure 1a). The planting distance was of 0.25 m between the 2 rows and 0.20 m between the plants in the same row. Each pot (48 cm × 18 cm × 16.3 cm) hosted 2 strawberry plants. The pots were made of UV-resistant treated plastic material to resist direct sunlight. They were fixed at a point in the irrigation drains so that they can resist high wind speed and other meteorological phenomena that may occur in the open field (Figure 1b). Each row had a length of 62 m and a total of 124 pots. Accordingly, 496 strawberry plants were cultivated in 2 rows. A distance of 2 cm was left between the pots and the irrigation drain to allow the irrigation water to flow easily from the pots.

Figure 1. Tabletop growing system: a) structure for supporting the pots; b) pots placed on irrigation drains

Preparing the substrate for strawberry cultivation

A substrate based on sphagnum peat (OPM 540 W, Kekkilä-BVB, Finland) was selected for the strawberry cultivar. The sphagnum peat contained limestone in order to decrease the pH at a value of 5.9 and had a NPK concentration of 1 kg/m^3. The required volume of sphagnum peat substrate for all 248 pots was 1.98 m^3. Before filling the containers, the sphagnum peat was crushed by hand, so that it was as loose as possible to facilitate the spread of the roots throughout the substrate (Figure 2).

Figure 2. Filling pots with peat:
a) crushing the peat; b) pots with crushed peat

Selecting the seedling material

'Albion' variety is a typical day-neutral strawberry cultivar and produces fruit regardless of day length when treated appropriately (Shaw et al., 2006). Because of their small dimensions (15-40 cm), the strawberries could be grown successfully in pots, greenhouses or in large outdoors spaces (Tudor et al., 2014).

Preparing and planting the strawberry plants

Dormant cold-stored plants of the 'Albion' cultivar were obtained from a commercial nursery (SC Strawberry Plants SRL, Tămășeu, Bihor, Romania). Before planting, the plants were kept at 1°C in plastic bags, firmly closed at the top to prevent mold and premature development of the strawberry leaf rosette. The roots were shortened to a length of about 10 cm, spread by hand to support their development, and immersed in a mixture of water and peat to prepare them for planting (Figure 3). After preparing the plants, they were planted in the pots and irrigated throughout the day.

Figure 3. Preparing and planting the strawberry plants in the experimental field

Irrigation system installation
For strawberry, overhead and/or trickle (drip) irrigation systems are used in both conventional and organic growing technologies. A drip irrigation system (Figure 4) was used in this study. Drip irrigation involves dripping water onto the soil at very low flow rates (2-20 L/h) from a system of small diameter plastic pipes (emitters). The irrigation system consisted of a main pipe, branched into 2 pipe of 16 mm diameter each, and arrow drip emitters attached to each strawberry plant (as seen in Figure 4). The irrigation system was first set-up to fill the peat with enough water to plant the strawberry plants. After planting, the irrigation system was set to water 4 times a day to facilitate plants growth under optimal conditions.

Fertilization requirements
Strawberry plants were fertilized according to their requirements and development of phenophases. The general recommendation for N-P_2O_5-K_2O application rate is about 155-104-283 kg/ha for a high yield production (Haifa Group, 2022). Being an experimental field, different fertilization methods were applied considering the nitrogen requirements for optimal plant growth and development.

Diseases and pest control
To ensure a good protection of the strawberry field, it is usually necessary to perform several treatments to combat diseases and pests. According to Hoza (2000), 3 treatments should be applied, *i.e.*, one against foliar diseases and fruit rottenness, one against pests, and one against fungi. Depending on the type of cultivation chosen (organic or conventional), different substances and products can be selected and applied.

Figure 4. Drip irrigation system

FIRST YEAR PRELIMINARY RESULTS

After the first year of experiment, the tailored growing system successfully resisted the weather variations, proving its durability and the possibility of its use in future research.
The irrigation scheme (Figure 4) proved to be effective, providing the plants with the necessary water. Moreover, it reduced water loss, being environmentally friendly and also decreasing the costs.
'Albion' strawberry plants were successfully grown in pots (Figure 5), they developed vigorously and bore fruit throughout the experiment. The fruit production obtained using this growing technology was in accordance with the data specified by the producer of 'Albion' variety. Regarding the

percentage of plant rooting, in the first days after planting, 98.5% of the plants survived and started to develop. At the end of the season, 81.4% of the plants had grown and developed.
The plants began to bear fruit in June 2022 (2 months after planting), with a production peak in autumn (September 2022), but fruiting continued even at lower temperatures (November 2022). In this experimental climate, the main stress factors were the high temperatures during the summer (when the substrate temperature exceeded 30ºC) and direct solar radiation. The solutions to these problems can be the optimization of the amount of irrigation water (depending on plant phenological stages and climate conditions) and the implementation of a shading system (to protect the plants from direct solar radiation, reduce the temperature in the pot, and prevent the sunburn of the fruits). Another advantage of the system is the presence of pollinating insects, more insects being near the pots than at ground level.

Figure 5. Strawberry plants development and fructification (06.06.2022-27.06.2022)

For an ecological approach, different plant species (*e.g.*, asparagus, beans, peas, spinach, lettuce, garlic, onion, horseradish, rhubarb) can be sown under the raised beds, which can improve soil fertility by fixing nitrogen. Companion plants (flower strips, trap plants or cover crops) are sources of nectar for antagonists and living mulches. These plants can increase biodiversity and strawberry crop productivity by minimizing the development of pests in the field, attracting beneficial entomophagists, for example pollinators or predators. Even if the implementation of the growing system can be more expensive than that of the conventional systems, this technology can be very profitable due to the reduction of costs corresponding to water and fertilizer consumption, human resources, etc.

CONCLUSIONS

This study successfully implemented a tailored hi-grow tabletop technology for strawberry in the USAMV of Bucharest experimental open field. Based on the developed technology, the 'Albion' strawberry plants grew vigorously and bore fruit throughout the experiment. This growing technology will allow experimentation with different fertilization treatments and strawberry cultivars as well as an evaluation of abiotic and biotic stress on plant growth. Fertilizers obtained from marine residual materials, *i.e.*, residues of fish, rockweed (*Ascophyllum nodosum*), and organic blue mussels (*Mytilus edulis*), will be tested to grow strawberries. The technology can be easily adapted for other crops, *e.g.*, salad, spinach, aromatic plants. Future research will be conducted to improve the technology with a shading system, which will increase the efficiency and production.

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ENVIRONMENTAL CONCERNS REGARDING THE OCCURRENCE OF NEONICOTINOID INSECTICIDES IN BERRY FRUITS

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Abstract

Goji berry (Lycium barbarum) and wolfberry (Lycium chinense) became popular nowadays due to their supposed health benefits. Conventionally grown goji berries are often treated extensively with pesticides that led to high levels of pesticide residues in the imported products in Europe and in the USA. Among the insecticides, neonicotinoid type active ingredients have frequently been found in wolfberry products of Chinese origin, which occasionally exceeded the corresponding official maximum residue limit (MRL) values. In addition to imidacloprid and thiamethoxam, much older and obsolete active ingredients have also been detected, e.g. carbofuran. The negative effects of neonicotinoids on pollinators, particularly bees, led to the ban of 3 neonicotinoids (clothianidin, imidacloprid and thiamethoxam), whereas acetamiprid is still in use in Europe. Along with the strict EU regulations, legal violations occurred related to pesticide residues in the imported goji berry products, which led to an increase in the level of official control on imports (2018/941) in the EU. Paradoxically, the corresponding MRL (EC 396/2005) e.g. for acetamiprid has been increased.

***Key words**: neonicotinoid insecticides, ban, goji berry, wolfberry.*

INTRODUCTION

Nowadays goji berry (*Lycium barbarum*) or wolfberry (*Lycium chinense*) became popular in developed countries due to their supposed health benefits. The fruit of these two closely related species (Figure 1) have been long used in the cuisine of various nations, and also as an ingredient in traditional medicine in Asia. It is cultivated mostly in China, but also in other East Asian countries (Japan, South Korea), and plantations have been established worldwide in the last decades.

Figure 1. The fruits of two closely related species, goji berry (*Lycium barbarum*) and wolfberry (*Lycium chinense*)

PESTICIDE RESIDUES FOUND IN GOJI BERRY OR WOLFBERRY

Conventionally grown goji berries are often treated extensively with pesticides (insecticides and fungicides) that led to the high levels of pesticide residues determined in the imported products in the United States and in Europe. From the beginning of this century, high levels of insecticides including fenvalerate, cypermethrin and acetamiprid, and fungicides including triadimenol and isoprothiolane have been detected by the US Food and Drug Administration in some imported wolfberries and wolfberry products of Chinese origin.

