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THE EFFECT OF BACTERIAL BIOPREPARATIONS ON THE PRODUCTIVITY OF PEPPER CULTIVATION IN THE PEDO-CLIMATE CONDITIONS OF THE VEGETABLE RESEARCH AND DEVELOPMENT STATION BUZĂU

EFECTUL BIOPREPARATURILOR BACTERINE ASUPRA PRODUCTIVITĂȚII CULTVĂRII ARDEIULUI ÎN CONDIȚIILE PEDO-CLIMATICE ALE STAȚIUNII DE CERCETARE-DEZVOLTARE PENTRU LEGUMICULTURĂ BUZĂU

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Abstract
The present article has the role of presenting the results obtained within the pepper culture, on the VRDS Buzău lots. An experiment was performed in which two types of biological fertilizers were tested simultaneously, in parallel with a chemical fertilizer, on an area of approx. 3 ha. Based on the experiments, certain parameters were established in order to highlight the effects of organic fertilizers on the culture of bell peppers and their contribution on the productivity and well-being of the previous mentioned culture. After analyzing and interpreting the data (both standard-Excel program and statistical-program Anova) it was concluded that the activity of live bacterial cultures in the content of the two biological fertilizers led to a substantial increase in both secondary parameters and a increase in the most important parameter, namely agricultural productivity.

Keywords: *increasing agricultural productivity, biological fertilization, live bacterial cultures.*

Rezumat
Articolul are rolul de a prezenta rezultatele obținute în cadrul culturii ardeiului, pe loturile SCDL Buzău. În stațiune a fost efectuat un experiment în care s-au testat simultan două tipuri de îngrășăminte Rom-Agrobiofertil NP, în paralel cu un îngrășământ chimic, pe o suprafață de cca. 3 ha. Pe baza experimentelor au fost stabiliți anumiți parametri pentru a evidenția efectele îngrășămintelor organice asupra culturii de ardei gras și contribuția acestora asupra productivității și bunăstării culturii menționate anterior. După analizarea și interpretarea datelor s-a ajuns la concluzia că activitatea culturilor bacteriene vii în conținutul Rom-Agrobiofertil NP a dus la o creștere substanțială atât a parametrilor secundari, cât și la o creștere a celui mai important parametru, respectiv productivitatea.

Cuvinte cheie: *creșterea productivității agricole, fertilizarea biologică, culturile bacteriene vii*

INTRODUCTION

The use of fertilizer products in agricultural crops is a beneficial source of supplementing the nutrients needed for the growth and development of both plants and an increase in agricultural production. However, often the fertilizer doses applied per hectare to agricultural crops are not respected (Khalid *et al*., 2009). Failure to comply with the applied fertilizer doses will lead to the occurrence of negative phenomena for soil, environment and agricultural crops, implicitly for human and animal health. Increasing the fertilizer doses per hectare and not respecting them will lead to the occurrence of soil acidification (Malusá and Vassilev, 2014). An acidic soil will lead to a deterioration of the active processes of the soil as well as of the beneficial fauna from its structure. Deterioration of soil fauna will lead to a decrease in bacterial colonies beneficial to the soil. This aspect will be a negative factor for the germination of seed material, for the growth and development of agricultural crops but especially for obtaining quantitative agricultural productions (Tejera *et al*., 2005).

The decrease of the bacterial colonies in the soil will bring with it a decrease of the humification processes, of the decomposition and solubilization processes of the complex compounds in the soil as well as favoring the leaching and appearance of the complex compounds in the soil (in large quantities) (Ravikumar *et al*., 2007). The increase of complex compounds in the soil will lead to a decrease in pH (below pH 7), which will lead to an increase in soil acidity. On acidic soil, crops will not reach their maximum potential in productivity (Ininbergs *et al*., 2011).

In order to restore the beneficial flora of the soil, the processes carried out in the soil and to restore the amount of organic matter in the soil, agricultural specialists have proposed a number of emerging technologies for the negative effects that excess fertilization has brought with it. Specialists have proposed a new fertilization technology, namely the use of live biological fertilizers (live bacterial cultures or bacterial biopreparations) (Yang și Hoffman, 1984). These fertilizers are biodegradable so they are not a polluting factor for agricultural ecosystems. Bacterial culture combinations can be diverse.

They can be used both as fertilizers and as biological insecticides, insecticides or herbicides. Research in crops has shown that the use of bacteria as fertilizers and PPPs activates various mechanisms such as nutrient synthesis and the production of phytohormones that feed and support plants in the growth, development and productivity phases of the crop (Glick *et. al*., 2007). Another important process favored by the activity of bacterial cultures is the mobilization of soil compounds. This process involves an action of bacteria on insoluble compounds in the soil and their solubilization so as to ensure a balance of basic nutrients for plant food and support the basic processes of plants: photosynthesis and chemosynthesis (Fu *et al*., 2010).

The use of bacterial biopreparation technologies in agricultural crops plays an important role in plant protection. Some bacterial cultures give plants a protection against pedo-climatic stress, a resistance to the attack of diseases and pests as well as conferring a protection on environmental factors (drought, heavy rainfall, cold, etc.). The use of these bacterial products as fertilizers as well as plant protection products has been shown to have great potential in growing, developing, maximizing agricultural production, in restoring and greening the soil and its beneficial flora, the role of these biological fertilizers being to address a green, sustainable agriculture and achieving high, healthy, nutrient-rich productivity, beneficial to human and animal health (Revillas *et al*., 2000).

The use of microorganisms as fertilizer and plant protection products in agricultural crops in Romania, has led to an increase in agricultural productivity of farmers, productions that represent a substantial profit for them. The mechanisms of action of microorganisms (PGPR mechanisms) are described in the following figure (Dweipayan *et.al*., 2016) (Figure 1):

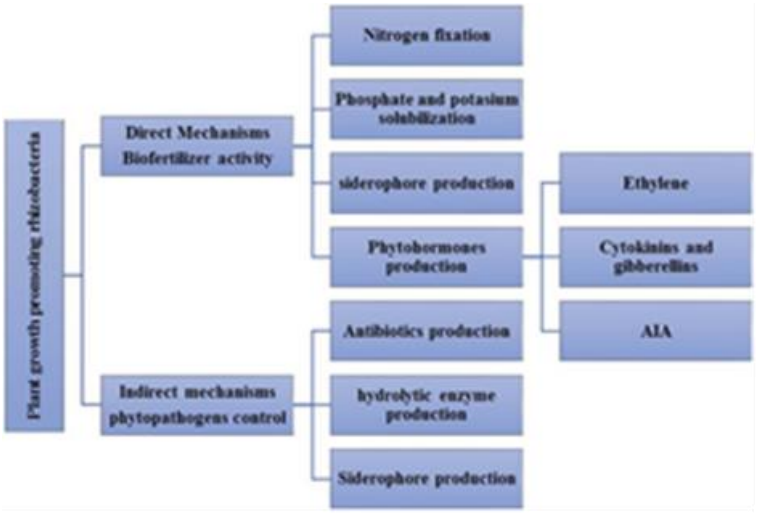

Figure 1. PGPR mechanisms of plants / Mecanismele de acțiune ale plantelor

Nitrogen is the basis of plant processes. The nitrogen cycle in plants is the essence in the processes of photosynthesis and chemosynthesis, this element being necessary in the formation of amino acids, proteins, in order to grow and develop plants. The bacterial species used in biological fertilization products (bacterial biopreparations) contain atmospheric nitrogen-fixing microorganisms, the most representative species like *Azospirillum spp., Azotobacter spp*. and *Bacillus spp*. (symbiotic bacteria associated with cereals and legumes), Frankia (actinorizale plants) (Ravikumar *et al*., 2007; Ininbergs *et al*., 2011).

MATERIALS AND METHODS

The research was carried out at Vegetable Research-Development Station Buzău. At the research center, for the cultivation of peppers, **Galben Superior** variety, three lots were established: V1-biologically fertilized lot 1-product fertilization Rom-Agrobiofertil NP (equal mix of live bacterial cultures of *Azotobacter chrooccum, Azospirillium lipoferum* and *Bacillus megaterium*),V2-biologically fertilized lot 2-Azoter product (equal mix of live bacterial cultures of *Azotobacter chroococcum, Azospirillum brasilense, Bacillus megaterium* bacteria),V3-chemically fertilized lot-product Complex NPK 16:16:16 (250 kg/ha).

The experiment was performed in the 2019-2020 period. The data obtained within the three batches were collected, statistically processed and, based on the results obtained from their modeling, the effectiveness of each product could be proven. The two biological fertilizer products, the chemical fertilizer product and the batches where these products were tested were used as working materials. Methods of observation, data collection, analysis, interpretation of data and methods of their dissemination were used as working methods.

RESULTS AND DISCUSSION

Following the pepper culture experiment, certain parameters were established to differentiate between the fertilizer products tested. Data interpretation was performed both by the Excel program (Table 1) and by the Anova statistical calculation program (Table 2), as follows:

Between the three lots there are differences between the established parameters, differences highlighted both between the two biologically fertilized lots and between the biologically fertilized lots and the chemically fertilized one. The most important parameter is crop productivity. Production showed a substantial increase of approx. 9.63% (V1 vs V2), 66.67% (V1 vs V3) and 52.03% (V2 vs V3). Based on these data, it was demonstrated that the effectiveness of bacterial cultures in the composition of biological fertilizers led to a substantial increase in pepper plants in the two biological groups.

Table 1. Results data parameters pepper culture (Excel program) / Rezultatele parametrilor înregistrați la cultura de ardei

Objectives	Parameters			Differences			
	V1-Rom-Agro	V2-Azoter	V3-NPK	V1 vs V3	V1 vs V2	V2 vs V3	V2 vs V1
Plant height (cm)	46.33	49.33	45.22	2.45	-6.08	9.09	6.48
Plant diameter (cm)	44.89	42.67	39.89	12.53	5.20	6.97	-4.95
Number of leaves	142	120.11	108.11	31.35	18.22	11.10	-15.42
Leaf length (cm)	17.68	17.48	16.58	6.63	1.14	5.43	-1.13
Leaf width (cm)	6.88	7.08	6.89	-0.15	-2.82	2.76	2.91
Number buds	25.56	19.22	18.56	37.72	32.99	3.56	-24.80
Number of fruits	6.89	5.44	6.56	5.03	26.65	-17.07	-21.04
Number of flowers	7.78	5.44	6.33	22.91	43.01	-14.06	-30.08
Stem diameter (cm)	1.11	1.11	1.16	-4.31	0.00	-4.31	0.00
Total production (t / ha)	20.5	18.7	12.3	66.67	9.63	52.03	-8.78
		Azoter parameters> Rom-Agrobiofertil NP parameters					
		Rom-Agrobiofertil NP parameters> Azoter parameters					

Fig. 2. The degree of branching of bell pepper plants, **Galben Superior** variety (organic fertilized lot, Rom-Agrobiofertil NP) / Gradul de ramificare al plantelor de ardei gras, soiul **Galben Superior** (lotul fertilizat organic cu produsul Rom-Agrobiofertil NP)

From Figure 2 we can see that the effect of bacterial cultures in the composition of the two biological fertilizers was much more beneficial than the effect of chemical fertilizer. This effect was visible both on the number of inflorescences on the plant but especially on the effect of bacterial cultures on agricultural production. At the same time, the experiments performed on the seed material (with the two biological fertilizers) showed that the bacteria in their content have the ability to produce enzymes and antioxidants (catalase, peroxidase, etc.) that have important roles in plant phenophases and plant protection, stress, acid rain, reactive oxygen (superoxide, hydrogen peroxide etc).

At the same time, in order to support the previous arguments, the statistical data from Excel were modeled with the help of a statistical program, namely Anova. Following the modeling of the data, it was proved that the effect of bacterial cultures was beneficial on agricultural productivity as well as on the amount of soil elements. After modeling the data through the Anova program, the following values were obtained (Table 2):

Table 2. Results data parameters in pepper culture (Anova program) / Parametrii rezultatelor obținute cu programul Anova la cultura de ardei

Anova: Two-Factor Without Replication						
SUMMARY	***Count***	***Sum***	***Average***	***Variance***		
Plant height (cm)	3	140.88	46.96	4.52		
Plant diameter (cm)	3	126.45	42.15	9.20		
Number of leaves	3	370.22	123.41	295.28		
Leaf lenght (cm)	3	51.74	17.25	0.34		
Leaf width (cm)	3	20.85	6.95	0.01		
Number buds	3	63.34	21.11	14.94		
Number of fruits	3	18.89	6.30	0.58		
Number of flowers	3	19.55	6.52	1.40		
Stem diameter (cm)	3	3.38	1.13	0.00		
Total production (t/ha)	3	51.5	17.17	18.57		
V1- Biologically fertilized lot 1 (Rom-Agrobiofertil NP fertilization product)	10	319.62	31.96	1739.18		
V2- Biologically fertilized lot 2 (Nitrogen fertilizer product)	10	286.58	28.66	1289.22		
Chemical fertilized lot (control lot - Complex 16:16:16 x 250 kg / ha)	10	260.6	26.06	1039.60		
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Rows	36097.27	9	4010.81	140.27	8.3128	2.456281
Columns	175.00	2	87.50	3.06	0.071788	3.554557
Error	514.70	18	28.59			
Total	36786.97	29				

After the end of the harvesting campaign, as a result of the increase of the productivity of the pepper crop on the biologically fertilized lots, a series of soil samples were taken in order to carry out an agrochemical mapping. Due to the lack of funds, it was decided to take samples from the control group (chemical fertilizer) and biologically fertilized lot 1 with the fertilizer product Rom-Agrobiofertil NP. Following pedological analyzes in a reference laboratory, the following changes in soil structure were identified (Table 3):

Table 3. Soil sampling mapping results (biologically fertilized lot 1 vs chemically fertilized lot) / Rezultatele cartografierii solului în lotul fertilizat biologic, comparativ cu lotul fertilizat chimic

Samples	pH	Humus	Nt	P_{AL}	P_{AL}[1]	K_{AL}
Sample 1 - control lot (chemical fertilization product Complex NPK 16:16:16)	8.04	2.59	0.191	562	275	460
Sample 1 - biologically fertilized lot 2 (biological fertilization product Rom-Agrobiofertil NP)	8.01	2.59	0.185	570	287	404
Biological vs chemical differences	**-0.37**	**0.00%**	**-3.14%**	**1.42%**	**4.36%**	**-12.17%**
Sample 2 - control lot (chemical fertilization product Complex NPK 16:16:16)	8.09	2.53	0.186	590	275	484
Sample 2 - biologically fertilized lot 2 (biological fertilization product Rom-Agrobiofertil NP)	8.14	2.53	0.182	611	270	424
Biological vs chemical differences	**0.62%**	**0.00%**	**-2.15%**	**3.56%**	**-1.82%**	**-12.40%**
Sample 3 - control lot (chemical fertilization product Complex NPK 16:16:16)	6.61	2.53	0.365	317	302	569
Sample 3 - biologically fertilized lot 2 (biological fertilization product Rom-Agrobiofertil NP)	6.54	2.47	0.359	310	297	563
Biological vs chemical differences	**-1.06%**	**-2.37%**	**-1.64%**	**-2.21%**	**-1.66%**	**-1.05%**

CONCLUSIONS

The use of excess chemical fertilizers in agricultural crops has led to a deterioration of the soil flora and a decrease in crop yields. The replacement of chemical fertilizers with biological fertilizers, which identify live bacterial cultures, had the effect of restoring the flora damaged by the action of chemical fertilizers, increasing the parameters of agricultural crops, especially their productivity and the amount of mineral elements in the soil. The use of these biological fertilization products in Romanian agricultural crops was an important step for Romanian agriculture. At first, farmers were reluctant to use these products because they were and still depend on chemical fertilization. The application of these products in agricultural crops has allowed farmers to open a new horizon, a horizon that has led to their transition from a conventional agriculture to a sustainable, ecological, environmentally sustainable agriculture.

Replacing chemical fertilizers with organic fertilizers was a great advantage for farmers in Romania and beyond. The beneficial bacteria from the content of biological fertilizers ensured the restoration of the soil (following its degradation), its recolonization with beneficial bacteria as well as an increase and maximization of agricultural production and obtaining healthy crops with a much better quality. At the same time, these bacterial biopreparations have the effect of lowering the pH of the soil through various pH regulation mechanisms. The decrease of the soil pH is an important aspect because the lands affected by this phenomenon can be reintroduced in the crop production circuit, which for farmers will represent a larger area, which will bring a substantial profit for them.

Bacterial cultures also have the effect of greening the groundwater and breaking down complex compounds into soluble compounds, easily assimilated by plants. Bacterial cultures in the composition of organic fertilizer products increase the amount of protein elements in plants and, implicitly, in agricultural production. Thus, farmers will get a higher production, much richer in beneficial elements for human and animal health. The use of microorganisms in agricultural crops has led to a balance of soil electrolyte balance, balancing soil processes and the very fast and beneficial mediation of energy exchange between soil, environment and agricultural ecosystems. Another important aspect in the use of microorganisms in agriculture is their use as a treatment for

seed material. Inoculation of the seed material in the three bacterial cultures from the Rom-Agrobiofertil NP product led to a much faster germination of the seed material, protection of the seeds from some pests in the soil and fixation of the planting material in the soil, a much better rooting and much better plant development in the soil.

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YIELD POTENTIAL OF SOME EGGPLANT CULTIVARS AND ADVANCED HOMOZYGOUS LINES

POTENȚIALUL PRODUCTIV AL UNOR SOIURI ȘI LINII AVANSAT HOMOZIGOTE DE PĂTLĂGELE VINETE

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Abstract
*Within the Research and Development Institute for Vegetable and Flower Growing Vidra, in 2024 was carried out an experience in which were studied 4 cultivars (**Luiza, Belona, Eleonora** and **Tudora**) and 10 advanced homozygous lines of eggplants from the collection of the Breeding and Seed Production Laboratory. The genotypes were compared in the comparative crop field. Physiological and biometric measurements were carried out, in order to establish the main phenophases of growth and development and the yield potential of each cultivar and line studied. Some genotypes present valuable characteristics such as high yield potential, while others stand out for their earliness. Due to its various properties, the studied material has the potential to be used in eggplant breeding programs.*

Keyword: *breeding, eggplant, yield potential*

Rezumat
*În cadrul Institutului de Cercetare-Dezvoltare pentru Legumicultură și Floricultură Vidra, în anul 2024 a fost realizată o experiență în care au fost studiate 4 soiuri (**Luiza, Belona, Eleonora** și **Tudora**) si 10 linii avansat homozigote de pătlăgele vinete din colectia Laboratorului de Ameliorare și Producerea Semințelor, care au fost comparate în câmpul de culturi comparative. Au fost realizate determinări fiziologice și măsurători biometrice, fiind determinate principalele fenofaze de creștere și dezvoltare și potențialul de producție al fiecărui soi și linie studiată. O parte din genotipuri prezintă ca însușiri valoroase un potențial ridicat de producție, în timp ce altele se remarcă prin timpurietatea lor. Prin diferitele proprietăți, materialul studiat are potențial de a fi folosit în programele de ameliorare la pătlăgelele vinete.*

Cuvinte cheie: *ameliorare, pătlăgele vinete, potențial de producție*

INTRODUCTION

The consumption of fresh, cooked or preserved vegetables is increasing worldwide, which calls for varieties and hybrids with remarkable fruit production and quality characteristics, resistance to pathogens and environmental stress factors. The breeding of plant resources in general, the creation of new genotypes in particular, is a constantly changing process, being determined mainly by market demands and changes in biotic and abiotic factors (Scurtu *et al*., 2016; Vînătoru *et al*., 2019). Worldwide, the breeding of solanaceous fruit species has experienced a particular boost. As proof, there is a very large number of cultivars listed in the official catalogs of European vegetable-growing countries (Scurtu, 2014). In the context of current climate change and the orientation towards sustainable and organic agriculture, ensuring long-term food security, the cultivar must be tolerant to adverse environmental factors (thermal and water stress) and biotic factors (Scurtu *et al*., 2016; Vînătoru *et al*., 2019). The year 2024 was the warmest in the history of measurements in our country (https://hotnews.ro/vara-lui-2024-a-fost-cea-mai-calda-de-cand-exista-date-meteo-in-romania-cat-de-mare-a-fost-diferenta-fata-de-cele-mai-racoroase-veri-1788484).

Eggplant is a heat-loving species, which makes them recommended in these conditions, but it must also be taken into account that they have high humidity requirements, due to its tropical

origin, which must be ensured through irrigation system (Munteanu, 2003). These characteristics are due to its tropical origin (Tudor *et al*., 2009). Eggplant is the most demanding species among the solanaceous vegetables when it comes to environmental factors. Its are warm climate plants, requiring a minimum of 125 days to produce. Temperatures of 20-25^{O}C are optimal for anthesis and pollination of plants. In Romania, eggplant is an important crop, being cultivated both in the open field and in protected areas, and it is considered that both consumption and the areas cultivated with this species will continue to grow in the coming years (Scurtu *et al*., 2020). In our country, the species finds optimal cultivation conditions in the area around Bucharest (Vînătoru *et al*., 2019).

In 2024, the Official Catalogue of Cultivated Plant Varieties in Romania included 19 eggplant cultivars, of which 13 were open-pollinated cultivars and six were hybrids (https://istis.ro/wp-content/uploads/2024/07/ISTIS-CATALOG-OFICIAL-2024.pdf).

In Romania, cultivars with black or dark purple fruit, with an ellipsoidal or pear shape, are preferred, but in recent years, preferences for lighter colored varieties, such as white or purple, have also increased (Vînătoru *et al*., 2019).

The breeding objectives for eggplants are more than they seem at first glance.

In all agricultural and horticultural species, the most important breeding objective is yield potential and the various parameters that define it, a valid aspect also for eggplant. The highest productivity is achieved by hybrid cultivars. However, obtaining valuable hybrids depends on parents. Therefore, obtaining valuable and productive open-pollinated cultivars is also of vital importance in breeding (Kumar, 2018).

The shape and color of the fruit is important, with consumers having different and changing preferences for these parameters (Vînătoru *et al*., 2019). The shape of the fruit varies greatly, from globular to ovoid or elongated, straight or curved. The color of the fruit varies greatly from one cultivar to another, from white to dark purple or black, they can have shades of green and may have lighter stripes (Tudor *et al*., 2009).

Some of the most important objectives of eggplant breeding are represented by various characters related to fruit quality.

Fruit bitterness, due to several chemical compounds present in the fruit (including solasonine), is one of the most important aspects of fruit quality breeding in eggplant. In general, preferences are directed towards less bitter cultivars. Also, the content of anthocyanins and phenolic acids is a variety characteristic and is sought, depending on preferences, both low-content and high-content cultivars. Some of these compounds can induce a bitter taste during preparation, but have important therapeutic value (Daunay, 2008).

Another objective is to adapt resistance to pedological and atmospheric drought (Chapman, 2020). Even though they are plants that tolerate high temperatures well, they have high requirements for soil and atmospheric humidity (Tudor *et al*., 2009).

A very important breeding objective in eggplant is resistance to biotic and abiotic stress factors. Of these, the most important is increasing tolerance to soil-borne diseases, such as Verticillium wilt (*Verticillium dahliae*) (Șovărel and Costache, 2018; Chapman, 2020) and Fusarium wilt (*Fusarium oxysporum* f.sp. *melongenae*) (Șovărel and Costache, 2018).

The purpose of this work is to evaluate the performance of four varieties and ten advanced homozygous lines from the Research and Development Institute for Vegetable and Flower Growing Vidra collection, in terms of productive potential, under the specific pedoclimatic conditions of the Ilfov area.

MATERIALS AND METHODS

The experience took place in the open field, at the Research and Development Institute for Vegetable and Flower Growing Vidra, in 2024. Seedlings from four cultivars and 10 advanced homozygous lines were used as biological material. The cultivars used in experience were **Luiza,**

Belona, Eleonora and T**udora**. Table 1 presents the phenotypic description of the four cultivars and the 10 advanced homozygous lines used in the present experiment.

Tabel 1. The main phenotypic characteristics of biological material / *Principalele caracteristici fenotipice ale materialului biologic*

Nr. crt.	Genotype	Plant vigor	Fruit shape	Fruit color
Cultivars	**Luiza**	medium	ellipsoid	very dark violet
	Belona	medium	pear shaped	white
	Eleonora	medium	ellipsoid	dark violet
	Tudora	big	club shaped	dark violet
Homozygous lines	**L 2**	big	ellipsoid	dark violet
	L 24	big	ellipsoid	dark violet
	L 31	medium	ellipsoid	dark violet
	L 228	medium	ellipsoid	dark violet
	L 43	medium	ovoid	light violet
	L 51	medium	cylindrical	medium violet
	L 59	medium	obovate	light violet
	L 33	medium	globular	white
	L 34	big	pear shaped	white
	L 245	small	ovoid	white

Two lines (L33 and L34) are characterized by white flowers, while the other genotypes have purple flowers (Figure 1).

Figure 1. Flowers at eggplant genotypes – white flower (left) and purple flower (right) / *Flori la genotipurile de vinete – floare albă (stânga) și floare violetă (dreapta)*

The seedlings were produced in alveolar pallets, with 70 cells, with a volume of 50 ml each cell. Fertilized peat, the Domoflor Mix product, to which 1% perlite was added, was used as

substrate. Since sowing was carried out separately, one seed in each alveolus, replanting was eliminated, avoiding the stress generated by this technological sequence on the plants.

The seedlings were planted in the open field on 18th of May, 2024, on mulched ground, with a distance of 70 cm between rows and 40 cm between plants. The experimental variants were arranged in randomized blocks, and every variant had three replications.

The plants were irrigated by drip irrigation, with a watering rate of 50-60 m^3/ha, every three days, the watering rate being adapted depending on the precipitation.

Several fertilization works were carried out. The first fertilization was carried out one week after planting, with the product Solfert 11.52.5 (12 kg/ha), for better rooting of the plants. The second fertilization was carried out with ammonium nitrate, in a quantity of 35 kg s.a per hectare. Another fertilization was applied with Solfert 20.20.20 (12 kg/ha), during fruit setting and one with Solfert 10.5.40 (12 kg/ha), during the fruit ripening period.

According to the National Meteorological Administration of Romania, the summer of 2024 was the warmest summer in Romania, from 1901-2024, with an average temperature in the country of 24.2^{O}C (https://hotnews.ro/vara-lui-2024-a-fost-cea-mai-calda-de-cand-exista-date-meteo-in-romania-cat-de-mare-a-fost-diferenta-fata-de-cele-mai-racoroase-veri-1788484). Figure 2 presents the values for atmospheric temperature, relative air humidity and precipitation amount for the period May - September 2024, in Vidra, Ilfov county.

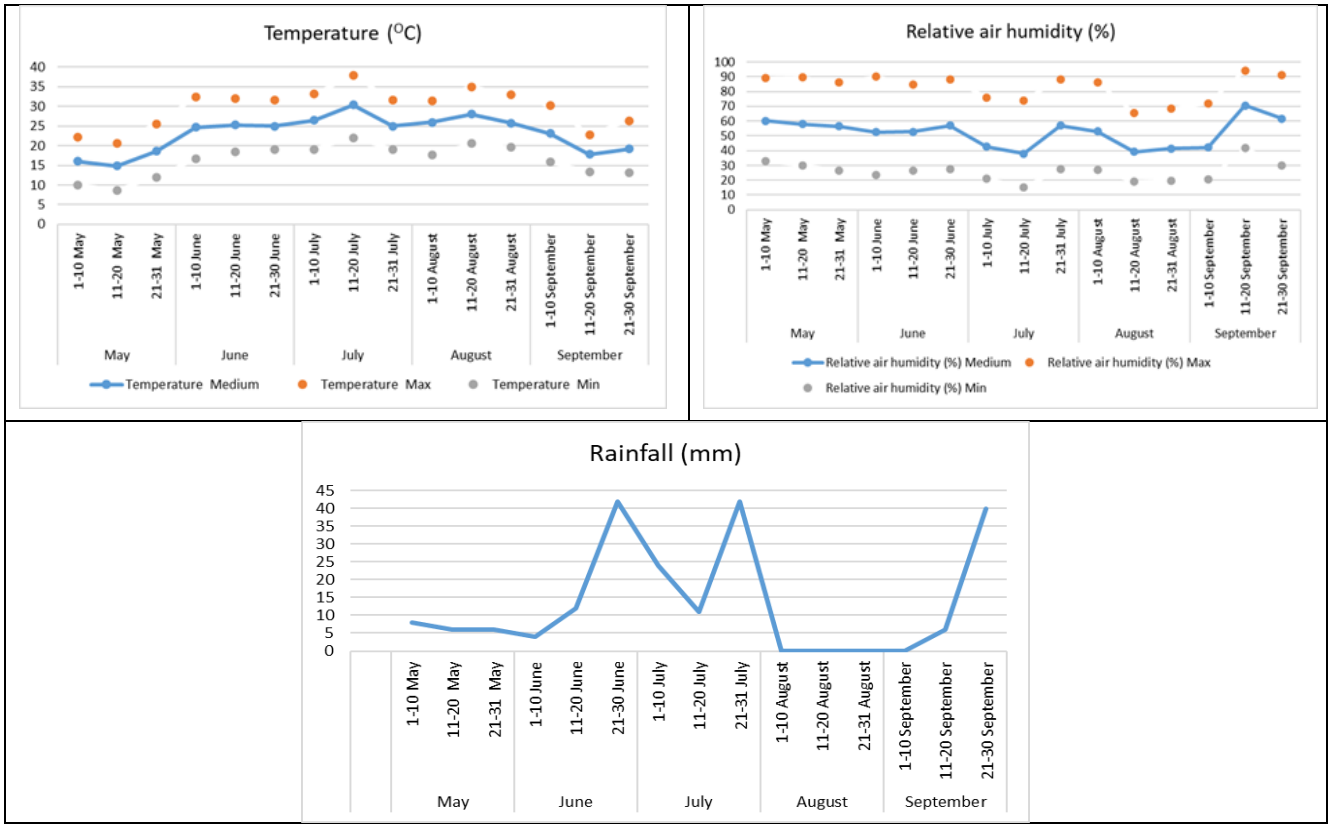


Figure 2. Atmospheric temperature, relative air humidity and precipitation amount for the period May - September 2024, in Vidra, Ilfov county / *Temperatura atmosferică, umiditatea relativă a aerului și cantitatea de precipitații în perioada mai - septembrie 2024, în Vidra, județul Ilfov*

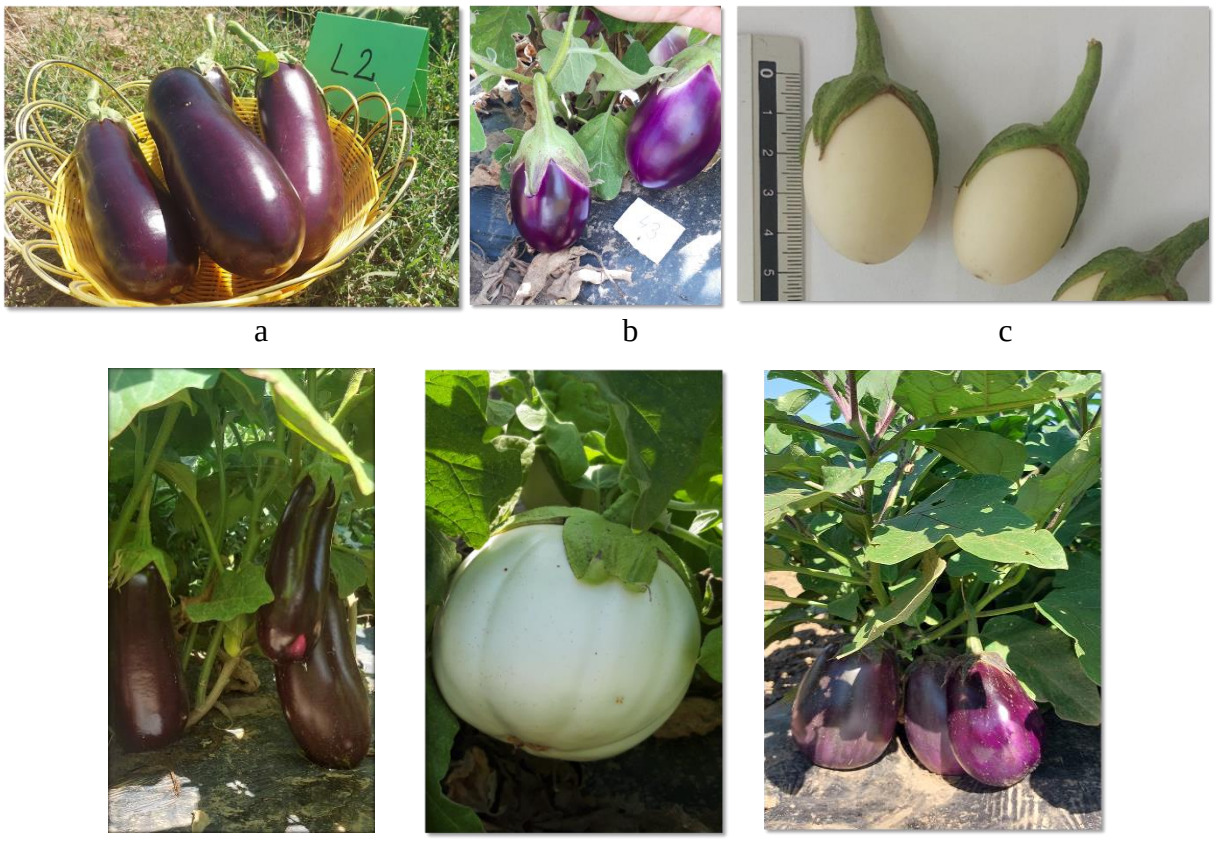
During the growing season, the plants were monitored to determine some of the most important phenological development stages. The moment of flowering (BBCH 61), beginning of fruit setting (BBCH 63) and the moment when the first fruit reached technological maturity (BBCH 71) were determined, these being expressed in number of days from seedlings emergence.

The eggplant harvest was carried out in stages, at the technological maturity of each genotype. The first harvest was carried out at the technological maturity date, followed by two more harvests. No fruit was harvested from any diseased plants, and fruit affected by blossom end rot were removed and not used in the measurements. Fruit that met commercial requirements were harvested, counted and weighed, in order to calculate the yield, the number of fruits/plant and fruit weight.

a b c

Figure 3. Eggplant cultivars studied (a – Luiza, b – Belona, c - Tudora) / *Cultivare de pătlăgele vinete studiate (a – Luiza, b – Belona, c - Tudora)*

In Figure 3, the variety of shapes and colors of three of the eggplant cultivars studied in the experiment can be observed. These meet the current requirements of the Romanian market and are appreciated by consumers.



a b c

d e f

Figure 4. Eggplant homozygous lines studied (a – L2, b – L43, c – L245, d – L24, e – L33, f – L59) / *Linii homozigote de pătlăgele vinete studiate (a – L2, b – L43, c – L245, d – L24, e – L33, f – L59)*

The shapes and colors of some of the advanced homozygous lines studied can be observed in Figure 4. Even if some of them do not meet market requirements of Romanian consumers, these

lines may come with other valuable traits, such as earliness or resistance to various diseases, especially soil diseases.

Statistical interpretation of the data was performed using a software program, IBM SPSS, version 26, the significance of differences being expressed with the Duncan multiple range test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Figure 5 presents the most important phenological development stages for the four cultivars and the 10 eggplant genotypes studied. It is observed that these parameters vary greatly depending on the genotype. This aspect is important, because some genotypes can be used to improve the earliness of other lines or cultivars.

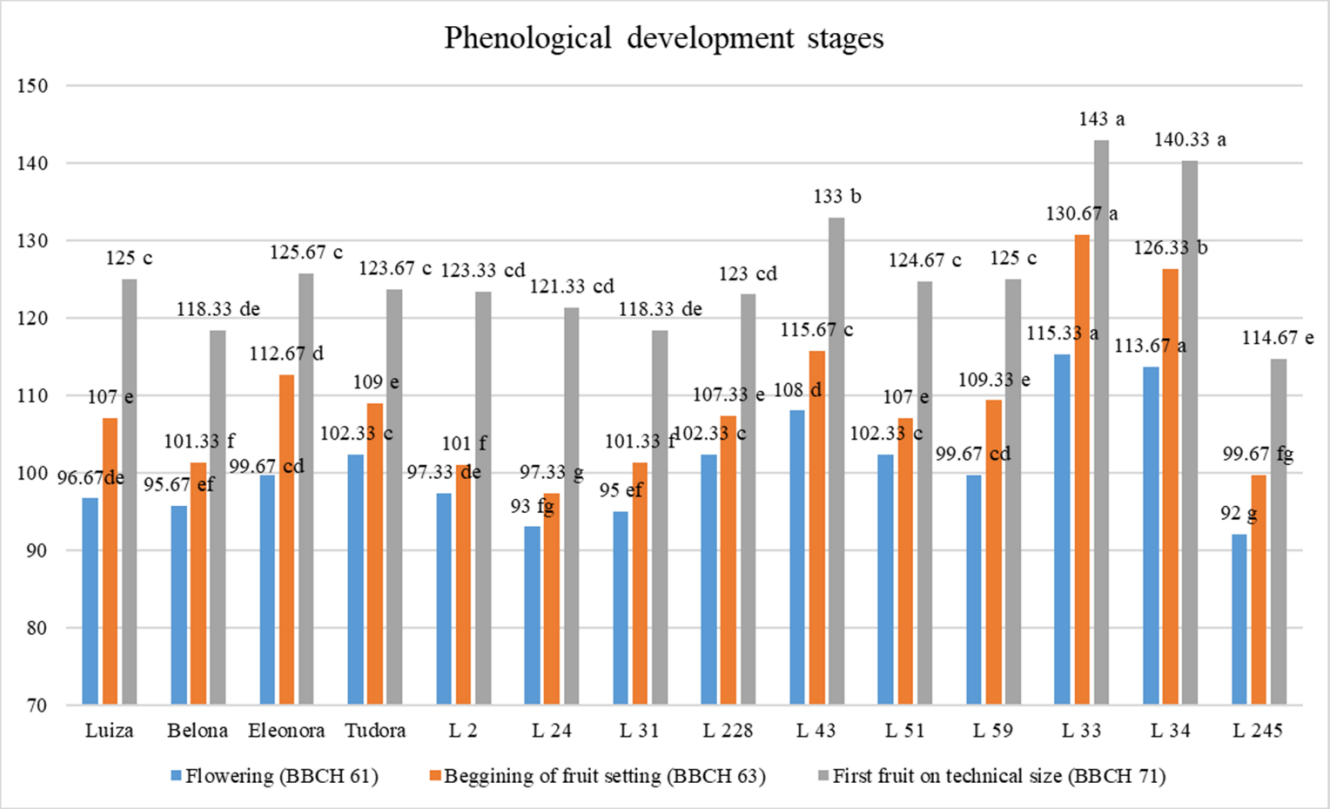


Figure 5. Phenological development stages at the studied eggplant cultivars and genotypes (days) */ Stadii de dezvoltare fenologică la soiurile și genotipurile de vinete studiate (zile)*

Flowering began after a number of days that varied between 92 and 115.33 days. The earliest to flower was the advanced homozygous line L 245, and the latest, the line L 33, both having white fruit. The values of this parameter varied a lot, with an average value of 100.93 days.

Among the cultivars, the earliest flowering was observed at **Belona** (95.67 days) and **Luiza** (96.67 days). The flowering was significantly earlier compared to the **Eleonora** and **Tudora** cultivars, which flowered at 99.67 days and 102.33 days, respectively.

Of the lines studied, five bloomed less than 100 days after emergence: L 245 (92.00 days), L 24 (93.00 days), L 31 (95.00 days), L 2 (97.33 days) and L 59 (99.67 days). The other five lines (L 228, L 51, L 43, L 34, L 33) flowered after an interval between 102.33 and 115.33 days.

Some genotypes are noted for their early flowering, which is advantageous in climatic conditions such as those of 2024, when there were many days with very high temperatures and

severe drought. It is good that the flowering period takes place before the beginning of the period with hot temperatures. When temperatures are too high and drought is too severe, there is a risk of flower abortion (Munteanu, 2003).

The beginning of fruit setting varies greatly depending on the genotype, with values ranging from 97.33 days to 130.67 days, with an average of 108.98 days.

All cultivars began fruit setting after a period of over 101.33 days, the first being the Belona cultivar (101.33 days), and the last, the **Eleonora** cultivar (112.67 days). Of the lines, two were very early, with fruits forming after a period of 97.33 days (L 24), and 99.67 days (L 245), respectively. Some lines required a very long period until the first fruit set, 126.33 days (L 34) and 130.67 days (L 33). Most lines needed an interval of 101.00 - 115.67 days until the first fruit set.

Also, the first fruit reached the technical size after a time interval that varied significantly between 114.67 and 143 days, with average of 125.67.

Among the cultivars, the earliest in terms of technical maturity was the **Belona** cultivar, in which the fruits could be harvested after 118.33 days from emergence, a significantly lower number compared to the other three cultivars, whose harvest began after a number of days that varied between 107.00 days (**Luiza**) and 112.67 days (**Eleonora**).

Two of the studied lines showed a high precocity in the technical maturity of the fruits: L 245 (114.67 days) and L 31 (118.33 days). Five of the lines could be harvested after an interval of 121.33 - 125.00 days (L 24, L 228, L 2, L 51 and L 59). Three of the lines were late, the technological maturity being after a number of 133.00 (L 43), 140.33 (L 34) and 143.00 days (L 33).