The European Food Safety Authority (EFSA) report on pesticide residues in food found in 2018 in the EU (EFSA, 2020) specified exceedance rates of the maximal residue limit (MRL) and quantification rates for processed food products (excluding baby foods); quantified residue levels below the MRL were found in nearly half (46.9%) of the goji berries, and residue levels were above the MRL in a quarter of the samples analyzed. Non-compliance rate for goji berries from China were 13% in 2018. A similar EFSA report for 2019 (EFSA, 2021a) found 45.8% quantification rate for goji products, and MRL exceedance rate was 4.2%. Processed goji berry products containing multiple quantified residues were detected in 55% of samples up to 5 ingredients in 2018 (EFSA, 2020), but an individual goji berry sample originated from China contained residues of as many as 28 different pesticides, which was likely due to mixing of different batches. Some non-approved EU substances (e.g. nicotine, anthraquinone, carbofuran) have also been found in goji berries originated from third countries. In the subsequent year (EFSA, 2021a), half of the analysed goji berry samples were without quantified residues, whereas six of the 48 samples contained one residue and multiple residues were detected in 18 samples, among them five were found to be contaminated with more than five residues. According to the provisions of Regulation (EC) No 669/2009 on import controls, in 2018, certain food products from China (tea, goji berries, broccoli), were subject to an increased level of official controls for certain pesticides at the point of entry into the EU territory.

A survey conducted in the Czech Republic with imported goji berry, reported multiple residues in all samples, and in some cases a total of over 20 different pesticide active ingredients were found (Czech Public Health Institute, 2017). These results are in accordance with those found in Germany, where only the two organic products and two of the 24 conventionally grown goji berry samples met the legal requirements (Hacker et al., 2010). Among the insecticides, neonicotinoid type ingredients (mainly imidacloprid, thiamethoxam and acetamiprid) were frequently found in some wolfberries and wolfberry products of Chinese origin, which occasionally exceeded the corresponding MRL. Particularly high detection frequencies were reported in Germany, where contamination rates for acetamiprid and imidacloprid were found 92% and 65% of the samples, respectively (Hacker et al., 2010). Similar results were obtained in the Czech Republic, as mentioned already, where according to the report of a public health institute, acetamiprid and imidacloprid were found in detectable amounts in all goji berry samples analysed (Czech Public Health Institute, 2017). In addition to imidacloprid and thiamethoxam, several old and outdated insecticide active ingredients (e.g., carbofuran, myclobutanil, carbendazim) were also detected, that have long been banned in the European Union (EU) (Hacker et al., 2010).

A national summary report on pesticide residues measured in Czech Republic in 2017 (EFSA, 2019a) also confirms multiple residues in goji berry samples (21 and 24 compounds). Detections notified in the EU Rapid Alert System for Food and Feed (RASFF) were related to propargite and carbofuran in goji from China, and also 2,4-D and acetamiprid were found at lower levels.

In Germany, only 6 out of 26 samples studied met the legal provisions in force set for pesticide residues (Hacker et al., 2010). Legal violations were mainly related to acetamiprid residues as its level exceeded the MRL in 77% of the conventionally grown goji berry samples, but MRL violations were also found for chlorothalonil and propargite. In addition, 11 pesticide residues per sample were detected in an average. In total, 38 different pesticides were found, and pollution rates for the most often found 17 active ingredients ranged from 23% to 92%. There is no point in using such a large number of pesticides, thus it indicates that farmers are not sufficiently advised by experts on the use of plant protection products (PPPs), or the cultivation areas are extremely polluted by pesticides.

High detection frequencies together with the high number of pesticide residues detected per sample have led to increase the levels of official control on imports of certain feed and food of non-animal origin in the EU, including goji berry as well (EU 2018/941). In contrast, however, the corresponding MRL (EC 396/2005) e.g., for acetamiprid changed from the original 0.01 mg/kg value to 0.1 mg/kg. According to EU regulation (Commission Regulation, 2018), the same MRLs apply to goji berry or wolfberry products as to tomato. After modification of the existing MRLs for acetamiprid in various crops (EFSA Journal, 2021), the corresponding MRL determined for tomato is currently 0.5 mg/kg. The same value was in force for imidacloprid as well, but now the MRLs for tomatoes has been lowered to 0.3 mg/kg (Commission Regulation, 2021)

Organochlorine pesticides also appear to still being used in certain regions of the world in commercial wolfberry cultivation to mitigate infestation by insects. As by China's Green Food Standard, some amount of pesticide and herbicide use is permitted, the organic claims for berries might differ from the European standard. A recently published study (Zhang et al., 2022) on the levels of major hazardous pesticides and metals in *L. barbarum* from China concluded that metal exposure is more hazardous than pesticide exposure. Only dichlorvos was detected at low levels from the six pesticides (dichlorvos, omethoate, cypermethrin, fenvalerate, malathion, and deltamethrin, nonetheless dichlorvos being the most hazardous of them) measured in supermarket samples. Deltamethrin was not detected in any sample, whereas the levels of other active ingredients were considerably below their MRLs in the plantation samples. Health risk assessment found low values, however removal rates of pesticides by washing and drying as well as consumption modes will substantially affect the results that should be further investigated.

More pesticide active ingredients were monitored in a study (Xing et al., 2022), where 200 wolfberry samples were analysed, and 23 pesticides were detected in 83.5% of the samples. Acetamiprid, carbendazim and imidacloprid were detected in 79%, 50% and 43% of the samples, and pyridaben, acetamiprid and difenoconazole exceeded the MRL the most frequently, in 19.5%, 12% and 11% of the wolfberry samples, respectively. Acetamiprid, imidacloprid carbendazim, pyridaben, propargite and thiamethoxam were detected in samples from all regions. Thus, neonicotinoids (imidacloprid, acetamiprid and thiamethoxam) were major components in all three categories (most frequent, high MRL exceedance rate, wide-spread occurrence). Nonetheless, the accessed risk posed by chronic and acute dietary intake of pesticide residues in wolfberry were low.

Another recently published study (Kim et al., 2021) on pesticide residue monitoring also confirmed that there is no significant health risk for consumers via consumption of *Lycium chinense*. Pesticides were detected in 14 of 15 total samples, and the number in each sample ranged from 2 to 21 different pesticide active ingredients with an average of 10.3 kinds per sample. In total, 42 different pesticide active ingredients were detected, including 27 insecticides and 15 fungicides. The most commonly detected pesticides were pyraclostrobin and tebuconazole (64.3% of 14 samples), but other ingredients (e.g. dinotefuran, imidacloprid) were also frequently found. The highest concentration was measured for boscalid (3.14 mg/kg), and the maximum residue concentration of insecticides ranged between 0.009 and 0.369 mg/kg. Regarding the mode of action, among the detected insecticides, neonicotinoids were the most predominant type, followed by pyrethroids.

NEONICOTINOIDS

Due to the high amounts and wide-scale use of the earlier applied neonicotinoids, they became ubiquitous contaminants in surface water and in the soil (Morrissey et al., 2015; Mörtl et al., 2020; Goulson, 2013). As these are systemic pesticide active ingredients, they readily translocate from the soil into the xylem fluid, spread throughout the entire plant, and their residues appear in the fruits, flowers, the nectar, the pollen, and the guttation fluid as well. Guttation is the exudation of droplets of xylem sap that appears on the tips or edges of the leaves. Berries (e.g., strawberry), but also tomato, corn and some grasses or weeds (e.g., red poppy) often and intensively guttate (Figure 2). Neonicotionoids and other systemic pesticides, which were uptaken by the roots from the surrounding polluted soil or irrigation water (Mörtl et al., 2016, Mörtl et al., 2020), can be detected in the guttation droplets. Analysis of the guttation liquid indicate the presence of pesticides residues; however, the exact concentrations depend on numerous factors including species, pollution level of the environmental area, soil properties and meteorological conditions as well.

The presence of chlothianidin was proven in the guttation drops of corn plants up to two months upon exposure, but thiamethoxam could also be easily detected over a month (Mörtl, 2020). In contrast, according to a study

(Li et al., 2019a) conducted with the commercial formulation of dinotefuran and thiamethoxam and goji berries in northwest China, the dissipation of neonicotinoids in goji seems to be fast. Thiamethoxam dissipated in the goji plant ecosystem with half-lives about one day in goji berry, whereas DT_{50} values ranged between 2.04 and 4.25 days in soil. The final residues of thiamethoxam were <0.005-0.382 and <0.005-1.120 mg/kg in goji berry and soil, respectively.

Figure 2. Guttation drops on leave edges of strawberry, tomato and corn plants

REGULATION OF NEONICOTINOIDS

Negative effects of neonicotinoids reported on non-target insect species, particularly on bees, led to the restriction of the use of imidacloprid, thiamethoxam and clothianidin in the EU (The European Commission, 2013), and then all their applications were banned except their use in closed greenhouses (The European Commission, 2018a; 2018b; 2018c). Acetamiprid is still in use in Europe as the EU's food safety authority (EFSA) established low risk to bees, but it was also banned together with thiacloprid from 2018 for example in France (France government, 2018). Based on a conclusion by the EFSA (EFSA, 2019b), the European Commission has not renewed the authorisation of thiacloprid in 2020 due to environmental concerns related to the use of this pesticide, particularly its impact on groundwater, but also related to human health, in reproductive toxicity. Currently, only acetamiprid is approved as a neonicotinoid active substance in the EU for the use in PPPs from the five neonicotinoid insecticides that were most often used. The agricultural sector that has been hit most hard by the ban of neonicotinoids is probably sugar beet cultivation. That is why Ministerial Order provisionally authorised the use of sugar beet seeds treated with neonicotinoid PPPs in 2021 in France. Defra in UK approved an emergency authorisation to use neonicotinoid-treated seed due to the risk posed by virus yellows for the 2022 sugar beet planting season, growers will be allowed to use seed treated by a PPP containing thiamethoxam. Emergency authorizations of the use of neonicotinoids on sugar beet have been issued by EU Member States e.g., Belgium, Croatia, Denmark, Finland, France, Germany, Lithuania, Poland, Romania, Slovakia, and Spain in several cases, and have been evaluated by EFSA (EFSA 2021c). This has triggered a debate on the definition and stricter requirements for the justification of any emergency authorization in the EU (Epstein et al., 2022).

In 2021, the EU Agriculture and Fisheries Council (AGRIFISH) established a specific protection goal of 10% as the maximum permitted level of colony size reduction resulting from exposure of honeybees to pesticides (EC, 2021). This has been communicated as a step towards the protection of bees, but in reality, it is a drastic reduction of the rigor in pollinator protection and appear to contradict ongoing EU sustainability goals (the EU Green Deal, the EU Biodiversity Strategy, the Farm2Fork Strategy), and may incapacitate

current effort halting and reverting the decline of pollinators (EU Pollinators Initiative).
The review of neonicotinoid insecticides by the US Environmental Protection Agency (US EPA) has been postponed to this year for the ingredients mentioned before with exception of thiacloprid but completed with dinotefuran. Its approval was voluntarily cancelled by registrant, as it is considered to be an endocrine disruptor and is, therefore, proven to be harmful to the hormonal balance of humans and animals. The banned ingredients were applied mainly as seed coating in different crops, but other application modes (e.g., spray) were also widespread in the past (Jeschke et al., 2011). Seed coating, as a preventive pest management practice for field crops (e.g., maize, sunflower, cotton), does not comply with the principles of integrated pest management (IPM) (Goulson, 2013), therefore new alternative pest control strategies were developed to substitute these banned ingredients (Furlan et al., 2021).

ALTERNATIVE METHODS

Before using any systemic insecticides in crop protection, the risks of a pest outbreak and the yield benefit should be assessed. Small damaging attacks of some soil pest such as wireworms are often compensated by the plants, and the effect of insecticides on yield and on the net income may be negligible. In some cases, increasing habitat complexity/ diversity to provide refuges and alternative hosts and food resources to predators and parasitoids may be effective. Upon high risks, alternative methods have been proposed. Among others the use of biological tools (e.g., attract-and-kill strategies) against soil pests, mating disruption based on the use of synthetic sexual pheromones, exclusion netting or natural-derived insecticides instead of synthetic chemicals were also tested. The main drawback is that these methods, in contrast to the prophylactic uses of highly toxic pesticides such as neonicotinoids, require a complex treatment. They are generally suggested to be applied in combination with or without low-risk pesticides for organic farming and IPM practices.
After the ban of neonicotinoids in the EU, increased use of certain outdated active ingredients (e.g., chlorpyrifos) has unfortunately been also observed, despite the fact, that the Green Deal aims to a 50% reduction of pesticide usage and a 25% increase in the proportion of organic farming by 2030 (Green Deal, 2022). These restrictions and withdrawals, of course, do not apply at other regions of the world, where these ingredients are still frequently used to prevent damages caused by insects, but this definitely affects the acceptability considerations in the RASFF system.
Among the methods in the pest control of goji berry, alternative to chemical crop protection using neonicotinoids or other PPPs, artificial defoliation against gall mites was also assessed (Li et al., 2019b). The procedure proposed enabled almost a complete defoliation, and *A. pallida* galls also fell off from the affected plants along with the defoliation process, and subsequent regenerated foliage escaped from mite attack. After defoliant application, the densities of mite galls decreased by more than 80% compared with those found in the pesticide treatment. Worthy of note, that the defoliation formulation consisted of a suspension concentrate containing the persistent diuron and the moderately persistent thidiazuron herbicide active ingredients. These generally are not approved in EU, only diuron is used in Bulgaria.

ORGANIC PRODUCTION IN EUROPE

Except for China and other Asian countries, as Malaysia, current goji production at a commercial scale initially began at the beginning of the century in Brazil, Canada and in the United States as well. The interest in Europe was skyrocketing after the intensive promotion of goji as a "superfruit" and the market demand increased very fast. In the European history of goji, legal violations related to the goji berry products, attempting to avoid the strict EU regulations, have unfortunately often occurred. The increasing demand for fresh goji berries in Europe and the pesticide residue problems with imported products on the European market also contributed to one initiative in Portugal to find out the best production processes for growing goji berries in Europe. In the frame of an EIP-AGRI project (Inspirational ideas: European goji berries, 2020.), cultivars of *L. barbarum* in organic production have been established, components in the fruits are monitored, and agricultural practices as well as soil and climatic conditions will be evaluated. There appears to be a growing interest in goji berry cultivation in other European countries, for example in Italy, Greece, Lithuania, Poland, and Romania. For instance, in Italy, as analysis performed at the border customs, found alarming levels of pesticide residue on the imported goji berries, mainly from China, the Italian farmers started to grow goji by their own, since 2007. In 2016, the Italian goji production reached 50 tons, making Italy the largest European producer (Knowles, 2016). In 2017, the area planted with goji was over 35 ha (15 ha in Veneto and 20 ha in Calabria), with a total of 60,000 plants, still being the largest farm in Europe (Zordan, 2016). Today, the Goji growers are mainly located in Calabria, Veneto, Puglia, Lazio and Tuscany. The Italian Capodaglio fresh goji berries are produced under the Organic Forest certification, the sustainable farming method promoting respect for natural ecosystems, genetic and biological cycles, and biodiversity (Zordan, 2016). Another Italian producer, Agricultural Company Leggero Luca, Canavese, Villareggia (Toscany) has now 3.5 ha of organic certified plantations, including a specialized nursery recognized and authorized by Piedmont regional office, and certified as Organic, that produces varieties of *L. barbarum* originally coming from the Ningxia region, China (ItalGoji, 2022). The same organic trend is apparent for the production of goji berry in Romania. The first company planted 2 ha of goji in 2011, with a variety develop by themselves, Erma, in Ciuperceni, Satu Mare. Their business grew and diversified with processed products, so the total planted area reached 11 ha in 2019 (GojiLand Romania, 2022). In Brașov area, another company started in 2014 the cultivation on 2.5 ha, with the Kronstadt variety, another variety registered in Romania and later diversify its activity both in developing their own brand and a certified organic nursery producing two of the Romanian varieties, that they later registered in the Official catalogue of cultivated plant varieties from Romania. Today, in Romania are registered 7 goji berry varieties in the *Official catalogue of cultivated plant varieties from Romania*, namely: 'Erma', ‘Transilvania’, ‘Kirubi', '‘Kronstadt‘, ‘Bucur’, ‘Sara’ and ‘Anto’, all being registered from 2017 to 2021 (ISTIS 2017, 2018, 2019, 2020, 2021). In Spain, GojiVital, S.L. started with 12 ha of goji plantation in the south of Spain (40,000 plants), since 2011, the company being GlobalGAP certified (GojiVital, 2022). Farmers from Poland have developed a special breeding technology for Goji berry, in greenhouses, the yield being increased starting the first year and fruiting period extended. The JB1 variety was produced *in vitro*, by a European company named Bio Tree Ltd (Biotree, 2022). In Hungary, goji cultivation is only at a start, triggered by improved yield performance of the variety Góliáth introduced in 2014. Beside small family orchards, goji berries are commercially cultivated and propagated in a 0.5 ha plantations at Ásotthalom (Csongrád-Csanád county).

CONCLUSIONS

The growing demand for goji berry as a healthy fruit is in contrast with its quality regarding its contamination with pesticides. Control activities of imported products, including pesticide residue analysis by RASFF, showed extensive amounts of residues and high numbers of pesticide active ingredients in goji berry. Among these contaminants, neonicotinoids, especially acetamiprid and imidacloprid, have frequently been found in shipment lots of imported goji berry from third countries. The latter active ingredient is currently banned in the EU, and other pesticides not approved in the EU have also been often detected.