Even though the constant warming trend is also observed in our country, cultivars with a shorter number of days until the fruit ripening for consumption are preferable, so that as many fruits as possible can be harvested before the first frost (Munteanu, 2003).

Table 2 presents data on the production parameters studied for the 14 eggplant genotypes that were the subject of the experiment.

Tabel 2. Yield per hectare, number of fruits on plant and fruit weight of eggplant genotypes/ *Producția la hectar, numărul de fructe pe plantă și greutatea fructelor la genotipurile de vinete*

	Genotype	**Yield/ha** *kg/ha*	**Number of fruits/plant** *fruits/plant*	**Fruit weight** *g*
1	**Luiza**	43.93 bc	4.08 cd	308.53 bcd
2	**Belona**	38.27 def	5.17 b	211.62 e
3	**Eleonora**	37.60 ef	3.92 de	274.67 d
4	**Tudora**	46.94 b	4.11 cd	332.16 abc
5	**L 2**	34.56 fg	3.67 de	270.12 d
6	**L 24**	53.59 a	4.89 bc	313.14 bcd
7	**L 31**	42.39 cd	4.56 bcd	270.42 d
8	**L 228**	38.59 def	3.92 de	282.52 cd
9	**L 43**	15.40 i	4.13 cd	106.74 f
10	**L 51**	26.23 h	4.53 bcd	166.59 e
11	**L 59**	32.78 g	3.60 de	262.01 d
12	**L 33**	31.42 g	2.55 f	354.71 ab
13	**L 34**	39.82 de	3.11 ef	369.75 a
14	**L 245**	8.60 j	8.14 a	30.35 g

The yield varied significantly, with values ranging between 8.6 and 53.59 kg/ha, with an average of 35 kg/ha.

The four cultivars created at RDIVFG Vidra have high productivity, being acclimatized and improved in our country, in the environmental conditions specific to the area around the Romanian capital. The four cultivars have productions ranging between 37.6 and 46.94 kg/ha. The most productive cultivars are **Tudora** (46.94 kg/ha) and **Luiza** (43.93), with no significant differences between the two. Compared to them, the other two cultivars, **Belona** and **Eleonora**, gave significantly lower yields, of 38.27 kg/ha and 37.6 kg/ha, respectively.

Among the advanced homozygous lines, the highest yield corresponded to the L24 line, with a value of 53.59 kg/ha. The next line, L31, gave a yield of 42.39 kg/ha, and L 34 (39.82 kg/ha) and L 228 (38.59 kg/ha) did not differ significantly. The L2, L59 and L L33 lines had significantly lower yields, ranging from 31.42 kg/ha (L33) to 34.56 ka/ha (L2).

The lowest yields were given by lines L 51, L 43 and L 245. The values ranged from 8.6 kg/ha (L 245) to 26.23 kg/ha (L51). Even so, these lines can be used in breeding for other traits, such as characteristic earliness, or fruit shape and color.

The number of fruits per plant varied greatly, between 2.55 and 8.14 fruits/plant, with an average of 4.31 fruits/plant.

Among the cultivars created at RDIVFG Vidra, the highest number of fruits per plant characterizes the cultivar **Belona**, with an average number of 5.17 fruits/plant. By comparison, the other two cultivars have a significantly lower number, ranging between 3.92 (**Eleonora**) and 4.08 (**Luiza**) fruits/plant.

In the case of advanced homozygous lines, the lowest number of fruits per plant was given by L 33 (2.55), and the highest number of fruits per plant, by L 245 (8.14). However, most lines were characterized by a number of fruits per plant close to the average of 4.31 fruits.

Fruit weight is a varietal characteristic and varied significantly. Values ranged from 30.35 to 369.75 g, with an average of 253.81 g.

In the case of the four cultivars studied, the fruit weight varied significantly between 211.62 g (**Belona**) and 332.16 g (**Tudora**). The fruits, in the case of the **Luiza** cultivar, had an average weight of 308.53 g, and there were no significant differences compared to the **Tudora** cultivar. In the case of the **Eleonora** cultivar, the fruit weight value of 274.67 was significantly higher than that of the **Belona** cultivar, but significantly lower compared to **Tudora**.

In the case of the lines from the institute's collection, the fruit mass varied within very wide limits, with values between 30.35 g (L 245) and 369.75 g (L 34). Three of the advanced homozygous lines studied had fruits with an average weight greater than 300 g: L 24 (313.14 g), L 33 (354.71 g) and L 34 (369.75 g). Four of the lines had a fruit weight between 262.01 - 282.52 g: L 59 (262.01 g), L 2 (270.12 g), L 31 (270.42 g) and L 228 (282.52 g).

CONCLUSIONS

1. Some of genotypes show high fruiting earliness. These can be used in breeding to shorten the fruiting period, so that as many fruits as possible can be harvested by autumn, when the ambient temperature drops and the fruits grow more slowly.
2. Some homozygous lines show high production potential. This character makes them valuable for creating hybrid cultivars.
3. Some homozygous lines show large fruits. In general, market demands are directed towards heavy fruit.
4. Special attention must be paid to the breeding process, as the genetic material may also have undesirable traits.

ACKNOWLEDGMENTS

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BIOCOMPOSITES FROM RED GRAPE BY-PRODUCTS: A SOIL FERTILIZATION APPROACH

BIOCOMPOZITE DIN SUBPRODUSE DE LA PROCESAREA STRUGURILOR ROȘII: O ABORDARE PENTRU FERTILIZAREA SOLULUI

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Abstract
The study aims to valorize the nutritive and bioactive compounds found in by-products resulting from red winemaking (pomace and wine lees from the Băbească neagră grape variety) for the formulation of a new biofertilizer with enhanced nutritional and functional properties. Red grape pomace is rich in organic and mineral compounds that serve as nutrients and bioactive compounds, positively influencing plants and soil microorganisms. To increase biotic potential, the wine lees biomass was processed through different treatments (ultrasound and thermal treatments), resulting in the release of bioactive compounds (proteins, polyphenols, named postbiotics) and parabiotics (dead cells), positively impacting the soil's microbiome. The results confirm that pomace and the treated wine lees biomass are valuable natural ingredients for formulating a new composite biofertilizer by combining them with slag, dolomite, and cement kiln dust (CKD), in proportions that provide standardized final product characteristics.

Rezumat
Studiul vizează valorificarea compușilor bioactivi din suprodusele (tescovină și drojdie reziduală), rezultate la vinificarea strugurilor roșii (soiul Băbească neagră) pentru formularea unui nou biofertilizator cu proprietăți nutritive și funcționale îmbunătățite. Tescovina din struguri roșii este bogată în compuși organici și minerali, cu rol de nutrienți și compuși bioactivi, cu influență benefică asupra microorganismelor din sol. În vederea creșterii potențialului biotic, biomasa de drojdie a fost procesată prin diferite tratamente (ultrasunete, tratament termic), obținându-se eliberarea compușilor bioactivi (proteine, polifenoli, denumiți și postbiotice) și paraprobiotice (celule moarte) cu impact pozitiv asupra microbiomului din sol. Aceste rezultate confirmă că tescovina și drojdia reziduală tratată sunt ingrediente naturale valoroase pentru formularea unui nou biofertilizator compozit prin combinarea acestora cu zgură, dolomit și praf de electrofiltru (CKD), în proporții care conferă caracteristici standardizate produsului final.

Keywords: *Băbească neagră; red grapes; pomace; wine lees; postbiotics; parabiotics, biotication; biocomposites; new biofertilizers*

Cuvinte cheie: *Băbească neagră; struguri roșii, tescovină; drojdie reziduală; postbiotice; paraprobiotice, bioticare; biocompozite.; fertilizatori noi*

INTRODUCTION

The proper management and valorisation of secondary products resulting from winemaking (pomace and residual lees) are essential for sustainable development from the perspective of the circular economy. According to data published by the Food and Agriculture Organization (FAO), with an estimated production of more than 79 million tons in 2018, grapevine culture is one of the most widespread fruit crops worldwide. Pomace is the main by-product of wine production, which is thought to account for about 75% of all grape production. Depending on the grape variety and

wine-making process, pomace represents 15-30% of the total weight of grapes processed, containing skins, pulp, seeds, and stems (Martínez Salgado *et al.*, 2019; Antonić *et al*., 2020).

To improve the soil microbiome, which is involved in soil bioremediation, and the nutrition, growth, vitality, health, and safety of plant biomass, winery by-products can be utilized as natural, beneficial ingredients for the formulation of green biofertilizers (Troilo *et al*., 2021).

Pomace is considered a valuable natural resource with many valorisation opportunities due to its composition, which is rich in nutritious and bioactive substances, including polyphenols, organic acids, fatty acids, proteins, vitamins, etc. (Almanza-Oliveros *et al.*, 2024). In its use in the formulation of biofertilizers, the physico-chemical properties are considered, i.e. the moisture after pressing 20 - 30% (g/g), high organic content consisting of macronutrients and micronutrients with high bioavailability, C:N ratio 40(45):1, pH values between 3.0-6.0, low electrical conductivity etc. (García-Lomillo & González-San José, 2017).

The residual yeast (lees) that results after the grape juice fermentation is a solid fraction mainly consisting of yeast cells (*Saccharomyces cerevisiae*), organic acids (mainly tartaric acid), insoluble carbohydrates, inorganic salts, polyphenols, proteins etc. Thus, lees are a valuable source of bioactive compounds (proteins, phenolic compounds, dietary fibers, antimicrobials etc.), which have functional properties (Maicas & Mateo, 2021).

Postbiotics and parabiotics are the emerging concepts associated with the functional characteristics of fermented products, having health-promoting properties. The postbiotics are defined as the complex mixture of metabolic products secreted by viable cells in cell-free supernatants, such as proteins, enzymes, peptides, organic acids, vitamins, etc. The parabiotics are the inactivated microbial cells (intact or ruptured, containing cell components) or crude cell extracts (i.e. with complex chemical composition) (Nataraj *et al.*, 2020). In this context, the concept of postbiotication means the increase the biotics (postbiotic, parabiotics) content by physico-chemical and biochemical processing of viable cells (Pihurov *et al.*, 2024).

In this study, the organic (nutrients and bioactives) and mineral composition of the red pomace and lees obtained from *Băbească neagră* grape variety vinification were characterized in the perspective of formulation of an innovative yeast-bioticated biofertilizer. In this context, the residual wine lees biomass was processed through different physico-mechanical treatments to release the valuable postbiotics (proteins, polyphenols) and to obtain the parabiotics (non-viable cells), which will be used for the biofertilizer formulation, with a positive impact on the soil microbiome.

MATERIALS AND METHODS

Raw by-products and reagents

The unfermented red grape pomace (*Vitis vinifera*) from the *Băbească neagră* variety and the residual wine lees were collected at Bratu winery, located in Odaia Manolache village, Vânători commune, Galați County (45º33′27.5182″N, 28º0′21.7552″E). The raw grape pomace was stored at -20°C until use, then dried at 35°C with hot air convection, until a moisture content of 8% was achieved and then ground and stored in vacuum-sealed bags under refrigeration (4°C). The residual wine lees produced during the fermentation of the same grape juice was collected and freeze-dried (-42°C, 0.11 mbar) to a constant mass. All chemicals used were of analytical grade and were obtained from the Sigma-Aldrich company (Steinhelm, Germany).

Red grape pomace composition analysis

The organic and mineral composition of dried pomace was analysed by using Fourier-transform infrared spectroscopy (FTIR) with an ISRpirit-T FT-IR spectrometer equipped with a built-in QATR-S type ATR accessory, DLATGS detector, and KBr beam splitter in the range of 4000-400 cm^{-1} at room temperature, with a resolution of 2 cm^{-1}, and X-ray fluorescence spectroscopy (XRF OLYMPUS Vanta V Model VCR-CCC-A3-E) (Castro *et al*., 2025).

For bioactive compounds assay, a quantity of 0.5 g of dried red pomace was subjected to solid-liquid extraction, assisted by ultrasounds, using 4.5 mL of 70% ethanol and 0.5 mL of glacial acetic acid. Ultrasound-assisted extraction was performed in a water bath with sonication at 30°C for 30 minutes, followed by centrifugation at 6000 rpm for 10 minutes at 4°C. The extract was characterized by determining the total phenolic content (TPC), total flavonoid content (TFC) and the total monomeric anthocyanin content (TAC) (Serea *et al.*, 2021).

Lee's biotication and composition assays

The organic and mineral composition of freeze-dried lees was analysed by using Fourier-transform infrared spectroscopy (FTIR) with an ISRpirit-T FT-IR spectrometer equipped with a built-in QATR-S type ATR accessory, DLATGS detector, and KBr beam splitter in the range of 4000-400 cm^{-1} at room temperature, with a resolution of 2 cm^{-1}, and X-ray fluorescence spectroscopy (XRF OLYMPUS Vanta V Model VCR-CCC-A3-E) (Castro *et al.*, 2025).

To acquire biotics, postbiotics (chemical compounds) and parabiotics (non-viable cells), various mechanical and physical treatments of lees according to **Figure 1** were attained. After treatments, the samples were processed by centrifugation at 6000 rpm for 10 minutes. The supernatants were collected, filtered through 0.22 µm pore size syringe filters, and stored under refrigerated conditions for subsequent analysis of bioactive compounds such as proteins and phytochemicals (total phenolic content - TPC; total flavonoid content - TFC; and the total monomeric anthocyanin content - TAC) (Cotârleț *et al*., 2025).

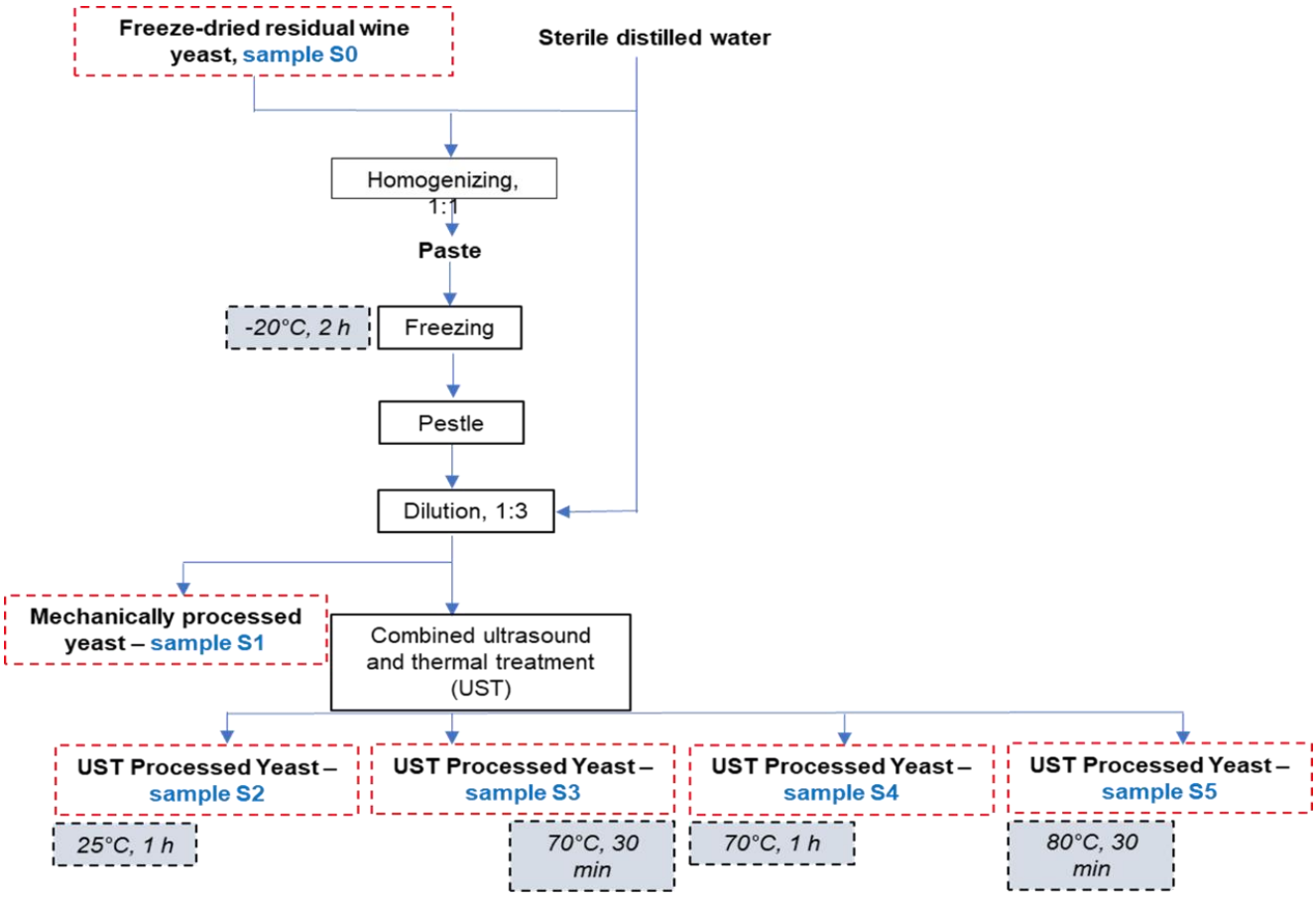

Figure 1. Red wine *Băbească neagră* lees biotication by release of postbiotics and obtaining parabiotics (Cotârleț *et al*, 2025) / Bioticarea drojdiei reziduale de vin (*Băbească neagră*) prin eliberarea postbioticelor și obținerea paraprobioticelor (Cotârleț și colab., 2025)

The yeast cell viability was evaluated by the indirect counting method (Martins *et al*., 2022; Cotârleț *et al*, 2025), by using two specific media for cultivation, Potato Dextrose Agar (PDA) and

Dichloran-Glycerol Agar Base (DG18). The survival rate (SR, %) of yeast cells after treatments was calculated according to the following equation:

$$SR\ (\%) = \frac{Log\ N_{initial}}{Log\ N_{after\ treatments}} x100$$

Where,

$N_{initial}$ – number of colony-forming units (CFU) per mL in sample **S0;**

$N_{after\ treatments}$ – number of CFU per mL in samples **S1-S5**

Statistical analysis

The experiment was conducted in duplicate, and each analysis was performed in triplicate.

The results were expressed as means and standard deviation, and were analysed using one-way analysis of variance (ANOVA), followed by the Tukey test at a significance level of 5% ($p < 0.05$) by using the statistical software package Minitab version 19.

RESULTS AND DISCUSSION

Chemical composition of dried *Băbească neagră* pomace

FTIR and XRF are modern techniques used for advanced composition investigation of the dried pomace.

FTIR spectrum of dried pomace analysis is presented in Figure 2.

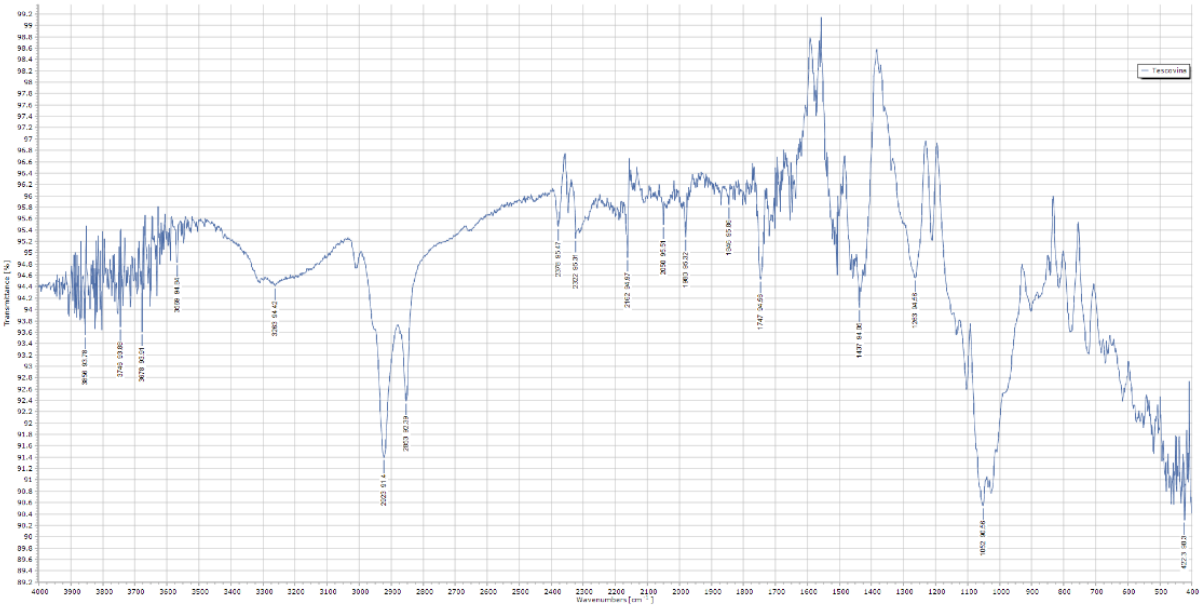

Figure 2. FTIR spectrum of dried *Băbească neagră* pomace / Spectrul FTIR al tescovinei (*Băbească neagră)* uscată

The FTIR spectrum shows various absorption bands indicating the presence of specific functional groups as follows:

- in the wavelength range between 3500-3300 cm¹, the peaks are associated with O-H stretching vibrations from hydroxyl groups;
- around the value of 2900 cm¹, the peak is attributed to C-H stretching vibrations from methyl and methylene groups, indicative of hydrocarbon compounds;
- at approximately 1650 cm¹, the peak indicates the presence of carbonyl (C=O) groups, specific for compounds such as proteins and lipids;

- around the value of 1550 cm¹, the peak indicates N-H and C-N vibrations, specific to proteins (amides);
- near the peak at 1400 cm^{-1}, C-H bending vibrations associated with methyl and methylene groups are present.
- at 1100 cm^{-1}, the C-O and C-O-C vibrations from carbohydrates and polysaccharides were identified;
- between 900-700 cm^{-1}, the peaks indicate the presence of aromatic rings and benzene compounds;
- at approximately 600 cm^{-1}, C-S vibrations are present, associated with thiolic or disulfide compounds.