Organic cultivation in the EU may satisfy the demand, and at the same time ensures the proper quality of the goji berry based products. The cultivated area with goji berry varieties is increasing yearly, Italy, Romania and Spain taking the lead of EU production, and the number of European registered varieties is constantly growing. The majority of producers are organic certified, but some pursue the ideal of having a chemical pesticide-free agriculture, and started gradually, first by adopting the

GlobalGAP certification, the set of standards for good agricultural practices. As consumer expectations regarding food quality are growing higher, it is expected that future pesticide residue analysis will show a decreasing trend of residues identified.

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USE OF DIFFERENT HORMONES ON *IN VITRO* PROPAGATION OF 'GISELA 5' CHERRY ROOTSTOCK

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Abstract

Gisela 5 is one of the most important dwarfing rootstocks for cherry (Prunus avium) in Central Europe, recommended in very high density plantations. It was obtained by crossing Prunus cerasus 'Schattenmorelle' x Prunus canescens and it is known for its high precocity, dwarfing and productivity. 'Gisella 5' is propagated clonally, using greenwood, soft or hardwood cuttings. In vitro cultures were established using vegetative shoots of 'Gisela 5', and after sterilization and inoculation of explants, multiplication mediums containing various concentrations of macroelements and phyto-hormones as BAP and GA3 were tested. Rooting capacity of the explants was observed on mediums with different salt concentrations and auxins as IBA, IAA and NAA.

***Key words**: auxines, 'Gisela 5', in vitro, micropropagation, rooting, rootstock.*

INTRODUCTION

'Gisela 5', a hybrid between *Prunus cerasus* and *Prunus canescens,* is one of the most important rootstocks for high density cherry (*Prunus avium)* plantings, in terms of the capacity of dwarfing, precocity and production (Zimmerman, 1994; Long & Kaiser, 2010).

Micropropagation of 'Gisela 5' cherry rootstocks maintains the advantages of clonal propagation, while adding on the benefits of micropropagation: possibility to produce high number of plants, production of pathogen-free planting material, all year-round production, genetic fidelity to mother plants.

Most used and efficient cytokinin used in micropropagation of 'Gisela 5' cherry rootstock is BAP, in concentrations of 0.3-0.5 mg/l, depending on the mineral composition of the medium used (Clapa et al., 2013; Borsai et al., 2020). BAP may be used alone, or in combination with gibberellins and auxins (Buyukdemirci, 2008; Thakur et al., 2016; Sharma et al., 2017). Clapa et al. (2013) efficiently propagated 'Gisela 5' cherry rootstock using MS medium supplemented with BAP in concentration of 0.3 mg/l and recommended the use of whole shoots as explants, explants no older than 1.5 or 2 months for both multiplication and rooting stages.

Borsai et al. (2020) recommended the use of MS or DKW medium with 0.3-0.5 mg/l BAP and 4 g/l agar for multiplication stage. Fallahpour et al. (2015), concluded that best results were obtained on WPM and DWK supplemented with 2 mg/l BAP, resulting in the highest percentage of shoot multiplication and number of shoots

When comparing different types of mediums, Nacheva and Gercheva (2009) concluded that sorbitol in the multiplication medium encourages the growth of the lateral buds and the further development of the shoots, when added in a proportion of sucrose: sorbitol 2: 1 or sorbitol: sucrose 1: 2. Most used hormones used in the process of rooting are IBA, in concentrations of 0.5-2 mg/l (Buyukdemirci, 2008; Fallahpour et al., 2015; Borsai et al., 2020), followed by NAA (Tariverdi et al., 2007). The mineral composition of the medium is also an important factor in rooting, as combinations of different concentrations of IBA or NAA with MS, DKW or WPM minerals give different results. Regarding the rooting stage, Fallahpour et al. (2015), recommends the use of WPM supplemented with 2 mg/l IBA, obtaining the

highest rooting percentage (93.70%), when compared to MS (53.1%) and DKW (14.0%). Tariverdi et al. (2007) obtained rooting percentages between 36.03% and 90.83% by supplementing the medium with different concentrations of NAA, the highest percentage being reached at a concentration of 6 mg/l NAA.
High rooting percentages of 94.74% were obtained on DKW medium supplemented with 1 mg/l IBA (Borsai et al., 2020). In the same study, Borsai et al. (2020) concluded that efficient *ex vitro* rooting of the shoots ca be obtained with floating perlite and 1 mg/l IBA with a percentage of rooting and survival of 96.15%. Sharma et al., 2017, established 100% rooting percentage on MS medium with normal salts concentration and 0.5 mg/l IBA, while rooting percentages were lower on mediums with IAA and NAA. Buyukdemirci (2008), observed that when using half strength MS in rooting stage, only the concentration of nitrates should be decreased. Potassium phosphate, magnesium sulphate, calcium chloride and microelements play an important role in rooting and decreasing MS nitrates resulted in the production of more roots per shoot. Thakur et al. (2016), concluded that the percentage of rooting can be increased by using two-step procedure for the rooting stage, with explants being immersed in liquid half-strength MS medium supplemented with 0.5 mg/l IBA, for 24 hours, in dark conditions and then cultured on hormone-free half strength MS.
Micrografting on Gisela 5 rootstock is possible and can be utilized for propagation of cherry threes with successful results (Exadaktylou et al., 2007).
The aim of this research was to develop and efficient procedure of multiplication and rooting of ‘Gisela 5’, using green wood nodal segments as starting plant material.

MATERIALS AND METHODS

Herbaceous green wood shoots of ‘Gisela 5’ were collected from Istrița Fruit Research & Development Station of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, in the period between April and June. Nodal segments with at least one bud were cut and sterilized with 70% ethanol for 25-60 seconds, 0.075-0.1% $HgCl_2$ for 7-8 minutes, followed by three rinses with sterile distilled water. Concentrations of disinfection solution and exposure time depended on the period of time when the plant material was collected, the material collected in the last period of the study requiring higher concentration and longer exposure times for successful disinfection. Explants were inoculated on hormone-free MS medium and transferred after two weeks of MS medium supplemented with hormones.

Multiplication

Media used for the multiplication stages was prepared accordingly to Table 1, with pH adjusted to 5.75 before sterilisation in the autoclave at 121°C and at an atmospheric pressure of 1.1 Bar for 20 minutes.

Table 1. Culture mediums used for multiplication stage

Basal salts	Hormones concentration		Agar concentration (g/l)	Sucrose concentration (g/l)
	BAP (mg/l)	GA3 (mg/l)		
DKW	1	0	7	30
DKW	1	0.1	7	30
MS	1	0	7	30
MS	1	0.1	7	30

Rooting

Apical shoots obtained on medium with added GA3, about 15-20 mm in length, were cultivated on MS medium with the concentrations of halved (X/2) or quartered (X/4) supplemented with 1 mg/l NAA, accordingly to Table 2.
Both media used for the rooting stage were prepared accordingly to Table 2, with pH adjusted to 5.75 before sterilisation in the autoclave at 121°C and at an atmospheric pressure of 1.1 Bar for 20 minutes.

Table 2. Culture mediums used for rooting stage

Basal salts concentration	NAA concentration (mg/l)	Agar concentration (g/l)	Sucrose concentration (g/l)	Activated charcoal concentration (g/l)
X/2	1	7	25	1
X/4	1	7	25	1

Acclimatization of explants

Acclimatization was carried 60 days after explants were transferred to the rooting mediums. The rooted shoots were removed from *in vitro* environment and the agar was carefully washed off the roots.

First 2-3 leaves from the bottom part of the explants were removed in order to reduce evapo-transpiraton off shots and roots were shorted to a maximum length of 5-6 centimeters. The shoots were placed in small pots, in sterilised substrate composed of peat, fine sand and perlite. The pots were covered with glass covers in order to keep the humidity high, and they were removed gradually, in order to help adapt the rooted shoots to the normal humidity level.

RESULTS AND DISCUSSIONS

Multiplication

Best results in term of shoot growth were recorded with the mediums supplemented with GA3, with 8.55 mm average shoot length on DKW and 8.31 mm average shoot length on MS (Figure 2). Highest number of shoots per explant were recorded on MS medium supplemented with GA3, average 10 shoots/explant, then DKW mediums, with no significant difference between them, 5.67 shoots/explant on the medium with GA3 and 5.60 shoots/explant on medium only with BAP and the lowest number of shoots per explant, 4.43, was recorded on MS supplemented only with BAP (Figure 3).

Figure 1. ‘Gisela 5’ explants in multiplication stage

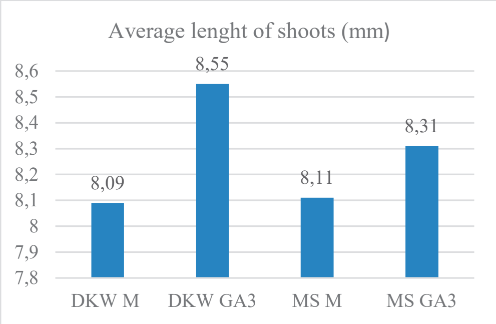

Figure 2. Average length of shoots (mm) on the four variants of mediums used for multiplication

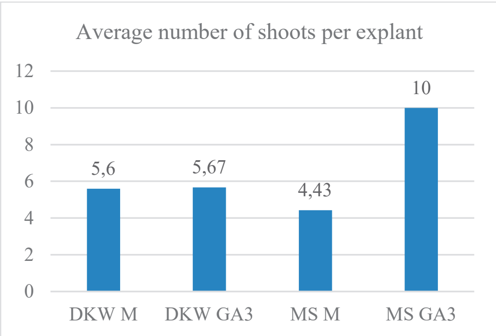

Figure 3. Average number of shoots per explant on the four variants of mediums used for multiplication

Regarding the number of leaves, best results were obtained on MS medium supplemented with GA3, 13.35 leaves/shoot, followed by MS medium with BAP only, 8.55 leaves/shoot. DKW medium showed similar results, 7.40 leaves per shoot on DKW with BAP and GA3 and 7.39 on medium DKW only with BAP (Figure 4).

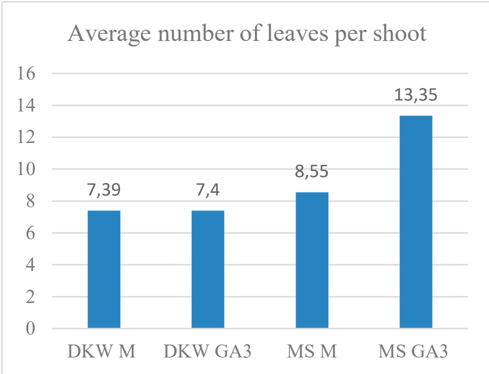

Figure 4. Average number of leaves per shoot on the four variants of mediums used for multiplication

Rooting and acclimatization

High percentages of rooting were achieved on both rooting mediums, with 83.33% on X/4 + 1 mg/l NAA and 76% on X/2 + 1 mg/l NAA.

Figure 5. Roots of shoots cultivated on X/4 medium

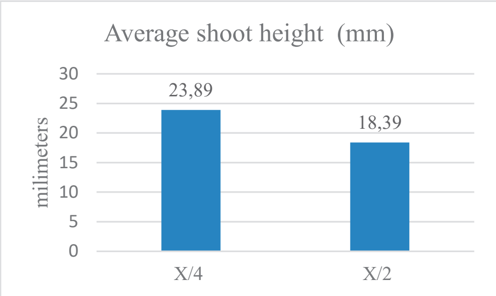

Figure 6. Average shoot height on the two variants of rooting medium

Medium with quartered concentration of salts (X/4) gave better results in terms of average height (23.89 mm on X/4 and 18.39 mm on X/2) (Figure 6), average leaf number (7.83 leaves on X/4 and 5.78 leaves on X/2) (Figure 7)

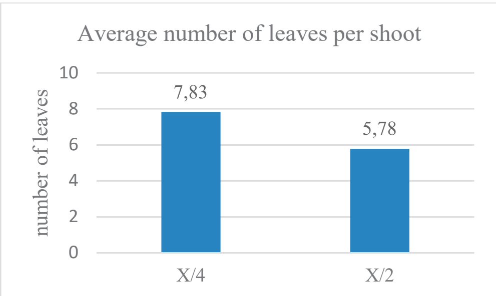

Figure 7. Average number of leaves/shoot on the two variants of rooting medium

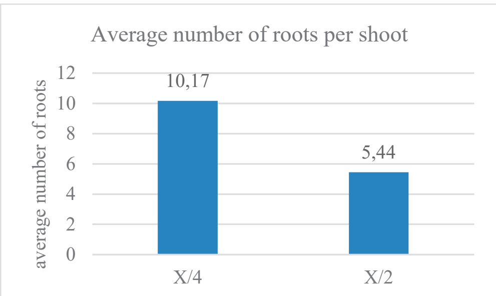

Figure 8. Average number of roots/ shoot on the two variants of rooting medium

Figure 9. Rooted shoot grown on X/4 medium, before acclimatization

Regarding root development and growth, medium X/4 showed better results in term of average number of roots (10.17 roots on X/4 and 5.77 roots on 5.44) (Figure 8). Average length of roots was slightly higher on X/2 (73.62 mm), compared to X/4 (71.67 mm), but we need to consider that the average number of roots on X/4 was much higher (Figure 10).

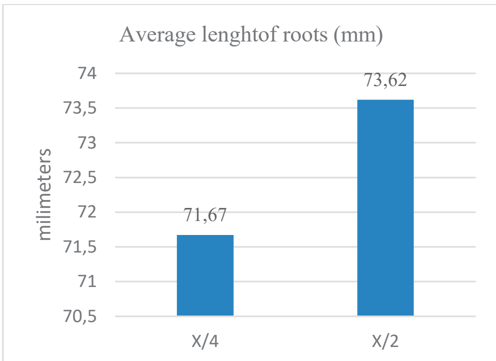

Figure 10. Average length of roots on the two variants of rooting medium

CONCLUSIONS

'Gisela 5' cherry rootstock can be successfully propagated using green wood shoots as a starting material.

Best results for multiplication stage were obtained on DKW medium supplemented with 1 mg/l BAP and 0.1 mg/l GA3. Rooting was successfully achieved on medium MS with quartered macro and microelements concentration, supplemented with 1 mg/l NAA and 3 g/l activated charcoal.

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CHARACTERISTICS OF SOILS FROM THE AREA OF XANTHI - NORTHERN GREECE FOR GROWING VINEYARDS AND KIWI

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Abstract

Due to the hot subtropical climate, agriculture in Greece specializes in growing various heat-loving crops - rice, cotton, olives, third in the world after Spain and Italy, citrus fruits, sesame, vines, special varieties of raisins and more. The aim of the present work is to determine to what extent the soils in the Xanthi region are suitable for growing kiwis and vineyards. Some soil indicators were determined such as particle size composition with FRITISH photosedimentograph, pH, humus content, total and active carbonates and digestible forms of N, P and K. It was found that the soils in the study area were developed on carbonate clays. In terms of particle size composition and their physico-chemical and agro-chemical properties, these soils prove to be suitable for growing kiwis and vines.

Key words*: soil properties, vineyards, Xanthi.*

INTRODUCTION

Greece specializes in the cultivation of citrus crops: oranges, tangerines, lemons, olives, perennials - vines, walnuts, almonds. About 1/3 of the production is exported. Large quantities of these fruits are processed into juices and are offered on the international market in original packaging.

Favorable soil and climatic conditions in Greece contribute to the cultivation of vineyards, which occupy 2.5 million acres of land. Wine and dessert grape varieties grow here. Seedless varieties for the production of raisins are also grown. Greece is the largest producer and exporter of raisins in the world.

Fruit growing is also well developed - grapes, melons, peaches and oranges are popular goods exported from Greece to European markets.

The country has a Mediterranean climate, mild and pleasant in winter, hot and dry in summer. Temperatures rarely fall below zero, but in the northern parts of the country, in the valleys of Struma and Mesta, cold air masses often come. Summer temperatures often exceed 40^0C, and in July and August sometimes the rains are completely absent. It is said that here the sun shines 3000 hours a year (Διευθυνση Γεωργιας Ξανθης).

In economic and geographical terms, the country is divided into 5 regions - central, northern, southern, western and eastern.

Northern Greece encompasses Aegean Macedonia and Aegean Thrace. It is forming as the second most important economic region of the country on the basis of the coal reserves in its western part, the are deposits in the Halkidiki peninsula and the agricultural raw materials: cotton, cereals and perennials in the coastal lowlands and fields.

The object of the present study is to assess the suitability of soils for the establishment of vines and kiwis in terms of soil and climatic factors characteristic of Xanthi located in the region of Northern Greece, region of Aegean Thrace.

MATERIALS AND METHODS

For the purpose of this thesis, 2 soil profiles were taken from the region of Xanthi. The soils are air-dried and prepared for analysis. The laboratory study includes preparation of soil samples for analysis, study of some soil and agrochemical properties of soils, in order to assess the suitability of the area for growing kiwis and vines.

The analyzes of the collected soil samples from the two profiles - № 1 kiwi, № 2 vineyards were performed by the following methods (Trendafilov et al., 2017):

• Particle size composition with FRITISH photosedimentograph;
• pH in H_2O - potentiometrically;
• The content of organic matter according to the method of Turin;
• Determination of total carbonates according to Scheibler;
• Determination of active carbonates by the Druino-Gale method;
• Assimilable K is determined in hydrochloric acid extract of 2n HCL;
• Mobile phosphates are determined by the double-lactate method of Egner-Reim;
• Ammonium and nitrate N in extract of 1% KCL.