The X-ray fluorescence (XRF) analysis of the pomace provides detailed data regarding the chemical composition. The pomace contains various elements derived from the soil, water, and the plant growth process. In the analysed dried red pomace, the following minerals were evidenced:

- Silicon concentration of 8.47%. The high concentration of silicon is common in plant biomass, reflecting the presence of silicates in the soil.
- Potassium concentration of 9.64%. Potassium is essential for plant growth and is frequently found in pomace, being crucial in the process of photosynthesis and water regulation.
- Aluminium concentration of 1.35%. Aluminium can come from the soil, being absorbed by plant roots. In large quantities, it can be toxic, but at these concentrations it is considered normal.
- Calcium concentration of 1.036%. Calcium is an important structural element in plant cells, and it is essential for cellular integrity.
- Iron concentration of 1.290%. Iron is vital for many biochemical functions in plants, including chlorophyll synthesis.

Some elements present in moderate concentrations have also been identified, such as:

- Phosphorus concentration of 780 ppm. Phosphorus is crucial for plant growth and development, being part of DNA and RNA molecules, as well as ATP.
- Sulphur concentration of 1000 ppm. Sulphur is important for proteins and enzymes biosynthesis. It is involved in the production of amino acids, proteins and enzymes.
- Manganese concentration of 670 ppm. Manganese is a trace element necessary for photosynthesis and other metabolic processes.
- Copper (concentration of 160 ppm. Copper is essential in small quantities, being part of some enzymes and playing an important role in the plant's metabolism.
- Zinc concentration of 106 ppm. Zinc is crucial for protein synthesis and plant growth.
- Titanium concentration of 1600 ppm. Usually, titanium does not have a significant biological role, but it can be present in the soils where grapes grow.
- Copper concentration of 160 ppm. Essential for numerous enzymatic and structural functions.
- Arsenic (concentration of 23 ppm. The concentration of arsenic is low, but its presence must be monitored due to its potential toxicity.
- Strontium concentration of 58 ppm. Strontium can be absorbed by plants in a similar way to calcium.
- Barium (concentration of 1900 ppm. Barium does not have a biological role in plants, and high concentrations may indicate soil contamination.

Other elements, such as magnesium, vanadium, and chromium, have no detection limits in tested conditions.

The XRF analysis of the pomace shows a typical composition of vegetal biomass containing essential elements for plant growth (K, Ca, P, Fe, Zn) and a few potential contaminants, in small

concentrations (As, Ba). The presence of these elements is directly correlated with the composition of the soil and the environmental conditions in which the grapes were grown.

Regarding bioactive components in the extracts of *Băbească neagră* grape pomace, a total polyphenol content of 42.35 ± 0.13 mg gallic acid/g dry mass, total flavonoids of 29.80 ± 0.25 mg catechin equivalents/g dry mass, and a total monomeric anthocyanin content of 1.49 ± 0.08 mg cyanidin-3-O-glucoside equivalents/g dry mass were determined.

The results are according to those reported by other authors (Serea *et al*., 2021; Spinei & Oroian, 2024), but the chemical composition of pomace is strongly influenced by several factors: genetically factors, geographical origin (soil quality, temperature etc), regional and seasonal variations, agricultural practices, vinification procedures, the moisture content (Wang *et al*., 2024).

The obtained results certified the potential of red *Băbescă neagră* pomace as a valuable ingredient for the formulation of the biocomposite biofertilizers.

***Băbească neagră* wine lees composition evaluation and biotication**

The freeze-dried lees composition was first analysed by FTIR and XRF assays. The results of the FTIR investigation are presented in Figure 3.

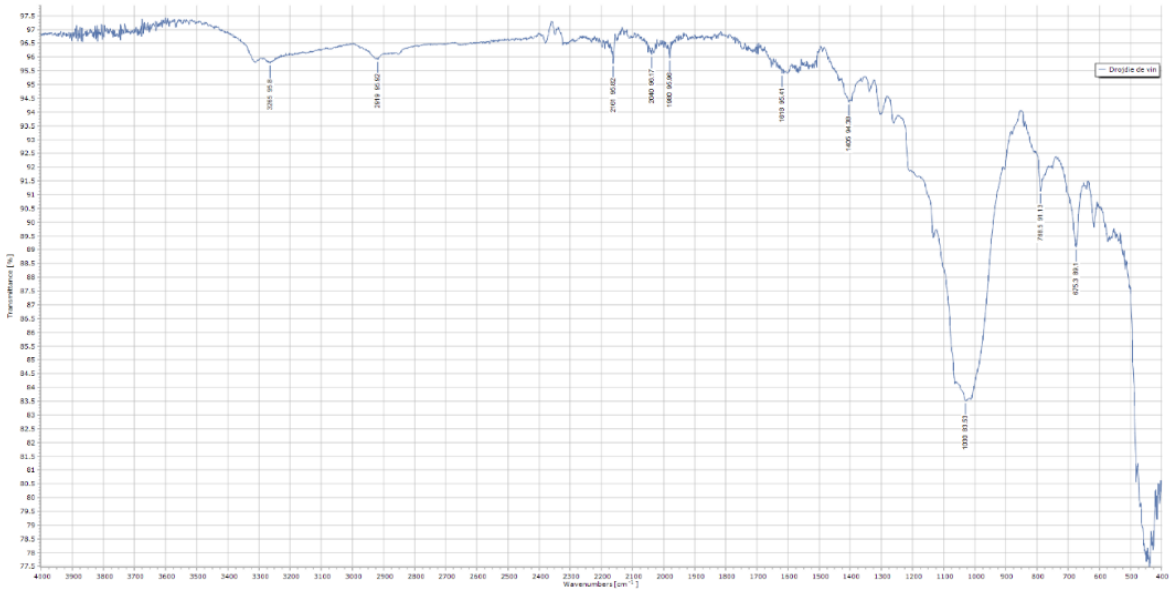

Figure 3. FTIR spectrum of freeze-dried lees (*Băbească neagră* wine) / Spectrul FTIR al drojdiei reziduală de vin (*Băbească neagră*) liofilizată

From the analysis of the obtained data, it is observed that the FTIR spectrum for lees presents several important peaks, each corresponding to a specific molecular vibration, which allows for the identification of important functional groups, as follows:

- at the wavelength around 3400 cm¹, this peak can be attributed to O-H stretching vibrations from hydroxyl groups and can be associated with the presence of water or hydroxylated compounds;
- at the wavelength of 2900 cm¹, it corresponds to the stretching vibrations of C-H in hydrocarbons (alkyl), indicating the presence of carbon chains;
- at the wavelength of 1650 cm^{-1}, this peak is typical for C=O (carbonyl) stretching vibrations in proteins and lipids;
- at the wavelength around 1550 cm^{-1}, this peak can be attributed to the stretching vibrations of N-H and C-N in amides (present in proteins);
- at the wavelength around approximately 1400 cm^{-1}, this band is specific to C-H bending vibrations from methyl and methylene;

- around the wavelength of approximately 1100 cm^{-1}, there are C-O and C-O-C stretching vibrations from carbohydrates (polysaccharides) and cellulose compounds;
- around the wavelength of 700 cm^{-1}, this peak can be attributed to C-H stretching vibrations in aromatic compounds and benzene rings;
- at the wavelength around 600 cm^{-1}, the presence of the peak can be attributed to C-S stretching vibrations from thiolic or disulphide compounds.

In the literature, FTIR spectra for *Saccharomyces cerevisiae* biomass and other types of yeast often show similar bands, reflecting their complex composition of proteins, lipids, carbohydrates, and other biomolecules. Proteins are confirmed by the presence of bands at 1650 cm^{-1} (C=O) and 1550 cm^{-1} (N-H, C-N). Lipids and fatty acids are identified by the peaks at 2900 cm^{-1} (C-H). Carbohydrates and polysaccharides are confirmed by the bands at 1100 cm^{-1} (C-O, C-O-C) (Binati *et al*, 2024).

The X-ray fluorescence (XRF) analysis of lees provides detailed information about the chemical composition of biomass. The presence of the following major elements is highlighted:

- Potassium concentration of 2.213%. Potassium is essential for the metabolism of yeast cells.
- Calcium concentration of 1.501%. Calcium plays a crucial role in cells' stability and their metabolism.
- Aluminium concentration of 4600 ppm. The aluminium may come from winemaking equipment or environmental contaminants.
- Silicon concentration of 6060 ppm. The concentrations of silicon reflect the presence of silicates and may originate from soil or contaminants in the winemaking process.
- Phosphorus concentration of 1530 ppm. Phosphorus is essential for cellular metabolism and for energy production in yeast cells.
- Sulphur concentration of 1590 ppm. Sulphur is involved in the synthesis of amino acids and proteins.
- Iron concentration of 900 ppm. Iron is important for many enzymes and cellular functions.
- Titanium concentration of 430 ppm. Titanium does not have a significant biological role, but it can be present as an impurity.
- Manganese concentration of 126 ppm. Manganese is an essential micronutrient for enzymatic activity.
- Copper concentration of 61 ppm. Copper is essential for numerous enzymatic functions.
- Zinc concentration of 37 ppm. Zinc is crucial for protein synthesis and yeast cell growth.
- Cadmium concentration of 29 ppm. Cadmium is toxic, and its presence indicates a potential contamination.

Magnesium, vanadium, and chromium had no detection limits in analysed conditions.

XRF technique provides non-destructive, multi-elemental analytical results with sensitivity levels reaching 10^{-8}g (depending on the element of interest), making it ideal for compositional evaluation for many research activities. The character's multi-elemental capability, satisfactory speed and efficiency, ease of automation, portability, and the ability to directly analyse solid samples without prior acid digestion are the primary attributes that have established it as a highly developed analytical tool for routine monitoring in various contexts. In addition, XRF can be effectively utilized for direct field analysis in agronomy research (Marguí *et al*, 2022). In the literature, there are no studies on applying this method for less' composition analysis. This study certifies that valuable information can be obtained valuable information about the elemental composition of lees biomass analysed as freeze-dried material.

To increase the bioactive characteristics of freeze-dried yeast biomass, a biotication strategy was tested to release the bioactive compound (postbiotics) and inactivate the cells (parabiotics).

Thus, the freeze-dried biomass was processed by physical and mechanical treatments according to Figure 1.

For the obtained biotic samples, the protein content and bioactive composition are presented in Table 1.

Table 1. The biotic properties of the treated red wine lees (adapted after Cotârleț *et.al*, 2025) / Proprietățile biotice ale drojdiei reziduale de vin tratată (adaptare după Cotârleț și colab., 2025)

Samples	Survival Rate (%)		Protein content,	TPC,	TFC, mg	TAC, µg
	PDA	DG18	mg protein/g DM	mg GAE/g DM	CE/g DM	C3G/g DM
S0	100.00^{A}	100.00^{A}	1.42 ± 0.002^{C}	2.03 ± 0.004^{B}	1.14 ± 0.28^{B}	49.00 ± 1.30^{A}
S1	80.65^{B}	73.36^{B}	1.49 ± 0.04^{C}	1.07 ± 0.004^{D}	0.64 ± 0.15^{C}	27.15 ± 0.60^{C}
S2	77.06^{C}	70.80^{C}	1.45 ± 0.05^{C}	0.98 ± 0.005^{D}	0.51 ± 0.01^{C}	23.27 ± 0.29^{D}
S3	64.34^{D}	0.00^{D}	1.85 ± 0.04^{B}	1.82 ± 0.22^{C}	1.11 ± 0.07^{B}	31.78 ± 0.38^{B}
S4	40.86^{E}	0.00^{D}	1.73 ± 0.04^{B}	1.93 ± 0.02^{C}	1.46 ± 0.05^{B}	11.12 ± 0.10^{E}
S5	23.66^{F}	0.00^{D}	2.37 ± 0.08^{A}	2.90 ± 0.13^{A}	2.96 ± 0.11^{A}	11.09 ± 0.46^{E}

Samples: *S0 – freeze-dried residual wine lees, S1- freeze-dried residual wine lees mechanically processed, S2 - freeze-dried residual wine lees processed by ultrasound-assisted method at 25°C/1 h, S3 - freeze-dried residual wine lees processed by ultrasound-assisted method at 70°C/30 min, S4 - freeze-dried residual wine lees processed by ultrasound-assisted method at 70°C/1 h, S5 - freeze-dried residual wine lees processed by ultrasound-assisted method at 80°C/30 min.* ***Phytochemicals:*** *Total polyphenol content (TPC); Total flavonoid content (TFC); Total monomeric anthocyanin's content; Statistically significant differences between the samples are denoted by superscript letters (A - F) with p <0.05, based on the Tukey test.*

By analysing the data from Table 1 can be seen that different treatments (mechanical and physical) caused the lysis of the cell walls from the perspective of obtaining formulas enriched in cellular bioactive compounds (proteins and phytochemicals) and parabiotics (non-viable cells). The ultrasound treatment combined with the thermal treatment at 80°C for 30 minutes, assures the highest level of total polyphenolic and protein content exogenously released and a high rate of non-viable cells (sample S5). For this sample (S5), only the TAC is the lowest, compared to the other samples, as a result of anthocyanins denaturation during thermal treatment at a high temperature (80°C) for a relatively long time (30 minutes).

CONCLUSIONS

1. The organic (nutrients and bioactives) and mineral composition of the red pomace and residual lees, resulting from *Băbească neagră* grape variety vinification, were characterized in the perspective of using these by-products as bioingredients for an innovative biofertilizer formula.
2. Both by-products from red grape winery are valuable raw resources with a beneficial composition (organic, inorganic, bioactives) for the formulation of biocomposite fertilisers in combination with other industrial wastes (slag, dolomite, and cement kiln dust).
3. In addition, residual wine lees biomass, obtained from the vinication of the same grape variety, was processed through different physic-mechanical treatments, to release the valuable postbiotics (proteins, phytochemicals) and to obtain the parabiotics (non-viable cells), with a positive impact on the soil microbiome and plants growth, vitality and safety assurance.
4. The main innovation for this research is the development of a strategy to obtain biofertilizers with improved functional properties, by exploiting the beneficial potential of red wine lees to be important sources of postbiotics (bioactives) and parabiotics (non-viable cells) in the modern context of metabiotics production and use for increasing the life quality and safety assurance on the axis soil-plants-consumers.

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THE ROLE OF SPENT MUSHROOM SUBSTRATE IN SUSTENABLE ORCHARD MANAGEMENT

ROLUL SUBSTRATULUI UZAT DIN CIUPERCI ÎN MANAGEMENTUL SUSTENABIL AL PLANTAȚIILOR POMICOLE

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Abstract

The global increase in mushroom consumption has led to a substantial rise in residual substrate production. For every kilogram of cultivated mushrooms, approximately 5–6 kilograms of secondary by-products are generated. Inadequate management of these residues contributes to environmental degradation through the depletion of soil resources, deterioration of soil quality, the release of potentially harmful compounds, and groundwater contamination beyond designated storage areas. In recent years, the growing emphasis on sustainable waste management has encouraged researchers and policymakers to investigate the potential applications of Spent Mushroom Substrate (SMS). This secondary by-product has been repurposed in various fields, including mushroom recultivation, animal nutrition, health enhancement, biodegradable packaging, construction materials, biofuel production, and enzyme synthesis. The application of SMS in agriculture, particularly in fruit tree cultivation, presents significant opportunities for enhancing sustainability and efficiency. Notably, the utilization of CO_2 emissions and thermal energy from mushroom cultivation within controlled orchard environments may further support plant growth and productivity. The integration of SMS into fruit production systems represents a promising strategy for improving soil fertility, mitigating environmental impacts, and advancing circular economy principles in sustainable horticulture.

Keywords: *spent mushroom substrate, fruit growing, circular economy, sustainable horticulture*

Rezumat

Creșterea consumului de ciuperci la nivel global a determinat o creștere semnificativă a cantităților de substrat rezidual. Pentru fiecare kilogram de ciuperci cultivate, rezultă aproximativ 5-6 kilograme de materiale secundare. Gestionarea necorespunzătoare a acestor reziduuri contribuie la degradarea mediului prin epuizarea resurselor de sol și implicit la deteriorarea calității solului, eliberarea de compuși nocivi și contaminarea apelor subterane dincolo de zonele desemnate pentru depozitare. În ultimii ani, accentul tot mai mare pus pe gestionarea sustenabilă a deșeurilor a determinat cercetătorii și factorii de decizie să analizeze potențialele aplicații ale substratului reutilizat din cultura ciupercilor (SMS). Acest material secundar a fost valorificat în diverse domenii, inclusiv recultivarea ciupercilor, nutriția animală, îmbunătățirea sănătății, ambalaje biodegradabile, materiale de construcție, producția de biocombustibili și sinteza enzimelor. Aplicarea SMS în agricultură, în special în cultivarea pomilor fructiferi, oferă oportunități considerabile pentru îmbunătățirea sustenabilității și eficienței. Utilizarea emisiilor de CO_2 și a energiei termice generate în timpul cultivării ciupercilor în sisteme pomicole controlate poate contribui suplimentar la stimularea creșterii plantelor și la sporirea productivității. Integrarea SMS în sistemele de producție pomicolă reprezintă o strategie promițătoare pentru îmbunătățirea fertilității solului, reducerea impactului asupra mediului și promovarea principiilor economiei circulare în horticultura sustenabilă.

Cuvinte cheie: *substrat uzat din cultura ciupercilor, pomicultură, economie circulară, horticultură sustenabilă*.

INTRODUCTION

Edible mushrooms are an important source of essential nutrients, being rich in proteins, fibers, vitamins, and minerals, while having a low caloric content. Their recognized health benefits have led to a sharp increase in global demand, turning mushroom cultivation and commercialization into a dynamic and fast-growing industry.

Global mushroom production has increased more than 30-fold since 1978, with Asia particularly China leading the market, accounting for approximately 90% of global output. At the same time, countries in the European Union, especially the Netherlands and Poland, as well as North America, have recorded significant growth in recent decades (Royse *et al*., 2017). Currently, over fifty species are commercially cultivated, with the most common belonging to the *Lentinula* (shiitake), *Pleurotus* (oyster mushrooms), *Auricularia* (wood ear mushrooms), and *Agaricus* (button mushrooms) genera, collectively representing around 74% of the global market.

Although mushroom cultivation is considered an environmentally friendly agricultural activity, it generates large amounts of spent mushroom substrate (SMS), also known as mushroom compost (MC). In China, this residue is also referred to as mushroom bran or residue (Chang, 2006). According to studies, approximately 5 kilograms of SMS are produced for every kilogram of mushrooms harvested (Medina *et al*., 2012). Improper management of this waste can have significant negative impacts on the environment.

The large quantities of SMS currently regarded as low-value waste pose a major challenge for mushroom producers, mainly due to the difficulty of disposal. The high costs associated with transporting a bulky material with low density and high moisture content, along with the difficulty of drying it, limit its practical reuse. Moreover, temporary storage of SMS often leads to uncontrolled anaerobic fermentation, resulting in greenhouse gas emissions, unpleasant odors, and leachate contamination of water bodies (Beyer *at al*., 2011). In the European Union, landfilling SMS is now prohibited under the Council Directive on biodegradable waste. Consequently, the current linear "take make dispose" approach, which treats SMS as mere waste, is increasingly incompatible with sustainable development goals.