Figure 1. Cromic-eutric cambisols

RESULTS AND DISCUSSIONS

Xanthi or Skecha (Greek: Ξάνθη, Xanthi, Turkish: İskeçe, Iskeche) is a city in northeastern Greece, in the region of Eastern Macedonia and Thrace, the center of dem Xanthi. It is located in White Sea Thrace, at the foot of Ruen Mountain (part of the southern slopes of the Rhodopes), /Soil Atlas, 2005/.
It is located in the western part of Thrace and borders on the north with Bulgaria - Rhodopes, on the west with Drama and on the southwest with Kavala on the west with the Rhodopes and on the south with the Thracian Sea. The region is characterized by a transitional Mediterranean climate, mild winters, warm and dry summers. Very low winter (- 7^0C, - 10^0C and less often - 12^0C) and very high summer temperatures (42^0C, and more often 37 and 38^0C) are observed for a short time. In winter there is a strong north-east wind (60-80 km/h). Annual rainfall is in the range of 800 to 900 mm/m^2, unevenly distributed, and sometimes for 4-5 months fall only 50-60 mm/m^2 (Οδηγος Ανατολικης Μακεδονιας και Θρακης).
Within the boundaries of the studied object, the soil difference according to the FAO classification (Boyadzhiev, 1994a; Boyadzhiev, 1994b) are Leached cinnamon forest soils (Cromic-eutric cambisols - Figure 1).
Climatic conditions in White Sea Thrace provide a long growing season and a long process of weathering and soil formation in the direction of fersialitization. Fersialitization weathering and soil formation in the past (during the Pliocene and the Old Quaternary), when these soils were mainly formed, was much more intense. At that time, the climate was better expressed in the Mediterranean, and the red colored materials on which the cinnamon forest soils are formed were mainly formed and deposited at that time (Yaranov, 1938).
Figure 2 shows the mechanical composition of the two soil profiles depending on the content of physical clay. It varies from medium sandy-clayey to clayey-sandy in profile 1 kiwi and heavy sandy-clayey in profile 2 vineyard. The heavy Particle size composition is explained by the presence of larger amounts of three-layer clay (Gyurov & Artinova, 2001).

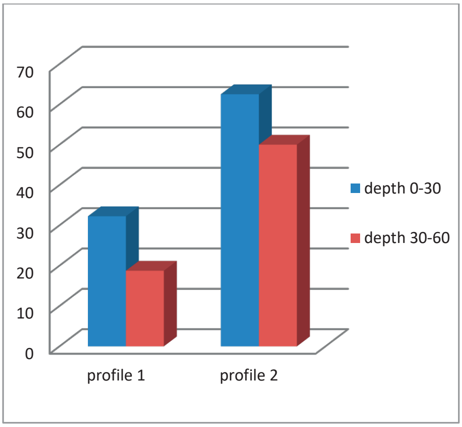

Figure 2. Mechanical composition (<0.01 mm, %) of soil profiles

In Figure 3 shows the reaction of the soil in both profiles. It is characterized as slightly alkaline in profile 1 and moderately alkaline in profile 2. Higher pH values in vineyard profile 2 are explained by the presence of $CaCO_3$.

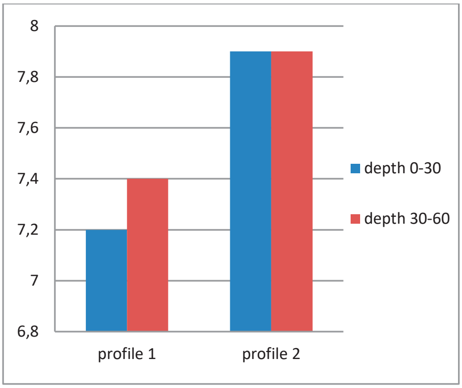

Figure 3. Soil reaction, pH (H_2O)

The high percentage of total and active carbonates is characteristic of this type of soils, as they are formed on carbonate soil-forming materials combined with the factors of weathering and soil formation **(**Figure 4) (Atanasov, 1987).
Figure 5 shows the organic matter content in%. The graph shows that according to the accepted classification for the stock of soils with humus, the studied soils of the two profiles are characterized by low to medium stock.
The content of digestible forms of nitrogen, phosphorus and potassium is presented in Figure 6. The studied soils in both profiles are weakly to medium stocked with nitrogen, poorly stocked with phosphorus and well stocked with potassium due to the presence of illite minerals.

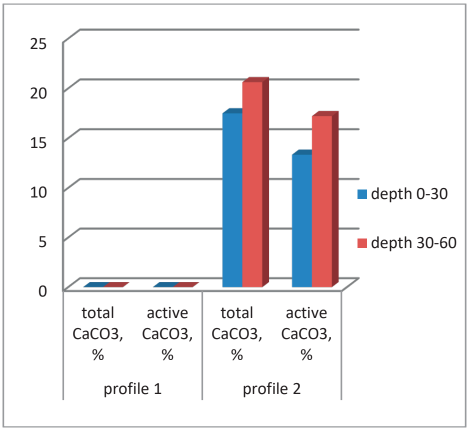

Figure 4. Wathering and soil formation factors

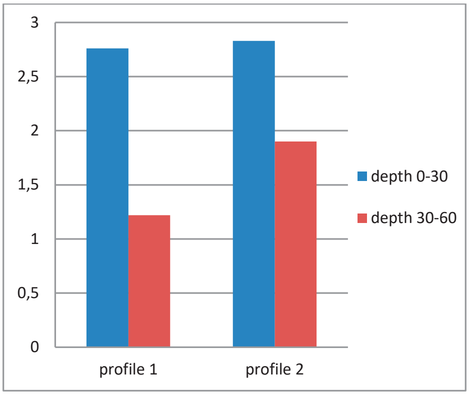

Figure 5. Organic matter (humus), %

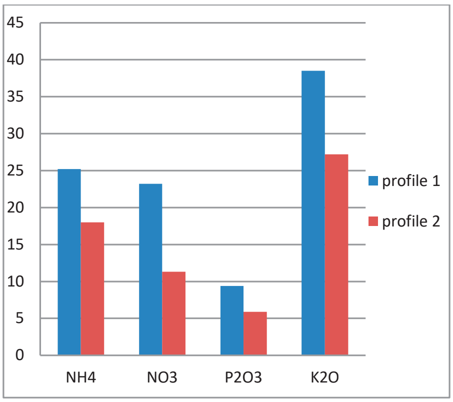

Figure 6. Digestible forms of nitrogen, phosphorus and potassium, mg/1000 g

Before the construction of the vineyard, storage and pre-planting fertilization must be performed. When young vines are stockpiled, they are not usually fertilized until they have started to bear fruit. However, if the vines have weak growth, they are fed with 8-10 kg of active substance nitrogen fertilizers per dka. In fruit-bearing vineyards, organic, phosphorus and potassium fertilizers are applied to the soil in autumn at the same time as deep plowing.

The main Greek vine varieties are:

Limino / Evros / - the cultivation of this variety is recommended on the territory of Evros, Xanthi, Drama, Serres, Kavala and Halkidiki, as well as in the region of Limnos, where the vines have a favorable climate.

Sefka / identical to the Bulgarian Shefka / is mainly grown in the region of Serres, very productive, resistant to diseases and drought.

Zoumiatiko or Damiatis / identical to the Bulgarian Dimyat /, distributed mainly in the area of Xanthi and Serres, a white variety of Balkan origin.

Pamidi identical to the Bulgarian Pamid, found in the region of Eastern Macedonia and Thrace. Pamida is easy to grow, very productive. Depending on the winemaking, white wines or rosettes are produced.

Mavroudi / identical to the Bulgarian Mavrud /. The name comes from the Greek word "black", derived from the deep, dark, ruby color of the grapes.

Keratsuda - red-green variety, common in northern Greece. It comes from an area near the Struma River in Bulgaria and Greece (Babrikov et al., 2005).

Actinidia (kiwi) originates from China, where there are natural habitats along rivers. A relatively new culture has recently spread to some Mediterranean countries.

Kiwi grows on fertile and moist soils. The plant is sown in spring and harvested in mid-October. Actinidia needs areas with mild winters and hot and humid summers to thrive. For this reason, it is cultivated in the Greek zone in Pieria, where it has been registered under the designation of origin (PGI) [2], since 2002 in the area of the Sperchios River in Phthiotis, where it is registered under the Protected Designation of Origin (PDO) (www.minagric.gr) and in Pella, Imathia, Mesolonghi, Chania, Rethymnon, Arta, Preveza and the delta of Pinios (Omolio, Pyrgetos, Aegean Sea). Kiwi fruit is rich in potassium, magnesium, phosphorus, fiber and trace elements. It also has more vitamin C than any other fruit. The presence of many vitamins makes kiwi the best means of protection against microbes. In addition, it eliminates the risk of clotting in the circulatory system and ensures proper digestion and proper bowel function. Finally, it helps improve vision thanks to the antioxidant lutein it contains.

Irrigation of the kiwi is extremely important. Although no strict scientific standards and deadlines for kiwi irrigation have been established, it can certainly be recommended that soil moisture be maintained at around 80-90% of FMC throughout the year. This can be achieved by watering the adult plants every 10-15 days at 50-60 l/m^2. Young plants are watered every few days with 5-10 liters of water. Mulching of the soil surface around the plants with well-decomposed manure or waste straw also gives a good effect (Lichev et al., 2020).

The use of organic and mineral fertilizers for kiwi fertilization supports the strong growth and nutrition of the rich fruit crop. In the case of fruit-bearing plants, manure is applied at once every 2-3 years in the autumn, spreading at a dose of 4-5 kg/m^2. At the same time it is fertilized with potassium and phosphorus fertilizers in doses of 30-40 g/m^2 and plowed with the autumn treatment. The plants are fed with nitrogen fertilizer in the spring, at a dose of 30-40 g/m^2 and again in July at a dose of 10-20 g/m^2.

CONCLUSIONS

Based on the study of soils in the region of Xanthi, northeastern Greece, as well as the climatic and soil-forming features characteristic of this region, we came to the following conclusions:

1. In terms of climate, the Xanthi region is suitable for growing kiwis and vineyards.

2. The soils in the area are developed on carbonate clays and limestones. With regard to the mechanical composition and their physico-chemical and agrochemical properties, these soils prove to be suitable for growing kiwis and vineyards by applying the necessary norms for

fertilization and agrotechnology of cultivation of the respective crops.

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PRELIMINARY STUDY ON THE INFLUENCE OF THE USE OF VERMICOMPOST AS A CULTURE SUBSTRATE ON THE QUALITY OF LETTUCE SEEDLINGS (*Lactuca sativa* L.)

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Abstract

The experiment was carried out at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture under laboratory conditions in 2020. During the COVID 19 pandemic, it took place in the university greenhouse, from the end of October until the end of November 2020. The biological material was represented by Red Oak lettuce cultivar, vermicompost (also known as vermicast), peat, zeolite and perlite. The paper aimed to present the benefices of using vermicompost in reducing nutrient solution and a higher yield. We tested 13 variants with 3 repetition each and used vermicompost in different percent as substrate such as 0%, 25%, 50% and 100% of pot volume. The vermicompost was produced by California Red Wigglers from converting two types of precomposted manure, horse and cattle over the period of 26 weeks. The vermicompost has a neutral PH 6.8-7.2 and does not burn the plant. The lettuce from variants where vermicompost was added, had a lower nutrient solution intake, this being an economic effect to reduce water consumption.

Key words: *vermicompost, peat, perlite, zeolite, lettuce.*

INTRODUCTION

Large quantities of animal manure and different organic waste are produced in farming and agricultural production along with sewage biosolids and food waste. If not disposed/treated properly these have the harmful potential of increasing water and soil pollution, globally. With the current world population of 7,999,420,420, we will reach 8 billion people by 15th of November 2022 and 9 billion by 2037 according to https://www.worldometers.info. It's harder and harder to dispose of our garbage in a safe and sustainable way for the environment. A sustainable process for waste and nutrient management is composting and/or vermicomposting. Over 50% of total wastes is organic and can be diverted from the landfills to compost and vermicompost facilities.

Edwards et al., 2011, in "Vermiculture Technology", presents a vermicomposting pilot program started in USA, in different colleges and universities such as The Evergreen State College located in Olympia, Washington, Southern Illinois University Carbondale located in Carbondale, Illinois, University of Oregon located in Eugene, Oregon, University of Massachusetts Lowell located in Lowell, Massachusetts.

Although composting and vermicomposting is happening since the beginning of life on Earth, researchers still don't have an exact definition for compost.

Comforter Compost, Banner Batches and The Complete Compost Gardening Guide use the word "compost" to describe a putrefaction, decay process.

Compost was one of the topics that has interested me since 2008 and over the years I came to the conclusion that you need 5 ingredients to make compost, such as oxygen, water, carbon, nitrogen and time. Adding worms to your compost system, will result in vermicomposting.

Blackburn, 2022, shows that vermicompost is the by-product of vermicomposting or worm composting. Vermicompost typically consists of unprocessed organic materials, microorganisms and worm castings.

Hernández et al, 2010, used vermicompost obtained from cattle manure as a substrate for growing lettuce and they observed that the plants presented a medium and small volume compared to variants cultivated in compost or urea fertilisation.

The substrate is very important for obtaining high quality lettuce seedlings (Draghici et al., 2016).

León et al., 2012, concluded that the volume of lettuce, number of leaves and nitrate content was influenced by applying different proportions of vermicompost to the nutrient solution.

Durak et al., 2017, in his study regarding the effect of vermicompost applied on lettuce, he showed that by applying 200-300 kg/ha of vermicompost, the growth parameters got better, soil quality improved and that lead to an optimal harvest.

Pleasant et al., 2008, mentions that the decomposition of organic materials process is over when you can see it visually and no fermentation odours come from the compost and/or vermicompost system.

Suthar, 2007, used the *Perionyx sansibaricus* (Perrier), a worm for vermicomposting and concluded that the most efficient conversion into vermicompost was from farm waste, sewage and different urban waste.

Tognetti et al., 2013, writes about the quality of vermicompost that it is influenced by the materials used to feed the worms.

Theourn et al., 2022, proves that the increasing of vermicast produced a higher number of active carbon (microorganism carbon energy source) also known as permanganate-oxidizable carbon (POXC).

Payal et al., 2006, did a study and evaluated the efficiency of *Eisenia foetida* (an epigeic worm) on processing organic waste from household, agricultural waste, sewage and fibre from the textile industry and they obtained a valuable vermicompost.

Munroe, 2009, writes about vermicompost's abilities to supress plant diseases and the concept of "soil food web".

Nancarrow et al., 1998, writes about how vermicompost/castings hold their nutrients wrapped around in a membrane. This helps the nutrients to be released slowly so the plant has access and availability as needed to it, over a period of time.

Vermicompost is a natural and organic product, that all farmers should use to improve their soil and production. Mala, 2022, remarks that this organic fertiliser is still unpopular among farmers.

The paper aimed to present the influence of vermicompost on pot substrate, the consumption of nutrient solution, as well as the difference of hight and number of leaves on lettuce.

MATERIALS AND METHODS

The experiment was carried out at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture under laboratory conditions, using the metric system.

The biological material was represented by Red Oak lettuce cultivar planted in individual pots for 30 days.

The vermicompost was produced over a period of 6 months from two type of manure, horse and cattle. Prior to feeding the worms, the manure was precomposted for 6 months. Then it was screen and feed to the worms every week, for 26 weeks. After this period, we sifted the worms from the castings (vermicompost) and used it under laboratory conditions for our experiment. The experimental variants consisted in the use of different nutrient substrates of vermicompost (from manure), peat, zeolite and perlite in percent of 0%, 25%, 50%, 75% and 100% also in different proportion (Table 1).

Table 1. The experimental variants

Variant	Substrate type
V1	Peat 100%
V2	Zeolite 100%
V3	Perlite 100%
V4	Vermicompost 100%
V5	Vermicompost 75% + peat 25%
V6	Vermicompost 50% + peat 50%
V7	Vermicompost 25% + peat 75%
V8	Vermicompost 75% + zeolite 25%
V9	Vermicompost 50% + zeolite 50%
V10	Vermicompost 25% + zeolite 75%
V11	Vermicompost 75% + perlite 25%
V12	Vermicompost 50% + perlite 50%
V13	Vermicompost 25% + perlite 75%

The pot experiment started on the 27th of October 2020 and ended on the 27th of November 2020. We used 13 varieties of substrate in different combination with 3 repetition each.

Appearance of lettuce seedlings in Figure 1.

Figure 1. Appearance of seedlings

RESULTS AND DISCUSSIONS

Over the course of 30 days, during seedling production, we used a different quantity of nutrient solution, depending on the growing medium. We discovered that for V1 where we used 100% peat for culture substrate, 562.67 ml/plant of nutrient solution was used. With the exception of V3 where we used 100% perlite as a substrate and 615.67 ml/plant nutrient solution. With 53.00 ml more then V1, 9% higher than the control variant (V1). Only V13 variant (vermicompost 25% + perlite 75%) had a significantly similar quantity of nutrient solution added, 562.33 ml.

In the case of using vermicompost at a rate of 100% (V4) the amount of nutrient solution absorbed by the plant was lower compared to the control V1. The amount administered was 466.67 ml with 96.00 ml less nutrient solution than V1, with a distinctly negative significance. We also found that when using vermicompost 75% + peat 25% (V5) the amount of nutrient solution compared to V4 in which only vermicompost was used, was 77.00 ml lower than V1 peat (control).