Efficient valorization of spent substrate is crucial for transitioning toward a circular economy within the mushroom industry. The composition and physicochemical properties of SMS are mainly influenced by the type of raw materials used to prepare the initial cultivation substrate. For species in the *Lentinula*, *Pleurotus*, and *Auricularia* genera, which together account for about 60% of global production lignocellulosic agricultural, forestry, and agro-industrial residues are commonly used. For *Agaricus spp.*, the substrate typically includes manure, along with additives such as cereal bran, legume flour, and mineral salts. During cultivation, these components are enzymatically degraded, and the released nutrients are used by the fungal mycelium for biomass development and fruiting body production. Significant mass losses have been reported: 26–46% of cellulose, 57–77% of hemicellulose, and 61–75% of lignin, depending on the species (Chen *et al*., 2022). Thus, the final composition of SMS strongly depends on the initial substrate and cultivated species (Wei *et al*., 2017), consisting mainly of plant cell wall components (lignin, hemicellulose, cellulose), residual fungal mycelium, carbohydrates, proteins, and minerals.

Although most growers prefer to replace the spent substrate with fresh material (Beyer *et al*., 2016), SMS still retains valuable agronomic properties. It can be used as an organic fertilizer rich in nitrogen for agricultural crops or as a growing medium for ornamental plants or hydroponic systems, as an alternative to peat moss (Oey *et al*., 2007). However, these applications involve additional costs and require pre-treatment or conditioning to improve substrate quality.

Multiple valorization pathways for SMS have been explored in previous research.

This paper is a review article that synthesizes national and international research findings regarding the use of spent mushroom substrate, with a particular focus on its applications in the sustainable management of fruit tree plantations. Based on a thematic analysis of the relevant literature, this article aims to:

- highlight the potential of SMS as a soil amendment and organic matter source in orchard systems,
- identify the benefits and limitations of its use in perennial cropping systems, and
- outline future research directions and practical solutions aligned with the principles of sustainable agriculture and the circular economy.

MATERIALS AND METHODS

Numerous studies have highlighted the positive effects of organic soil amendments on crop productivity and soil health through changes in physical, chemical, and biological soil properties (Paredes *et al*., 2016; Medina *et al*., 2012). Among the commonly used organic materials are municipal waste, manure, crop residues, and agro-industrial by-products, although many of these can contain hazardous compounds or plant pathogens.

Spent mushroom substrate (SMS), by contrast, is rich in nutrients and typically low in xenobiotics and heavy metals, making it suitable for agricultural application, either directly or after composting. Its properties vary depending on the raw materials used, the fungal species, and the cultivation technology applied, resulting in diverse outcomes when used in soils and crops (Paula *et al*., 2017).

This review draws on experimental findings from various studies that assessed SMS application across different cropping systems. For example, application of unknown SMS at 20 mg/ha combined with poultry manure at 10 mg/ha in sandy soils over 20 years increased organic matter content by 102–201% and water holding capacity by up to 251% (Lipiec *et al*., 2021). Annual applications of SMS (8–25 mg/ha) in vineyard soils improved inorganic nitrogen, organic carbon, total nitrogen, and labile organic fractions (Peregrina *et al*., 2012).

Incorporation of A. bisporus SMS (100 kg/ha) increased oxidable carbon, organic nitrogen, and available phosphorus more than a 1:1 v/v mixture with P. ostreatus SMS (Medina *et al*., 2012). Microbial and enzymatic activity, such as phosphatase activity, also improved with no negative effects on soil salinity or pH.

SMS combined with Bacillus amyloliquefaciens improved soil performance in Hibiscus sabdariffa more effectively than NPK fertilizers (Ngan *et al*., 2021). Fresh or sterilized SMS from F. velutipes applied in cucumber crops enhanced microbial biomass and dissolved organic carbon compared to NPK (Wang *et al*., 2021).

In lettuce, A. subrufescens and L. edodes SMS produced higher dry matter yields than NPK fertilizers (Paredes *et al*., 2016). SMS use has also shown higher yields than mineral fertilizers across various crops (Gobbi *et al*., 2015; Wuest *et al*., 2012).

Was to investigate the potential of using mushroom compost in fruit growing, particularly in the culture of fruit shrubs. In this sense, observations and determinations were made in the experimental field and confirmed by the chemical analyzes carried out in the agrochemical laboratory of RSFG Baneasa Bucharest (Dogaru *et al*., 2024).

RESULTS AND DISCUSSION

Influence on soil microbial communities and enzymatic activity

SMS positively influences microbial communities and enzymatic activity, essential for ecosystem function. However, immature SMS may limit agricultural use. Composting, either independently or co-composted with other organic waste, under controlled conditions can address this issue (Meng *et al*., 2017).

Organic Matter decomposition and compost quality

Research demonstrates that SMS promotes organic matter decomposition in mixtures with sewage sludge, pig manure, corn stalks, and cow dung (Paula *et al*., 2017).). Co-composting with crop residues, manure, sewage sludge, or biogas digestate has been shown to improve humification, reduce ammonia volatilization, immobilize heavy metals, and increase compost maturity (Meng *at al*., 2017). For example, composted A. bisporus SMS increased Lolium multiflorum yield by up to 300% compared to NPK (Abram *et al*, 2017). Composting Auricularia auricula-judae SMS with biogas residues and pig manure produced superior seedlings versus commercial substrates (Meng *et*

al., 2019). Compost mixtures of A. bisporus and P. ostreatus SMS enhanced baby lettuce yield, even under pathogen pressure *(Pythium irregulare*) (Hernández *et al*., 2021).

Enzyme addition or vermicomposting with earthworms can further improve compost quality by stimulating beneficial bacteria, increasing cation exchange capacity, lowering total carbon and C/N ratio, and promoting nitrate synthesis .

Crop yield and soil fertility enhancements

Composted A. bisporus SMS has increased Lolium multiflorum yield by up to 300% compared to NPK. Auricularia auricula-judae SMS co-composted with biogas residues and pig manure resulted in better seedling quality than commercial substrates (Meng *et al*., 2019). Composted blends of A. bisporus and P. ostreatus SMS improved baby lettuce yields even in the presence of Pythium irregulare (Hernández *et al*., 2021).

Beyond productivity, SMS enhances the nutritional and biochemical profiles of crops. Applications in tomato, basil, pepper, sugar, and melon increased chlorophyll, carotenoids, flavonoids, soluble sugars, essential oils, and antioxidant activity (Vahid Afagh *et al*., 2019, Medina, Paredes *et al*., 2009).

Advanced techniques to enhance SMS efficacy

Techniques such as enzyme addition or vermicomposting using earthworms further enhance compost quality by stimulating beneficial bacteria, increasing cation exchange capacity, reducing total carbon and C/N ratio, and promoting nitrate synthesis (Biswas *et al*., 2018; Singh *et al*., 2018).

Application in Orchard Systems

Numerous studies report the application of SMS in fruit-growing systems: vineyards (Peregrina *et al*., 2012), culture of fruit shrubs at the Moara Domnească Experimental Base (Dogaru *et al*.,2024), apple nurseries (Delver, 1982; AntSaoir *et al*., 2000) and tea plantations (Manivel *et al*.,1994),. Benefits included improved water retention, soil organic content, and reduced fertilizer dependency.

Although most studies target vegetables, the findings are transferable to orchards due to similar rhizosphere and soil dynamics. Orchards being long-term systems without crop rotation require inputs that regenerate soil biology. SMS can enhance soil fertility, pathogen suppression, and fruit quality in sustainable orchard systems.

Environmental integration and circular economy impact

Mushroom farms produce heat and CO_2 that can be used in greenhouses or orchard nurseries (Iglesias *et al*., 2025). SMS fits into circular economy strategies by reducing waste, supporting carbon sequestration, and lowering input costs (Medina *et al*., 2009). Nevertheless, attention must be paid to its salt content and compost maturity to prevent phytotoxicity (Wuest *et al.*, 1991; Beyer, 2015). Transport costs and farmer acceptance remain practical barriers (Zailani & Hamid, 2023).

Summary of experimental applications of SMS in agriculture

Table 1 summarizes the applications of spent mushroom substrate (SMS) found in scientific literature, presenting its origin, application methods, and the observed effects on soil and crops. These data support the conclusions previously discussed regarding the agronomic value of SMS.

Fig.1. Moara Domnească experimental field, 2024 / Câmpul experimental de la Moara Domnească, 2024

Table 1. Reuse of spent mushroom substrate (SMS) as soil amendment based on outcome reported in pertinent publications / Reutilizarea substratului uzat pentru ciuperci (SMS) ca amendament pentru sol, pe baza rezultatelor raportate în publicații pertinente

Origin of SMS	Type of SMS and incorporation rate to soil or field plot	Effects noted on the soiland/or crops after the use of SMS	Reference
NR	SMS (10-50%) and poultry manure (10-50%) mixed with saline soil in pots	Increase of nutrient availability and salt-tolerant PGPB observed in treated saline soils; using 10% poultry manure and 10% SMS significantly enhanced maize plant growth and yield	(Upadhyay, et al., 2022)
Agaricus bisporus	SMS integrates to the soil in dosess of 25 and 100 mg /ha (dry weight)	SMS in degraded vineyard soils enhanced dehydrogenase activity, respiration activity and soil microbial biomas	(Herrero, et al., 2022)
Agaricus bisporus and Pleurotus ostreatus	Composted A. bisporus SMS and P. ostreatus SMS 7:3 (v/v) to replace peat to pots	Higher yields of baby leaf lettuce, i.e. 3–7 times more than that obtained by peat (even under the pressure of the soil-borne plant pathogen Pythium irregulare	(Hernández, et al., 2021)
NR	SMS (20 Mg ha^{-1}) and chicken manure, applied to sandy soils every 1–2 years for 20 years	OM content increased; pH increased by 1–1.2 units, while soil bulk density decreased; the content of residual pores increased by 30–251%, and the fitted unsaturated hydraulic conductivity decreased	(Lipiec, et al., 2021)
NR	SMS (35 g) in soil-containing pots (1.5 kg)	Increased NPK and OM contents, soil PGPB, and soil enzyme activities; higher biomass and chlorophyll content obtained in Hibiscus sabdariffa in comparison to the use of mineral NPK (16:16:16) fertilizer	(Ngan, et al., 2021)
Flammulina velutipes	Fresh or sterilized SMS (5%, w/w) mixed with soil in glass jars	Total and dissolved OC, microbial biomass carbon and nitrogen, abundance and diversity of bacteria and fungi and enzyme activities were enhanced	(Wang, et al., 2021)
Agaricus bisporus	SMS (45 and 85 ton ha^{-1}) mixed with soil in pots	SMS promoted the presence of fungi in the highly connected fraction of the active microbial community	(Paula, et al., 2020)
Auricularia auricula-Judae	Composted SMS, biogas residues and pig manure 1:1:1 in seedling pots	Better seedling quality was obtained by using the SMS-based substrate than with the commercial seedling substrates	(Meng, et al., 2018)
Volvariella volvacea	Fresh, weathered, and carbonized SMS mixed with soil (1:2) combined with 0, 50 or 100% of the required rate of nitrogen fertilizer in pots	Weathered and carbonized SMS increased available N; fresh SMS immobilized various nutrients; high yields of pechay during first and second crop on weathered and carbonized SMS; fresh SMS led to high yields only during the third crop; yield was increased by N fertilizer only in weathered and carbonized SMS treatments	(Ultra , et al., 2018)
Agaricus bisporus	Composted SMS (5 to 75 g L^{-1}) in pots	SMS (as the sole fertilizer source) improved grass (Lolium multiflorum) yield up to 300% (with a concentration/dependent response) compared to the untreated control (with no NPK fertilization)	(Paula , et al., 2017)
NR	SMS used to supply 50% or 100% of the crop's nitrogen requirements	In contrast to mineral fertilizers, no increase in salt content was recorded when SMS was applied; similar lettuce and leek yields when either SMS or mineral fertilizers were used	(Gobbi, et al., 2015)
Pleurotus ostreatus	Fresh SMS incorporated (15–20 t ha^{-1}) during a period of four years to a depth of approx. 10 cm	SMS led to increase in porosity and fractal dimension, and caused strong development of a granular microstructure in the A horizon (15–20 cm) and a spongy structure in the B horizon (45–50 cm and 70–75 cm)	(Nakatsuka, et al., 2016)
Agaricus bisporus and Pleurotus ostreatus	SMS was incorporated to a soil depth of 30 cm, 1 month prior to planting; both organic treatments providing 100 kg/ha of N	SMS amendment on a calcareous clayey-loam soil resulted in higher oxidizable OC, organic N, extractable K, and available P compared to soil fertilized by 100, 22 and 208 kg/ha N P and K, respectively; the use of SMS provided lettuce yields similar to that obtained with mineral fertilizer	(Paredes, et al., 2016)
NR	SMS and peat moss alone or mixed (1:1, 1:2, and 2:1 v/v) with or without NPK fertilizer	SMS could replace up to 50% peat moss to support Chinese kale (Brassica oleracea) production; SMS alone cannot be used as growth medium because of its low nutrient content	(Sendi, et al., 2013)
Agaricus bisporus and Agaricus bisporus with Pleurotus ostreatus (1:1,v/v)	SMS-based treatments provided 100 kg ha^{-1} of N	SMS increased the oxidizable OC, organic N, available P, respiration rate, and phosphatase activity, while it did not affect pH, EC, catalase, and urease activities in soil cultivated with lettuce	(Medina, et al., 2012)
NR	Fresh or composted SMS applied annually for four years at rates of 8 and 25 Mg ha^{-1} (d.w.)	SMS led at increased OC, TN and labile organic forms as well as enhanced microbiological activity in a semiarid vineyard soil	(Peregrina, et al., 2012)
Agaricus subrufescens and Lentinula edodes	*A. subrufescens* SMS (5 to 40%, d.w.) and *L. edodes* SMS (5 to 25%, d.w.) mixed with soil in pots	SMS led to increase of water retention and enhanced the soil microbial population; when supplemented by 10% of A. subulatus SMS, lettuce dry weight increased by 2.2 and 1.3 times compared to the control and the NPK (44% N, 37% P_2O_5 and 48% K_2O) treatments; lettuce in L. edodes SMS did not perform equally well	(Ribas, et al., 2009)
NR	SMS distributed onto field plots with a manure spreader at rates of 22.5, 45.0, and 90 kg m^{-2}	Corn yields were significantly higher in SMS-amended plots, and the nitrogen content of both grain and stover was significantly higher than the control	(Wuest, et al., 1995)

Abbreviations used: NPK-nitogen, phosphorus, potassium; NR - not reported; OC - organic carbon; OM - organic matter; PGPB - plant growth promoting bacteria; TN - total nitrogen

FUTURE DIRECTIONS AND CONCLUSIONS

The cultivation of edible and medicinal mushrooms is a highly dynamic sector that has experienced remarkable development over the past decades. However, increased mushroom production leads to the generation of large volumes of mushroom substrate (SMS).

The accumulation of unused SMS, or its restricted use in low-added-value applications, threatens the long-term sustainability of the mushroom industry. Therefore, identifying efficient

valorization strategies for SMS is critical to ensuring sustainable sectoral growth. The research reviewed in this paper highlights the broad potential of SMS as a source of value-added products and ecosystem services.

Due to its nutritional and energetic value, SMS can be reused as a component in new cultivation cycles, provided that adequate treatment or supplementation is applied. Reintroducing SMS into the production of mushrooms either of the same species or different ones, has proven effective in maintaining yield levels, reducing production costs, and improving environmental performance.

The nutritional composition of SMS supports its use in the development of animal feed. Experimental data confirm its viability in the diets of poultry, ruminant, monogastric animals, and even in aquaculture and insect farming. However, limitations such as high fiber content and variable digestibility must be addressed. Research shows that through appropriate pre-treatment, both digestibility and acceptance by animals can be improved.

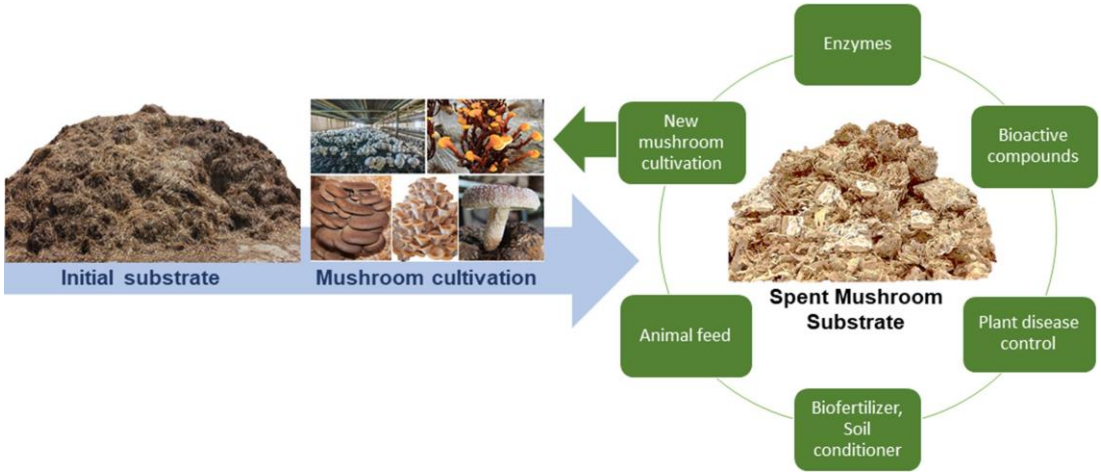

Fig. 2. Use of SMS in a circular economy / Utilizarea SMS în economia circulară
(Source/Sursa: Martin, C., Georgios I. Zervakis C, Shaojun X., Georgios Koutrotsiosc, and Knut Olav, 2023)

SMS also presents significant potential as a raw material in the creation of sustainable, bio-based, and low-cost agronomic products. Applied as a soil amendment or organic fertilizer, SMS improves soil structure and fertility, without causing salinization or acidification. Furthermore, it shows efficacy in controlling plant pathogens and in enhancing the content of secondary metabolites and nutrients in crops. Scaling up current research through field trials and pilot-scale demonstrations is essential to validate these results under real-world agricultural conditions.

Beyond agriculture, SMS contains extracellular enzymes secreted by fungi, enabling applications such as textile effluent decolorization, soil bioremediation, and wastewater treatment. Enzymes can also be extracted and refined for use in industrial and environmental processes. Moreover, SMS can support the growth of enzyme-producing microorganisms, and the resulting crude enzyme extracts can be purified and used in various value-added applications.

The extraction and application of bioactive compounds from SMS is an emerging field of great potential. These compounds may serve as sustainable ingredients in the nutraceutical, pharmaceutical, and functional food industries. The future development of a new generation of "mycotherapeutics" derived from SMS will depend on optimizing extraction protocols that preserve the properties of the target molecules and avoid degradation of other potentially valuable components.

Spent mushroom substrate has considerable potential in sustainable orchard and horticultural systems:

- SMS is a valuable by-product of an expanding agricultural sector.
- Its application improves soil fertility, structure, and biological activity.

- It aligns with circular economy principles and can be used in composting, biofertilizers, construction materials, energy, and as a growth medium.
- In controlled orchard systems, SMS enables the use of CO_2 emissions and heat, supporting EU renewable energy goals.
- SMS use reduces greenhouse gas emissions and promotes soil carbon sequestration.
- Standardization, improved logistics, and environmental risk mitigation are essential for broader adoption.
- SMS offers a sustainable alternative to conventional fertilizers, with added benefits for soil and crop quality.

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STUDY ON CONSUMER OPINIONS ON THE USE OF BIOTECHNOLOGIES IN AGRICULTURE

STUDIU PRIVIND OPINIILE CONSUMATORILOR PRIVIND UTILIZAREA BIOTEHNOLOGIILOR IN AGRICULTURA

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Abstract
Biotechnologies are playing an increasingly important role in food production, offering solutions to increase agricultural yields, reduce pesticide use and improve product quality. This study explores consumers' attitudes towards foods derived from biotechnology, their level of information and the factors influencing their purchasing decisions. Understanding consumer perceptions is essential for developing transparent policies and effective communication strategies in the field of food safety.

Keywords: *agricultural biotechnologies, genetically modified organisms (GMOs), consumer perception, food labeling, food safety*

Rezumat
Biotehnologiile joacă un rol din ce în ce mai important în producția de alimente, oferind soluții pentru creșterea randamentelor agricole, reducerea utilizării pesticidelor și îmbunătățirea calității produselor. Acest studiu explorează atitudinile consumatorilor față de alimentele obținute prin biotehnologie, gradul lor de informare și factorii care influențează decizia de cumpărare. Înțelegerea percepțiilor consumatorilor este esențială pentru dezvoltarea unor politici transparente și a unor strategii eficiente de comunicare în domeniul securității alimentare.

Cuvinte cheie: *biotehnologii agricole, organisme modificate genetic (OMG), percepția consumatorilor, etichetare alimentară, securitate alimentară*

INTRODUCTION

Biotechnology is a research and capital-intensive sector, one of the most rapidly developing fields globally. Its progress therefore depends crucially on a strong intellectual property protection framework and an effective competition regulatory system (Sharma et al., 2010).

Since the beginning of the 21st century, the potential of new biotechnologies to contribute to strengthening sustainable food security and increasing incomes in the agricultural sector has been the subject of extensive debate.

According to Chatterjee and Ghose (2010), agricultural biotechnology is expected to have a significant impact on ensuring long-term food security by increasing agricultural yields, reducing dependence on chemical inputs such as pesticides, reducing soil degradation, increasing the nutritional value of crops and, at the same time, promoting sustainable agricultural practices that contribute to protecting the environment for future generations.

Sehgal (2000) believes that biotechnology has considerable potential in increasing food production and promoting sustainable agricultural development, both on high-fertility and marginal lands. Given that seeds are the main vector for the transfer of biotechnological innovations in agriculture, the benefits of this technology can be exploited by farmers only to the extent that they have access to high-quality seeds.

Agricultural biotechnology offers promising prospects for improving food security in developing countries by increasing farmers' incomes and providing more affordable and

nutritionally superior food products to consumers. However, the realization of this potential is significantly limited by the restrictive regulatory framework imposed by the European Union, which has established rigorous policies on the authorization, production and marketing of genetically modified organisms (GMOs), including strict rules on imports of agri-food products that do not meet these standards (Anderson, 2010).

The objectives of the study were:

- identifying the level of consumer awareness about GMOs and agricultural biotechnologies;
- analysis of attitudes and level of trust towards these products;
- observing behaviors related to reading labels and making purchasing decisions;
- exploring perceptions of the role of authorities and information channels;
- assessing consumers' openness to accepting biotech products.

MATERIALS AND METHODS

The research was based on the application of a structured questionnaire, composed of 25 closed and semi-open questions. It was applied to a sample of 103 respondents, randomly selected from various regions of the country, both urban and rural. The questionnaire was divided into four thematic sections:

1. Socio-demographic data;
2. Knowledge about biotechnologies and GMOs
3. Behaviors regarding product labeling;
4. Opinions regarding the consumption of genetically modified products.

The data were centralized and analyzed using Microsoft Excel software, aiming to identify frequencies, simple correlations and major trends among respondents

RESULTS AND DISCUSSION

Regarding the profile of the respondents, the socio-demographic analysis showed that the majority of respondents are between 18 and 35 years old (over 60%), which suggests a relatively young and active population. The gender distribution was balanced, with a slight female predominance. The level of education is high, over 70% of the participants have university or postgraduate studies, which may positively influence the level of information and the ability to understand scientific concepts. Also, 60% of the respondents come from urban areas, where access to information is generally easier (Figure 1, 2).

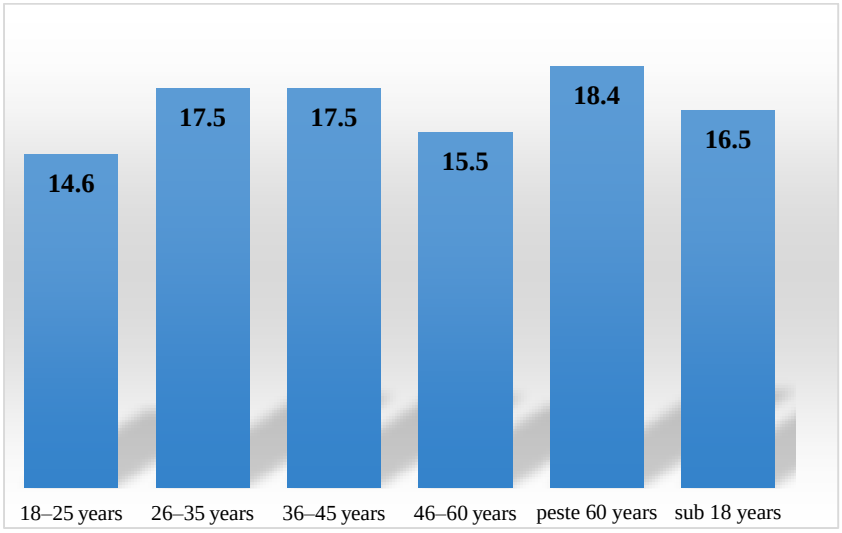

Figure 1. Distribution of respondents by age category / Distribuția respondenților pe categorii de vârstă
Source: processing based on questionnaire data / Sursa: procesarea datelor din chestionare

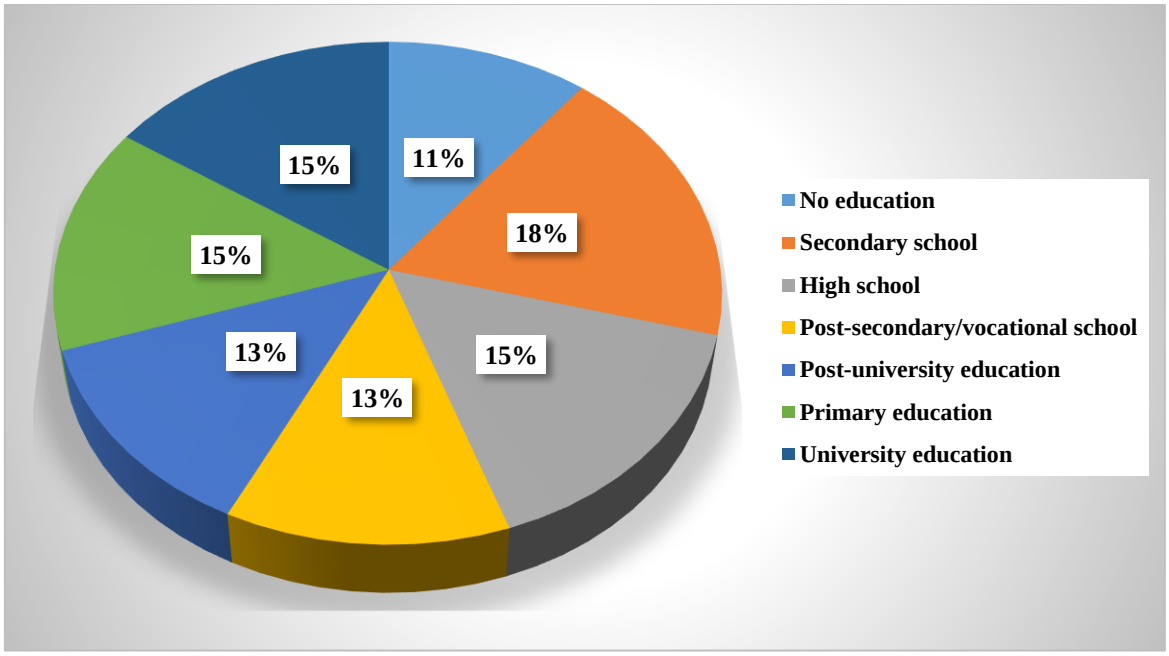

Figure 2. Distribution of respondents by level of education / Distribuția respondenților pe nivelul de studii
Source: processing based on questionnaire data / Sursa: procesarea datelor din chestionare

Questions regarding knowledge about GMOs revealed that over 80% of respondents had heard of their existence. The main sources of information were the internet (70%), television (50%) and formal education (25%). However, only 60% were able to provide a correct or partially correct definition of GMOs. The rest admitted that they did not fully understand the term or confused it with other concepts such as food additives.

Perceptions regarding the safety of genetically modified foods are divided: 40% consider these products safe, 35% perceive them as unsafe, and 25% have no clear opinion. The perceived advantages of GMOs include: increased production, increased resistance to diseases and pests, but also lower product prices. Among the disadvantages frequently mentioned are possible health effects, ecological impact, and the lack of conclusive long-term studies (Figure 4).

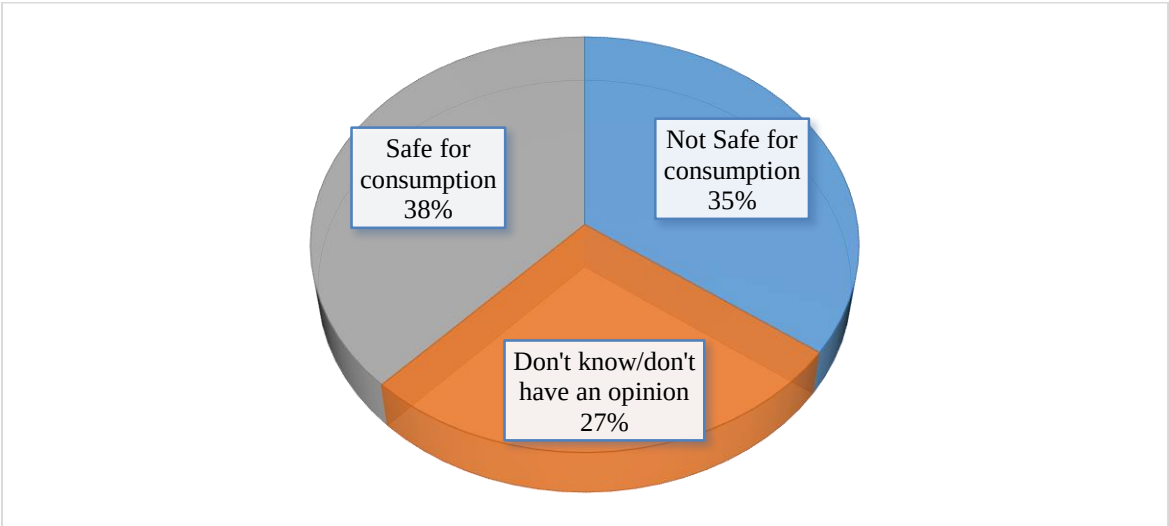

Figure 3. Respondents' perception of the safety of GMOs / Percepția respondenților privind siguranța OMG-urilor
Source: processing based on questionnaire data / Sursa: procesarea datelor din chestionare

Labeling-related behaviors. Regarding food labeling, over 55% of participants say they constantly check the information on the packaging. The most searched elements are ingredients, nutritional values, and the origin of the product. Only 35% specifically look for mentions of GMOs. A significant number (45%) say they have not noticed any labels regarding GMOs, and 78% believe that these mentions should be mandatory and clearly formulated. At the same time, 60% of respondents believe that current labeling does not provide sufficient information about the presence of GMOs in food products.

By age group, the situation is as follows (Table 1):

- **18–25 years old** (15 respondents) are distinguished by a strong presence in the categories with ***university*** (6) and ***post-secondary education*** (3). *These young people seem interested in the label, in line with the younger generation's concerns for healthy eating.*
- **26–45 years old** (36 respondents) is distinguished by a large number of people with high school education (6), a sign that it is a ***mature category,*** possibly involved in the decision to purchase food for the entire family.
- **over 60 years old** (19 respondents) has a heterogeneous behavior: they are present both among those ***without education*** (2) and among those with ***higher education*** (3). *Label checking among seniors is not negligible, but it is likely to be limited to basic elements (expiration, ingredients).*

-

Table 1. Distribution by age category regarding label checking / Distribuția pe categorii de vârstă în ceea ce privește verificarea etichetei

Source: processing based on questionnaire data / Sursa: procesarea datelor din chestionare

Age Category	Number of Responses (%)
under 18 years 16.5	16.5
18–25 years	14.6
26–35 years	17.5
36–45 years	17.5
46–60 years	15.5
over 60 years	18.4
Total	100.0

One of the key questions in the study was about the acceptance of genetically modified (GMO) products. The results showed a relatively low level of acceptance, with only 25% of respondents willing to accept their consumption. This figure suggests a significant reluctance of the population towards genetically modified products, which reflects the fears and uncertainties that exist among consumers.

On the other hand, 35% of respondents actively reject the consumption of GMO products. They are firmly against these products, raising concerns about food safety, the long-term impact on health and the environment, as well as the ethics involved in the use of biotechnologies in food. These people seem to be very reluctant to accept any argument in favor of the use of GMOs, considering them a risky choice, even in the face of more attractive economic possibilities.

In contrast, 30% of respondents would accept genetically modified products, but only in the absence of safer and more affordable alternatives. They are therefore more flexible, being willing to accept these products only for economic reasons or in conditions where there are no other viable options on the market. They could be positively influenced by the lower prices of GMO products, which could offer them a more affordable solution compared to the higher costs associated with natural or conventional products.

The reasons given by those willing to accept the consumption of genetically modified products are diverse. These include reduced costs for consumers, the possibility of more affordable

food, recommendations and studies carried out by specialists in the field of biotechnology, as well as the belief that GMO products do not pose major risks to health or the environment. These people rely on information from experts, considering that current technology is sufficiently advanced to guarantee the safety of these products.

Those who reject GMO products cite a number of reasons related to their safety. The main concern is the uncertainty about the long-term impact on human health and the environment. The lack of long-term studies and concrete evidence of their absolute safety contributes to consumer fears. Many of those who reject these products also highlight the lack of clear and transparent information about the process of genetic modification and the potential associated risks. In addition, there is a significant preference for natural, unprocessed products, perceived as healthier and safer. This reflects a broader trend of consumers seeking authentic, traditional foods and foods perceived as closer to nature.

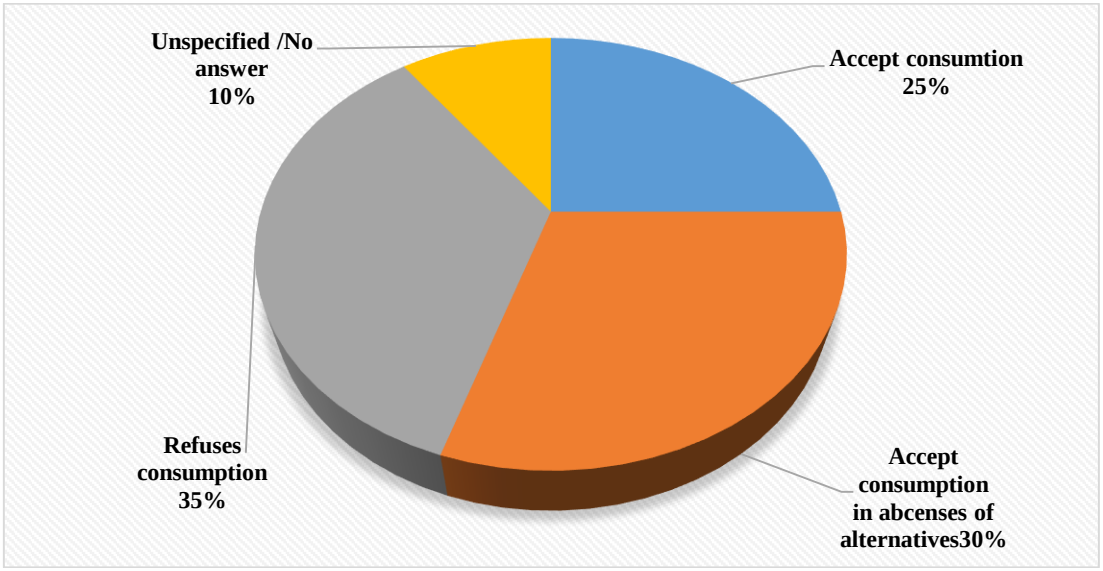

Figure 4. Respondents' perception of GMO consumption / Percepția respondenților față de consumul de OMG-uri
Source: processing based on questionnaire data/Sursa: procesarea datelor din chestionare

An important aspect highlighted by the study results is the low level of trust that citizens have towards the authorities that regulate the field of biotechnology. Thus, only 15% of respondents declared that they have full trust in the state institutions responsible for this field. This figure indicates a significant deficit of credibility, which can influence the public perception of the decisions taken at the governmental level and the regulations in force.

Amidst this lack of trust, most respondents choose to get their information from alternative sources. The most frequently used channels are traditional media (television, radio, newspapers) and, to an increasing extent, the internet, especially social networks and news websites. This trend highlights the need for more efficient and transparent communication from authorities, as well as the importance of verifying the quality and veracity of information available in the public space.

Regarding the responsibility for informing the public about biotechnology, the majority opinion is that this task should not fall exclusively on a single entity. Respondents stressed the need for a collective and coordinated effort, involving the state (through competent and transparent institutions), independent specialists (who can provide objective and scientifically substantiated opinions), non-governmental organizations (who can represent the interests of civil society) and even manufacturers in the biotechnology industry (who must assume an active role in ensuring correct and responsible information for consumers). This collaborative model is seen as essential for building a lasting relationship of trust between citizens and the actors involved in the development and regulation of biotechnology.

Regarding the cultivation of genetically modified organisms (GMOs) in Romania, the study revealed a diversity of opinions, reflecting a cautious and sometimes skeptical attitude of the public towards this practice. Approximately 40% of respondents believe that GMOs could be cultivated in the country, but only under strictly regulated and monitored conditions. These participants emphasize the need for a rigorous legal framework, which would ensure the safety of both the environment and the health of the population. They also believe that regulations should include rigorous control measures to prevent any potential risks related to the use of these technologies in agriculture. This group is open to the possibility of integrating GMOs, but only under the close supervision of the competent authorities.

Another significant group, 30%, is completely opposed to the cultivation of genetically modified organisms in Romania. These people are strongly against the use of GMOs, for various reasons, including concerns about food safety, the impact on biodiversity, and the long-term risks to the environment and health. Many of them believe that, even with strict regulations, genetic modification technology represents too risky an artificial intervention in natural ecosystems, and the potential economic benefits do not justify the possible irreversible damage. Some of them are also influenced by environmental movements and the global trend to promote a sustainable and natural agricultural model.

A percentage of 20% of respondents do not have a clear opinion on this topic, being undecided or simply uninterested in the topic of GMOs. They may either be uninformed about the long-term implications of the use of GMOs in agriculture, or simply do not consider this topic to be a major priority in the context of the economic, political or social problems facing the country.

Only 10% of respondents support the unconditional cultivation of genetically modified organisms. They are generally more confident in the potential of biotechnology technologies and believe that the economic and production benefits could outweigh any perceived risks. Many of them are influenced by scientific arguments that GMOs can contribute to more efficient agricultural production, more resilient to extreme climate conditions and better able to meet the demands of a growing world population. They believe that safety fears are unfounded, given that existing scientific studies have not identified major risks to human health (Figure 5).

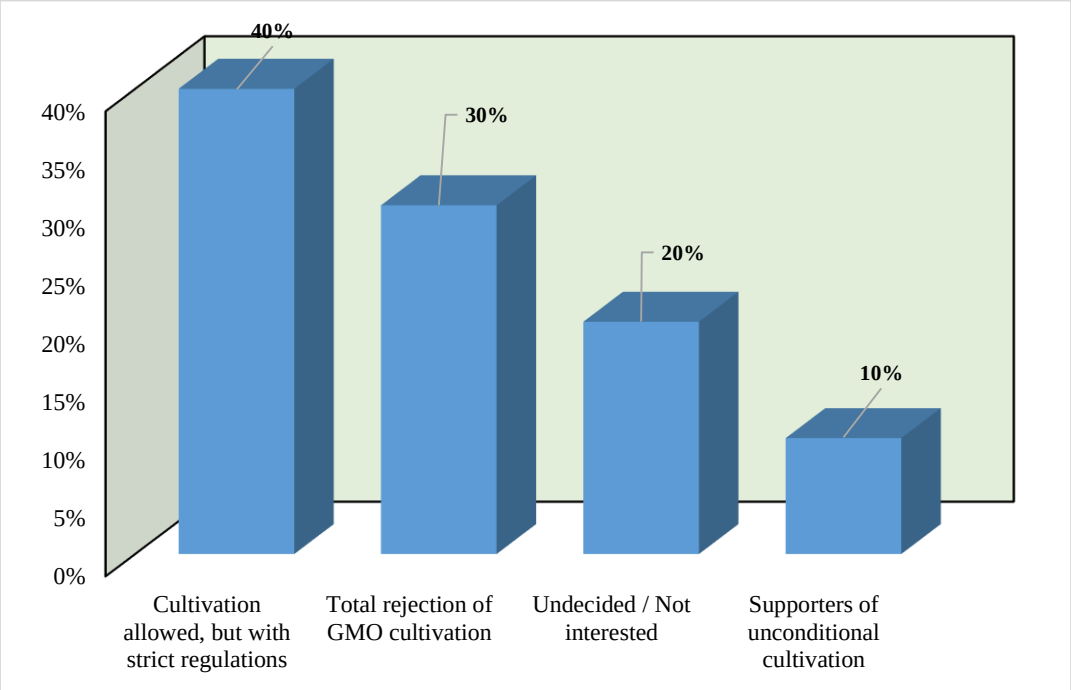

Figure 5. Respondents' perception of GMO cultivation / Percepția respondenților față de cultivarea OMG-urilor
Source: processing based on questionnaire data / Sursa: procesarea datelor din chestionare

Regarding the future of biotechnology in agriculture, the majority of respondents (over half) believe that biotechnology holds significant potential for the development of agriculture in the future. They are convinced that biotechnologies can play a key role in increasing agricultural production, especially in the face of global challenges such as climate change, frequent droughts and increased food requirements. However, these opinions are accompanied by a constant call for accurate and continuous information for the population. Respondents emphasize that, in order to gain public trust and successfully integrate biotechnologies into agriculture, it is essential that authorities, research institutions and companies in the field provide transparent and accessible information about the benefits and risks associated with their use. In addition, many participants suggest the need for educational campaigns to help citizens better understand biotechnological technologies, differentiate between myths and reality and make informed decisions regarding their use.