As the amount of peat in the mixture increased, the amount of nutrient solution used increased, but it was lower than V1-control. In the case of using zeolite in the nutrient mixture in a percentage of 100% (V2), we found that, on average, on the variant, an amount of 463.33 ml of nutrient solution was used, with a percentage of 82.35% less than the control (V1).

A similar aspect was found when vermicompost was used in combination with perlite (Table 2.)

Table 2. The influence of culture substrate on the intake of nutrient solution

VARIANTS	Nutritive Quantity (ml)	Difference (ml)	Difference (%)	Significance
V(0) average	514.41	-48.26	91.42	OOO
V(1)	562.67	0.00	100.00	Ct
V(2)	463.33	-99.33	82.35	OOO
V(3)	615.67	53.00	109.42	***
V(4)	466.67	-96.00	82.94	OOO
V(5)	**485.67**	**-77.00**	**86.32**	**OOO**
V(6)	**551.00**	**-11.67**	**97.93**	**OOO**
V(7)	**553.33**	**-9.33**	**98.34**	**OOO**
V(8)	423.33	-139.33	75.24	OOO
V(9)	522.67	-40.00	92.89	OOO
V(10)	525.33	-37.33	93.36	OOO
V(11)	427.67	-135.00	76.01	OOO
V(12)	527.67	-35.00	93.78	OOO
V(13)	562.33	-0.33	99.94	N

DL 5% = 4.640 DL 5% in % = 0.8246

DL 1% = 6.290 DL 1% in % = 1.1179

DL 0.1% = 8.440 DL 0.1% in %= 1.5000

The appearance of seedlings on experimental variants is shown in Figures 2 and 3.

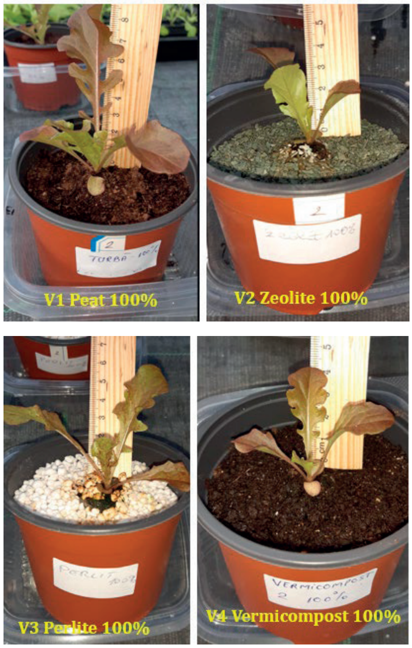

Figure 2. Appearance of seedlings on experimental variants

Figure 3. Appearance of seedlings on experimental variants

Analysing the height of the lettuce seedlings we found that at V1 control - peat 100%, the seedlings had a height of 10.66 cm, compared to the variants in which we used substrate of perlite, zeolite and vermicompost.

The smallest height of 8.67 cm was recorded at V10-Vermicompost 75% + perlite 25%. In the case of variants where vermicompost was used in combination with perlite and zeolite, the height of the seedlings was higher compared to the control variant (Figure 4.).

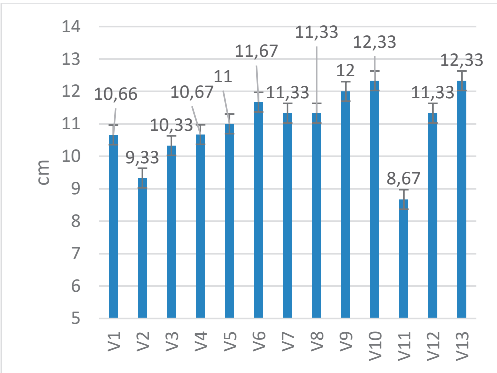

Figure 4. The height of lettuce seedlings after 30 days

It was noted that in the case of V2 in which we used zeolite substrate (V2), the height of the seedlings was 1.33 cm below the V1 - control, aspect noted as well in V3 - perlite, the difference being 0.33 cm, as well as in V11 (Vermicompost 75% + perlite 25%) 1.99 cm.

At the variant where we used 100% vermicompost, it showed a height close to the control version (V1). The highest height of the seedling was identified in V10 (Vermicompost

25% + zeolite 75%) and V13 (Vermicompost 25% + perlite 75%) 1.67 cm above the control V1 (Figure 5).

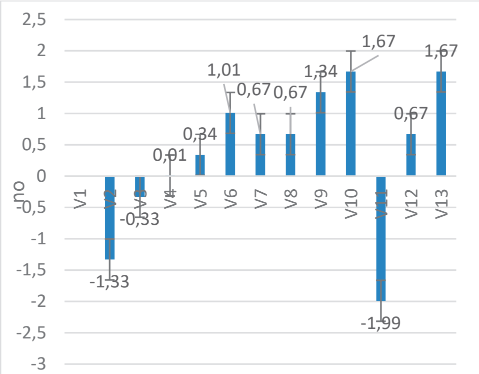

Figure 5. Difference from the control variant (V1 peat substrate) on the height of lettuce seedlings

Regarding the number of leaves we found insignificant differences from the control - V1 between the variants grown on the substrates of zeolite 100% and perlite 100% as well as between V7-Vermicompost 25% + peat 75%, V9-V13. Significant negative differences were noted in the rest of the variants (Table 3).

Table 3. Numbers of leaves for the lettuce seedlings

Variants	Number of leaves no.)	Difference (no.)	(%)	Significance
V(1)	4.33	0.00	100.00	Ct
V(2)	3.67	-0.67	84.62	N
V(3)	4.00	-0.33	92.31	N
V(4)	3.33	-1.00	76.92	O
V(5)	3.33	-1.00	76.92	O
V(6)	3.33	-1.00	76.92	O
V(7)	4.33	0.00	100.00	N
V(8)	3.33	-1.00	76.92	O
V(9)	3.67	-0.67	84.62	N
V(10)	3.67	-0.67	84.62	N
V(11)	3.67	-0.67	84.62	N
V(12)	4.33	0.00	100.00	N
V(13)	4.67	0.33	107.69	N

DL 5% = 0.910 DL 5% in % = 21.0000
DL 1% = 1.230 DL 1% in % = 28.3846
DL 0.1% = 1.660 DL 0.1% in % = 38.3077

CONCLUSIONS

Based on the results obtained, it can be estimated that the largest amount of nutrient solution was used in V3, a variant in which 100% perlite was used.

In the case of using vermicompost, the amount of nutrient solution used throughout the seedling's production was lower, the percentage being 82% nutritional solution compared to the peat-control V1, which means that the substrate retained a higher humidity, a longer time with economic effects to reduce water consumption.

Regarding the height of the seedlings, there were no significant differences, an aspect to be taken into account for the uniformity of the seedling but also for the economic establishment of the components used in the growing substrate.

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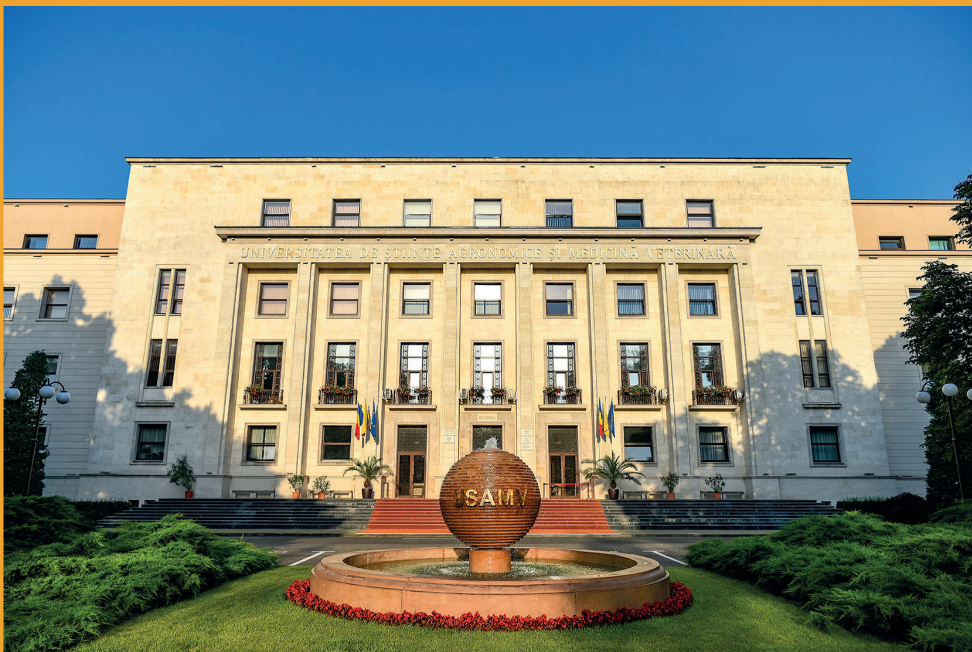

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