Thus, while biotechnology has considerable potential to revolutionize agriculture, there is still a significant need for dialogue and education to overcome public reluctance and create a regulatory framework that addresses safety and sustainability concerns.

CONCLUSIONS

1. Regarding the level of information and knowledge about GMOs, a significant percentage of respondents (80%) are aware of the existence of GMOs, but only 60% manage to provide a correct or partially correct definition of them. This suggests a lack of detailed understanding of biotechnology concepts, even among those who are informed. In addition, the main sources of information are the internet (70%) and television (50%), which indicates a significant dependence on media channels and a potential lack of objective scientific information.
2. The perception of the safety of GMOs is divided. Thus, 40% consider GMOs safe, 35% consider them unsafe, and 25% have no clear opinion. This highlights a general uncertainty among the population about the impact of GMOs on health and the environment, which can be a significant factor in influencing consumer decisions.
3. Regarding product labelling, the majority of respondents (55%) check food labels, but only 35% pay attention to GMO claims. There is a significant desire (78%) for these claims to be mandatory and clear. These findings suggest a clear need for stricter and clearer regulations on the labelling of genetically modified products, to facilitate informed consumer choices.
4. The study highlights a general reluctance to consume genetically modified products, with only 25% of respondents willing to accept them. This low percentage reflects concerns about food safety and long-term health and environmental effects. A significant group (35%) also categorically refuses to consume GMOs for safety and ethical reasons.
5. Regarding the cultivation of GMOs in Romania, 40% of respondents claim that they could be cultivated, but only under strictly regulated conditions. However, 30% are completely opposed, while 10% are open to unconditional cultivation. This indicates a cautious attitude, but also an interest in the possibility of strict regulation of the use of GMOs, which reflects a desire for a balance between innovation and safety.
6. Trust in the authorities regulating biotechnology is very low (only 15% of respondents have complete trust in them). This highlights a problem of communication and transparency, suggesting that a sustained effort is needed from the authorities to gain public trust and clarify regulations and processes related to GMOs.
7. Over half of respondents believe that biotechnology has significant potential for the future of agriculture, being seen as a solution to increase production and respond to global challenges such as climate change and food needs. However, these views are accompanied by a clear call for accurate and continuous information to ensure public confidence in the use of biotechnology.

8. The study suggests that while biotechnologies and GMOs are perceived as having the potential for more efficient agriculture, there is considerable reluctance to use them, largely due to uncertainty about long-term safety and a lack of transparency in regulations. It is essential that authorities, researchers and industry work together to provide clear, objective and accessible information about biotechnologies so that greater trust in these technologies can be built and their informed and responsible adoption in agriculture can be supported.

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THE USE OF ALGINATE AND NATURAL POLYMERS MIXTURES AS CARRIERS FOR MALOLACTIC BACTERIA CELL IMMOBILIZATION

UTILIZAREA ALGINATULUI ȘI A UNOR MIXURI DE POLIMERI NATURALI CA AGENȚI SUPORT PENTRU IMOBILIZAREA CELULELOR BACTERIENE MALOLACTICE

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Abstract.
Malolactic fermentation (MLF) is defined as the enzymatic bioconversion of malic acid into lactic acid, a biological process of reducing the acidity of wine performed by lactic acid bacteria (LAB). The use of immobilized bacteria provides important advantages compared to the classic free cell inoculation, improving cell stability and process yield. The current study aimed at the immobilization of an autochthonous LAB strain (Oenococcus oeni, strain 13-7) cells, by entrapping in 2% sodium alginate, 1% alginate/1% k-carrageenan and 1% alginate/1% gellan gum mixtures, analysing the physical characteristics of the beads and the bioconversion yield in three successive fermentation cycles. An additional layer of 0.1% gel with the same composition was applied to the biocatalysts after initial extrusion of gels in 0.2 M $CaCl_2$. The beads obtained were spherical, with a diameter between 3.39-3.62 mm. Inoculated in synthetic wine (12% vol. ethanol; 10^8CFU/mL), the immobilized LAB cells metabolized between 69-71 % of the malic acid in the initial fermentation cycle (10 days), the bioconversion yield decreasing by about 3% in the second cycle and by 10% in the final cycle of MLF. Blending of alginate (1%) with other natural polymers improved the structure of the beads, especially in combination with 1% gellan gum, the constant values of the optical transmittance at 550 nm (~99%) and the reduced number of cells released in the final fermentation cycle indicating a superior stability of the immobilization matrix compared to plain alginate beads.

Keywords: *sodium alginate, lactic acid bacteria, malolactic fermentation, polymeric gels, synthetic wine*.

Rezumat.
Fermentația malolactică este definită ca bioconversia enzimatică a acidului malic în acid lactic, proces biologic de reducere a acidității vinului realizat de către bacteriile lactice. Utilizarea celulelor imobilizate oferă avantaje importante comparativ cu inocularea clasică, îmbunătățind stabilitatea celulară și randamentul procesului fermentativ. Studiile efectuate au avut ca scop imobilizarea celulelor unei tulpini bacteriene lactice autohtone (Oenococcus oeni, tulpina 13-7), prin includerea în geluri de alginat de sodiu 2% şi mixuri de alginat 1%/k-caragenan 1% și alginat 1%/gumă gelan 1%, analizând caracteristicile fizice ale perlelor formate și randamentul de bioconversie, în trei cicluri succesive de fermentație. Un strat suplimentar de gel 0,1% cu aceeași compoziție a fost aplicat biocatalizatorilor după extrudarea prin picurare în 0,2 M $CaCl_2$. Perlele obținute au fost sferice, cu diametrul cuprins între 3,39-3,62 mm. Inoculate în vin sintetic (12% etanol; 10^8 UFC/mL), celulele bacteriene imobilizate au metabolizat între 69 și 71 % din acidul malic în primul ciclu de fermentație (10 zile), randamentul de bioconversie scăzând cu aproximativ 3% în al doilea ciclu și cu până la 10% în ciclul final. Combinarea alginatului (1%) cu cei doi polimeri naturali a îmbunătățit structura perlelor, în special în cazul gelanului 1%, valorile constante ale transmitanței optice la 550 nm (~99%) și numărul redus de celule eliberate în ciclul final de fermentație, indicând o stabilitate superioară a materialului suport.

Cuvinte cheie: *alginat de sodiu, bacterii lactice, fermentatie malolactică, geluri polimerice, vin sintetic.*

INTRODUCTION

In the winemaking practice, it is generally accepted that wine is the result of two biological fermentation processes, alcoholic and malolactic, determined by microorganisms that develop on grapes during their maturation period (Filimon *et al*., 2022). If in the alcoholic fermentation sugars are metabolized by yeasts and transformed into ethanol and carbon dioxide, in the case of malolactic fermentation (MLF), under the action of lactic acid bacteria (LAB), malic acid is decarboxylated to lactic acid and carbon dioxide, with the formation of variable concentrations of

secondary fermentation compounds. Beyond the reduction of acidity, carried out under optimal conditions, malolactic fermentation provides important benefits to wine quality, increasing microbiological stability (by exhausting the carbon source) and amplifying the aroma profile of the wine, increasing its complexity (Lerm *et al*., 2010). Considering all these aspects, malolactic fermentation became an essential stage of the technological process of winemaking.

Cell immobilization is one of the most interesting aspects of biotechnology, with the first research being carried out since the 1970s (Hansen and Cheong, 2013). Recent studies highlighted the advantages of using the immobilized cells in biotechnological processes: increase the stability of the bacterial cell, increase the performance of the process by developing continuous fermentation systems, facile separation of biomass and reduced production costs (Tao *et al*., 2022). Given the distinct metabolic particularities of main LAB species used in winemaking (especially *Oenococcus oeni*), research conducted in the last years aimed at identifying efficient support materials for cell immobilization, testing new immobilization techniques and identifying the relationships between the microbial cell and the carrier (Virdis *et al*., 2021). Moreover, was established that cell immobilization by entrapment in natural polymeric gels does not pose a risk of toxicity, the materials being intended for the food industry. In winemaking, the use of immobilized cells for the biological deacidification is based on the following reasons: increases the tolerance and fermentative capacity of bacterial cells under difficult conditions in wine (low pH, presence of ethanol and SO_2), rapid initiation of the process, the enrichment of the wine aroma the and the possibility of reusing the biocatalysts in successive operational cycles (Filimon, 2023). Sodium alginate is a food additive (E401), being the first material used for cell immobilization, a linear polysaccharide derivative of alginic acid, a component of the cell wall of brown algae containing 30 to 60% alginic acid. Sodium alginate can bind an amount of water 300 times higher than its weight, being used in food as thickener, emulsifier and gelling agent (Generally Recognized as Safe - GRAS by the U.S. Food and Drug Administration). When monovalent ions (sodium) are exchanged for divalent ions (calcium), a structural change occurs by passing from a low-viscosity solution to a gel matrix. Alginate is a valuable carrier agent due to its non-toxic nature, ability to form soft matrices, and capacity to encapsulate sensitive microorganisms such as lactic acid bacteria (LAB). Although recent research suggests that cell viability remains unaffected, alginate presents certain limitations, including susceptibility to acidic environments and structural degradation caused by calcium chelating agents, which may affect its protective barrier activity (Krasaekoopt *et al*., 2003; Totosaus *et al*., 2013). However, combining alginate with other hydrocolloids has proven to be an effective strategy for overcoming these mechanical and structural challenges. In recent studies, was often proposed to use alginate in association with other natural polymers (Totosaus *et al*., 2013) or inorganic compounds (Guzzon *et al*., 2012; Iurciuc *et al*., 2017). Gellan gum is an extracellular anionic heteropolysaccharide produced by *Sphingomonas elodea* (syn. *S. paucimobilis*), a food additive (E418) discovered since the 1970s. Gellan is composed of a repeating sequence of four sugars, which requires monovalent or divalent cations for gelation. It is commercialized as thickener or solidifying agent, replacing agar in microorganism culture media. Gellan type with a high degree of acylation produces soft and elastic gels, while weakly acylated forms form hard, firm, but more fragile gels. The main advantages of using gellan gum in cell immobilization are represented by its versatile texture, stability at high temperatures and at wide pH values (2-10), easy dissolution in water and compatibility for combination with other polymers (Kang *et al*., 2015). Carrageenan is the generic name for a group of polysaccharides with high gelling capacity, obtained by extraction from red algae (*Rhodophyta* sp., *Eucheuma* sp.). Carrageenan is used in food, pharmaceutical, and cosmetic industries as thickening, gelling or stabilizing additive (E407) (Wardhana *et al*., 2022). Among the known types of carrageenan, the k-type (kappa) is the strongest gelling agent, being the most used form in the immobilization of living cells or enzymes. K-carrageenan shows affinities for K^+, Ca^+, Na^+, Li^+ ions that increase the gelling capacity (Chen *et al*., 2002).

Considering the related aspects, the current studies aimed at obtaining improved alginate beads for LAB cell immobilization, by mixing the sodium alginate with other natural polysaccharides, like k-carrageenan and gellan gum, and testing the obtained biocatalysts in successive fermentative cycles. The experimental data are useful to researchers, winemakers and biotechnologists, who will be able to choose the best option for the initiation and control of MLF in order to achieve high quality wines.

MATERIALS AND METHODS

The autochtonous strain *Oenococcus oeni* (code 13-7) isolated from the wine microbiota at Research Development Station for Viticulture and Winemaking Iași, Romania (Filimon *et al*., 2022), was incubated in anaerobic conditions (under thin layer of liquid paraffin) in 10 mL of sterile MRS broth (De Man *et al*., 1960), at 28℃, for 3 days. The biomass was separated by centrifugation (10 minutes, 4500 RPM; centrifuge CNBH-600, MRC, Israel) and suspended in 10 mL of acclimation medium with 10% ethanol (Lerm *et al*., 2011). After incubation at 28°C, for 48 hours, the cells in the exponential growth phase adapted to the presence of ethanol were separated from the medium by centrifugation and the biomass was washed with sterile distilled water. Thus, primary inoculum was obtained as a cell suspension with an optical density of 1.25 AU at 600 nm (Specord 200 Plus UV-vis spectrophotometer, Analytik Jena, Germany), which corresponds to a cell concentration of 10^9 CFU/mL.

For the preparation of the immobilization support, sodium alginate (BosFood, Germany) was dissolved in distilled water, in a water bath at 80℃, at a concentration of 2% (1 g of powder dissolved in 45 mL of sterile distilled water). After autoclavation (121℃, 15 min), the 2% alginate solution was cooled in a water bath at 40℃. To obtain the immobilization matrix, 5 mL of the primary inoculum was added to the alginate solution, with continuous homogenization on a magnetic stirrer (40 RPM), obtaining a cell concentration of 10^8 CFU/mL. In addition to the plain alginate version (AL), two mixtures of 1% alginate with 1% gellan gum (Hampp Media, Germany; medium acylation) (AG) and 1% alginate with 1% k-carrageenan (Bara Ezquerra, Spain) (AK) were obtained using the same protocol as in the case of alginate. The homogeneous immobilization mixtures with a cell concentration of 10^8 CFU/mL were maintained at constant temperature (40℃) until the extrusion operations.

Using a 20 cm^3 medical syringe equipped with a sterile G_{23} needle (0.60×30 mm), the homogenized cell suspension was manually extruded by gradual dripping at constant pressure into a 0.2 M calcium chloride ($CaCl_2$) solution, the droplets immediately passing from the low-viscosity solution state to a dense gelled structure. Extrusion was made at laboratory temperature (18-20 ℃), under continuous homogenization (magnet stirrer), 1 hour. After repeated washing with sterile distilled water to remove excess calcium ions, the gel beads were resuspended in solutions containing 0.1% of the same polymer or mixture of polymers (without inoculum), at temperatures below 45 ℃, for 15 minutes, under continuous stirring, in order to obtain a secondary compact gel layer on the bead's surface. After washing with sterile distilled water, 8.50 g of double layer beads were inoculated in 100 mL synthetic wine (12% ethanol, pH 3.50, 3.5 g/L malic acid) (Bravo-Ferrada *et al*., 2014), ensuring a cell density of 10^8 CFU/mL. A fermentation cycle was established for 10 days, at 25℃, being performed three successive cycles. At the end of each cycle, the synthetic wine was collected for analysis, and after washing the beads with distilled water, a similar volume of synthetic wine was added over the beads. In parallel, a synthetic wine sample was inoculated with free cells of the same strain (*O. oeni 13-7*), at a similar cell density (10^8 CFU/mL), and incubated under the same experimental conditions as control.

Monitoring of the MLF process was performed by thin layer chromatography (TLC), using cellulose plates 10×20 cm (Merck, Germany) and a mixture of solvents as mobile phase: n-butanol: distilled water: acetic acid: bromophenol blue, in a ratio of 100:20:20:0.1 (v/v/v/w). The retention factor (Rf) for each acid was calculated as the ratio of the distance travelled by the compound to the

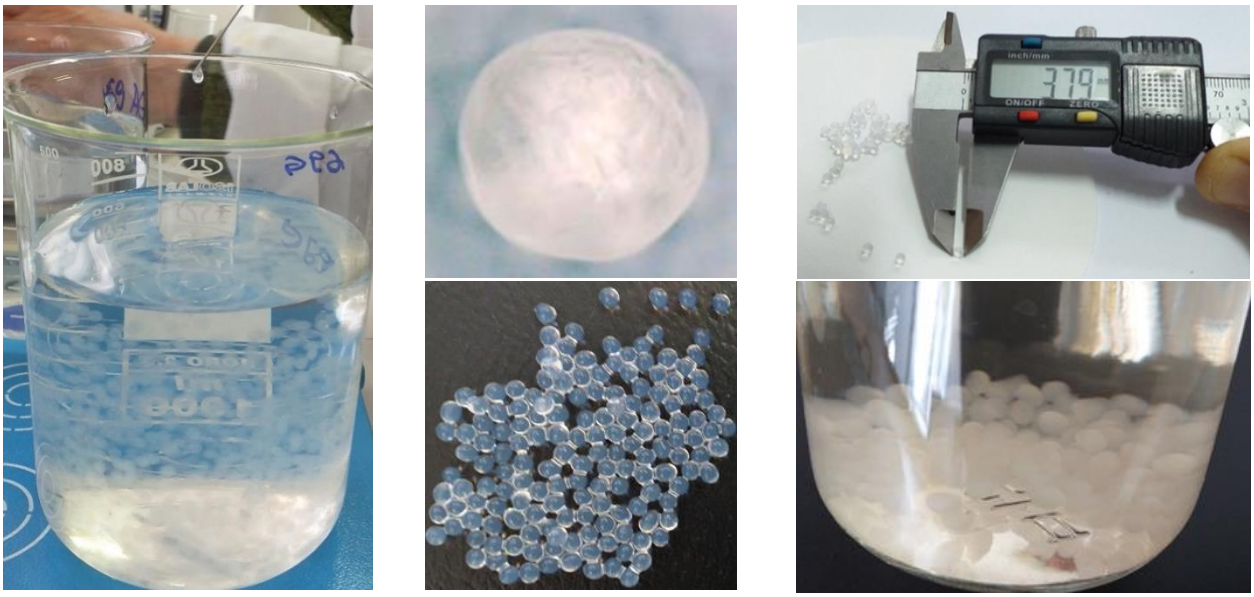
distance travelled by the solvent (Bele and Khale, 2011). The residual L-malic acid was determined using an enzymatic kit (BioSystems, Spain), involving the activity of L-malate dehydrogenase and glutamate-oxaloacetate transaminase (GOT) at 37 °C and reading the optical density at λ 340 nm. To highlight the cell releases from the immobilization matrix and beads stability, the transmittance (%) of the synthetic wine for each immobilization variant was determined at a wavelength of 550 nm, at the end of the incubation period. Low transmittance values indicate high turbidity of the analyzed media, due to both the release of cells from the biocatalyst beads and possible degradation of the polymer structure. Also, the number of bacteria released from the support were enumerated at the end of each fermentation cycle using spread plating on MRS agar (48 h, 28°C) supplemented with 1% $CaCO_3$, and reported as colony forming units per milliliter (CFU/mL). Determinations were carried out in three replications.

pH values were determined using a pH meter InoLab Level 1 (WTW, Germany). The diameter (mm) of 20 beads from each variant was determined using a digital vernier caliper (Powerfix, Germany), while their volume was determined by immersing 2 g of beads in distilled water, in 10 cm^3 graduated glass cylinder. Beads weight was assessed using an ATX 220 analytical balance (Shimadzu, Japan).

Analysis of variance ANOVA test was initiated to investigate significant differences between data in XLSTAT® statistical software. P values lower than 0.05 ($p < 0.05$) were considered to be significant. The method used to discriminate among the means was Tukey's test at 95% confidence level. Different letters indicate significant differences between data.

RESULTS AND DISCUSSION

The immobilization of bacterial cells in polymeric gels involved several complex operations: preparation of the bacterial cell suspension, preparation of the gel solutions, obtaining the cell suspension-immobilization gel mixture, extrusion and formation of gel beads, obtaining the additional layer of gel, inoculation into the fermentation medium and incubation. LAB cells, adapted to the presence of ethanol, were added to the polymeric suspensions under continuous stirring and extruded dropwise into the 0.2M $CaCl_2$ solution in order to achieve the gel state (Figure 1 a, b).



(a) **(b)** **(c)**

Figure 1. Obtaining the alginate beads by extrusion in $CaCl_2$ solution (a), their appearance (b) and characterisation (c) / *Obținerea perlelor de alginat prin extrudare în soluţie de $CaCl_2$ (a), aspectul (b) şi caracterizarea acestora (c)*

As established in preliminary studies, the additional gel layer applied to the biocatalyst beads provides additional protection to the peripheral cells and improve physico-mechanical characteristics of the beads. Thus, after obtaining the additional gel layer for each experimental variant, the beads were characterized and inoculated into synthetic wine (12% vol. ethanol) to perform MLF. The main physico-mechanical characteristics of the gel beads are showed in Table 1. Along with the weight and volume, bead sphericity was calculated as the ratio between the main diameters, and was determined the number of non-conforming beads resulting from the extrusion process (deformed, cracked, elongated). A diameters ratio closer to unity (1) indicates a high sphericity. The double layer beads showed a spherical shape (diameters ratio > 0.95) and a high uniformity, regardless of the gel composition (Figure 1 b, c).

Table 1. Physico-mechanical characterization of double layer biocatalyst beads obtained by entraping LAB cells in alginate and polymeric mixtures / *Caracterizarea fizico-mecanică a perlelor biocatalizator obţinute prin includerea celulelor bateriene în alginat şi amestecuri de polimeri*

Experimental variant		Diameter (a) (mm)	Diameter (b) (mm)	Sphericity (a/b)	Weight of 100 beads (g)	Volume of 100 beads (cm^3)	Non-conforming beads (%)
Code	**Composition**						
AL	2% alginate	3.39±0.18^{a}	3.54±0.16^{a}	0.96±0.03^{a}	1.70±0.02^{b}	1.80±0.02^{b}	1.67±0.58^{a}
AG	1% alginate/1% gellan gum	3.44±0.12^{a}	3.62±0.14^{a}	0.95±0.05^{a}	1.76±0.02^{a}	1.82±0.03^{ab}	1.00±0.00^{b}
AK	1% alginate/1% k-carrageenan	3.46±0.16^{a}	3.58±0.10^{a}	0.97±0.06^{a}	1.74±0.01^{ab}	1.86±0.02^{a}	1.33±0.58^{ab}

The diameter of the beads varied in the case of alginate between 3.39 and 3.54 mm, being slightly higher in the case of the mixtures of alginate with gellan or k-carrageenan (3.44-3.62 mm). The weight and volume of the beads varied significantly depending on the polysaccharide used, the plain alginate beads presenting the lowest weight and volume. Regarding the conformity of the beads obtained by extrusion, the percentage of non-conforming beads was the highest in the case of AL (1.67%), and the lowest in the case of the AG combination (1.00%).

Biocatalyst beads containing the bacterial cells were inoculated in synthetic wine (cycle 1), the progress of the MLF process being highlighted after 10 days of incubation by thin layer chromatography (TLC). TLC is a laboratory technique used to separate compounds from liquid mixtures by exploiting their differential affinity for a stationary phase and a mobile phase (solvent) (Santiago and Strobel, 2013). This qualitative method combines the advantages of a low cost and simple work protocol, with the accuracy and speed of obtaining results. After each 10-day cycle, the MLF was assessed by the presence of malic and lactic acids yellow spots on the chromatographic plates (Figure 2).

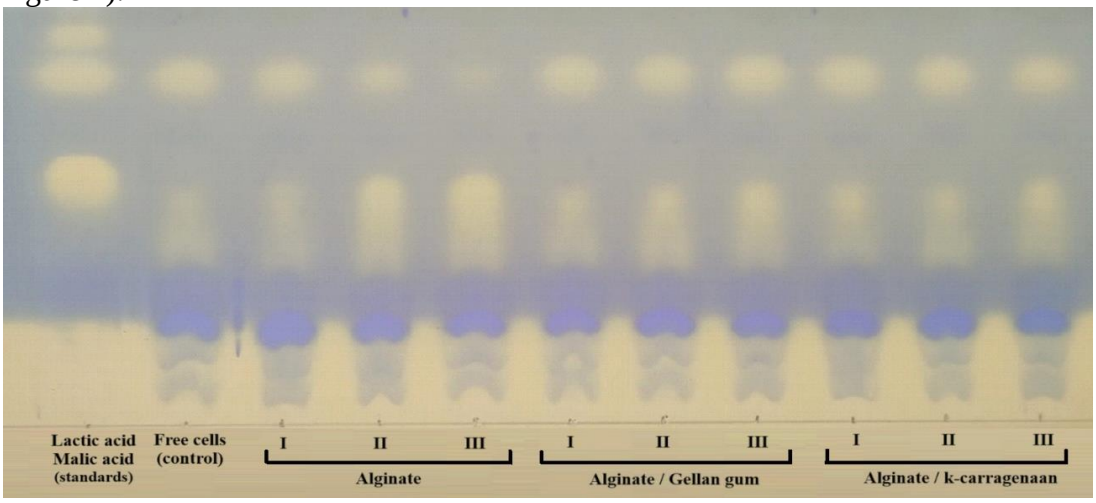


Figure 2. Monitoring the MLF process in synthetic wines by thin layer chromatography in three successive fermentative cycles with immobilized LAB cells / *Monitorizarea fermentației malolactice în vin sintetic prin cromatografie în strat subțire, în trei cicluri fermentative succesive realizate cu celule bacteriene lactice imobilizate*

The Rf values of the spots obtained by migrating the samples on the chromatographic plate were compared with those of standard solutions of malic and lactic acids (in similar concentration). The retention factors of the two acids, calculated at a migration front of 7.5 cm, were: Rf malic acid = 0.48 and Rf lactic acid = 0.75, respectively.

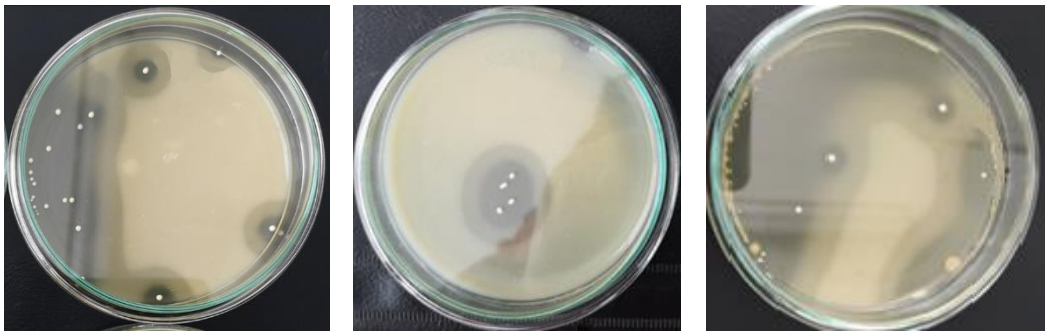
Determination of synthetic wine turbidity by measuring transmittance at 550 nm was used as a qualitative indicator of the mechanical stability and integrity of the beads, as previously proposed by Iurciuc *et al*. (2017). It is possible that at the end of the MLF, some polymer fragments or LAB cells may detach from the gel matrix and therefore diffuse in the medium, causing an increase in turbidity. Thus, turbidity of the synthetic wines varied significantly depending on the fermentation cycle. If in the first cycle of MLF the differences between the variants were non-significant, in the second cycle the synthetic wine of the AL variant presented a significantly lower transmittance compared to the mixed immobilization variants (97.86%) (Table 2).

Table 2. Transmittance of fermentation media after malolactic bioconversion with immobilized bacterial cells and residual malic acid concentrations for each cycle performed / *Transmitanţa mediilor de fermentaţie după efectuarea bioconversiei malolactice cu celule bacteriene imobilizate şi concentraţiile de acid malic rezidual pentru fiecare ciclu*

Experimental variant		Transmitance at 550 nm			Residual malic acid (g/L)		
Code	**Composition**	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 1**	**Cycle 2**	**Cycle 3**
AL	2% alginate	99.18±0.34^{a}	97.86±0.26^{b}	95.80±0.32^{c}	1.04±0.06^{b}	1.16±0.08^{b}	1.37±0.11^{a}
AG	1% alginate/1% gellan gum	99.50±0.18^{a}	99.59±0.12^{a}	99.48±0.16^{a}	1.03±0.04^{b}	1.09±0.05^{b}	1.29±0.08^{a}
AK	1% alginate/1% k-carrageenan	99.43±0.22^{a}	99.14±0.18^{a}	98.92±0.20^{b}	1.07±0.04^{b}	1.11±0.04^{b}	1.34±0.10^{a}

Regarding the alginate mixtures (AG and AK), no differences were found between the variants in the first two fermentation cycles. The third fermentation cycle led to an increase in the turbidity of the samples, respectively a decrease in transmittance by up to 4% in the case of AL, indicating a lower stability of the gel beads. Moreover, a higher standard deviation of AL samples was observed, as a result of an erratic behavior of alginate gels during successive cycles.

However, gel strength and the characteristics of the beads can be influenced by the concentration of the calcium ions, in this case, a concentration of 0.2 M $CaCl_2$ solution was used for all tested variants, to highlight structural differences. In this case, the turbidity of the environment can be influenced not only by the polymer fragments, but also by the cells which can diffuse from the gel beads. The number of viable LAB cells in the synthetic wine was determined by inoculating serial dilutions on Petri plates with MRS medium with 1% $CaCO_3$ (Figure 2). In the case of AL beads, the number of viable cells released from the support matrix at the end of the third fermentation cycle was 21±6 CFU/mL, and much lower in the case of AG combinations (4±2 CFU/mL) and AK (11±4 CFU/mL).



(a) (b) (c)

Figure 3. Determination of the number of viable cells in synthetic wine in the last fermentation cycle (MRS agar, with 1% $CaCO_3$): (a) alginate, (b) alginate/gellan, (c) alginate/k-carrageenan / *Determinarea numărului de celule viabile din vinul sintetic în ultimul ciclu fermentativ (MRS agarizat) (a) alginat, (b) alginat/gellan, (c) alginat/k-caragenan*

Although the mechanical stability of the beads was different, the fermentation process did not undergo significant changes in the first cycles of FML. The residual malic acid concentrations varied between 1.03 (AG) and 1.07 (AK) g/L in the first cycle, gradually increasing in the second and third fermentation cycles respectively, probably due to a gradual loss of LAB cells. Compared to immobilized cells, the control sample with free LAB cells (10^8 CFU/mL) showed a non-significantly higher malolactic activity, the amount of residual malic acid ranging between 1.02 – 1.06 g/L.

The percentage of malic acid metabolized by the immobilized LAB cells is showed in Figure 3, for each fermentative cycle. In the first cycle the percentage of malic acid metabolized varied between 69.43 (AK) and 70.57% (AG), gradually decreasing as successive cycles were carried out. In the case of AL variant, the percentage of malic acid consumed by the immobilized bacteria decreased by about 3% in the second cycle and by up to 15 % in the third fermentation cycle. The mixture of alginate with gellan gum led to a non-significant increase in the bioconversion activity of *Oenococcus oeni* strain. Also, the percentage of malic acid consumed in the second cycle of AG variant was about 2 % lower compared to the first cycle, and up to 10 % in the third fermentation cycle. The AK variant showed verry similar results to the AG variant. However, in the case of all immobilization variants, the percentage of metabolized malic acid decreased significantly only in the third fermentation cycle.

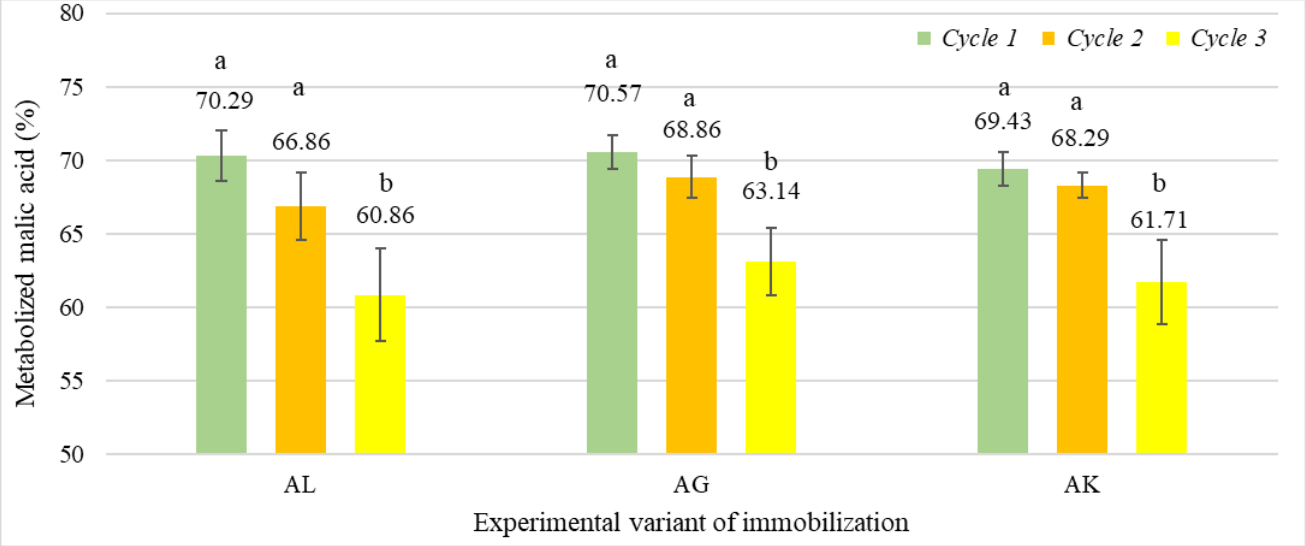


Figure 4. The percentage of malic acid metabolized in successive fermentation cycles by immobilized lactic acid bacteria (AL – alginate; AG – alginate/gellan gum; AK – alginate/k-carrageenan) / *Procentul de acid malic metabolizat în cicluri succesive de fermentație de către bacteriile lactice imobilizate (AL – alginat; AG – alginat/gumă gelan; AK – alginat/k-carragenaan)*

Considering the medium composition (12% ethanol; pH 3.50; 3.5 g/L malic acid) and the experimental conditions (10 days; 25°C; anaerobiosis), the pH of the synthetic wine inoculated with the biocatalyst beads has changed once malolactic bioconversion was performed. MLF deacidifies the wine by converting the strong diprotic malic acid to the softer monoprotic lactic acid, resulting in a decrease in total acidity and an increase in pH by about 0.3 units (Zoecklein *et al.*, 1999). Thus, in correlation with the concentration of metabolized malic acid, the pH of the synthetic wine increase by up to 0.26 units (in the first MLF cycle) (Table 3). The differences in pH were non-significant between the experimental variants.

According to Lee and Mooney (2012), the viscosity of alginate solutions increases as pH decreases, and reach a maximum around pH 3.0 - 3.5, as carboxylate groups in the alginate become protonated and form hydrogen bonds.

Table 3. Changes in synthetic wine pH during malolactic bioconversion conducted with immobilized bacterial cells in successive fermentative cycles / *Modificări ale pH-ului vinului sintetic la finalul bioconverisei malolactice efectuate cu celule bacteriene lactice imobilizate, în cicluri fermentative succesive*

Experimental variant		Initial pH	Final pH		
Code	Composition		Cycle 1	Cycle 2	Cycle 2
AL	2% alginate	3.50	3.71±0.07[a]	3.68±0.08[a]	3.61±0.09[a]
AG	1% alginate/1% gellan gum	3.50	3.76±0.02[a]	3.72±0.05[a]	3.70±0.06[a]
AK	1% alginate/1% k-carrageenan	3.50	3.72±0.04[a]	3.69±0.06[a]	3.67±0.08[a]

Calcium alginate beads, as any hydrogel, are constituted mainly by water (typically 96-99%) and tend to be dehydrated in contact with alcohols, such as ethanol (Torres *et al*., 2011). The presence of ethanol can cause damage to the alginate beads, such as shrinkage, deformation or drying. Thus, at the end of the three MLF cycles, the diameter of the beads was remeasured and their changes were determined (Table 4).

Table 4. Physico-mechanical characterization of double layer biocatalyst beads after performing three MLF succesive cycles / *Caracterizarea fizico-mecanică a perlelor biocatalizator după efectuarea celor trei cicluri succesive de fermentație malolactică*

Experimental variant		Diameter (a) (mm)	Diameter (b) (mm)	Sphericity (a/b)	Weight of 100 beads (g)	Volume of 100 beads (cm^3)
Code	Composition					
AL	2% alginate	3.28±0.14[a]	3.41±0.10[a]	0.96±0.05[a]	1.68±0.04[a]	1.70±0.06[a]
AG	1% alginate/1% gellan gum	3.35±0.10[a]	3.53±0.11[a]	0.95±0.04[a]	1.75±0.05[a]	1.74±0.03[a]
AK	1% alginate/1% k-carrageenan	3.38±0.12[a]	3.48±0.12[a]	0.97±0.04[a]	1.73±0.03[a]	1.75±0.03[a]

The diameter of the pearls decreased at the end of the three fermentation cycles compared to the initial data with a percentage between 2.3 (AK) and 3.5 (AL) %, but the sphericity of the pearls was not affected. Thus, the average diameter of the beads was reduced by 0.08 to 0.12 mm, with higher shrinkage recorded in the case of plain alginate (AL). Although the weight was slightly reduced, the volume of 100 beads decreased by over 5% due to the reduction in average diameter.

CONCLUSIONS

1. Due to the advantages it offers to wine quality, malolactic fermentation became an essential step in the winemaking process. Immobilization of lactic acid bacteria by entrapping in natural polymeric gels is a helpful strategy for improving cell stability and increasing the yield of the bioconversion process.

2. Double layer beads with spherical shape, high uniformity, and specific weight and volume were obtain by entrapping the cells of *Oenococcus oeni* strain 13-7 in 2% alginate, 1% alginate/1% gellan gum and 1% alginate/1% k-carrageenan mixtures. The malolactic bioconversion yield in synthetic wine was non-significantly higher in the case of alginate/gellan mixture (>70.5 % of the malic acid was consumed).

3. After the three successive fermentation cycles (10 days per cycle) the percentage of malic acid metabolized by the immobilized *O. oeni* cells gradually decreased for all tested variants, but more in the case of alginate (by about 3% in the second cycle, and by up to 15 % in the third fermentation cycle).

4. The mechanical stability and integrity of the plain alginate beads was affected after three fermentation cycles in synthetic wine, fact highlighted by the lower values of optical transmittance at 550 nm (<96%) and the higher number of viable cells released from the immobilization matrix.

5. In the presence of ethanol, the beads obtained by gel extrusion in $CaCl_2$ solutions, have reduced their diameter and implicitly their volume, especially in the case of plain alginate (−3.5% of volume), maintaining their weight and sphericity.

6. Considering the bioconversion yield and the constant physico-mechanical characteristics of double-layer gel beads after performing multiple fermentation cycles, the 1% alginate/1% gellan

gum mixture could be recommended for immobilization of lactic acid bacteria for wine inoculation, thus improving the alginate stability and increasing the efficiency of the process.

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PHENOLOGICAL RESPONSES OF PEA (*PISUM SATIVUM* L.) ACCESSIONS TO CLIMATIC VARIABILITY BETWEEN 2020 AND 2023

INFLUENȚA CONDIȚIILOR CLIMATICE ASUPRA FENOLOGIEI LINIILOR DE MAZĂRE (*PISUM SATIVUM* L.) ÎN PERIOADA 2020-2023

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Abstract
This study examines the phenology of different pea accessions from VRDS Buzău collection over four years (2020–2023), focusing on the impact of climatic conditions on key phenophases: emergence, flowering, pod formation, harvest maturity, and physiological maturity. Temperature and precipitation significantly influenced both the duration of the crop cycle and yield potential. In 2020, emergence occurred 14–26 days after sowing, with harvest maturity at 65–96 days. High precipitation delayed harvest and reduced yield. In 2021, slower emergence (18–34 days) and extended physiological maturity (71–116 days) were observed. The 2022 season, characterized by extreme drought and strong winds, led to delayed emergence and lower yields., In contrast, 2023 experienced faster emergence and uniform maturation due to favourable weather condition. These findings highlight the critical role of climate monitoring in optimizing pea production. Understanding the relationship between environmental factors and crop development is essential for improving agricultural efficiency and ensuring yield stability.

Keywords: *abiotic stress, climate resilience, crop phenology, environmental stressors, pea phenology*

Rezumat
Studiul examinează fenologia mai multor linii de mazăre din baza de germoplasmă a SCDL Buzău, în perioada 2020-2023, evidențiind impactul condițiilor climatice asupra principalelor fenofaze: răsărit, înflorit, formarea păstăilor, maturitatea de consum și maturitatea fiziologică. Variabilitatea temperaturii și precipitațiilor a influențat semnificativ durata ciclului de vegetație, determinând diferențe între linii. Astfel, în 2020, răsărirea a avut loc în 14-26 zile de la semănat, iar maturitatea de consum între 65-96 zile. Precipitațiile abundente în ultima fenofază au întârziat recoltarea și au redus producția. În 2021, răsărirea a fost mai lentă (18-34 zile), iar maturitatea fiziologică s-a atins în 71-116 zile. Anul 2022 s-a remarcat prin secetă și vânt puternic, provocând o răsărire întârziată și o scădere a producției. În 2023, condițiile mai favorabile au favorizat o răsărire rapidă și o maturizare uniformă. Rezultatele evidențiază rolul esențial al monitorizării climatice pentru optimizarea producției de mazăre, subliniind influența factorilor de mediu asupra dezvoltării plantelor și a randamentului final.

Cuvinte cheie: *adaptare climatică, dezvoltarea culturilor, factori de stres ambiental, fenologie, stres abiotic*

INTRODUCTION

The Leguminosae family (Fabaceae Endl.) is one of the most diverse plant families worldwide, encompassing hundreds of genera and a very large number of species, cultivated across all continents. Numerous species within this family hold significant economic importance, having been utilized since ancient times for various purposes, including human nutrition, animal feed, and as a natural fertilizer (Mikić et al., 2006; Jacobsen et al., 2012).

A representative species is the pea (*Pisum sativum* L.), extensively cultivated annual legume, especially in temperate regions, where it holds a vital role in agricultural systems. (Mikić et al., 2007; Mihailović & Mikić, 2010; Chețan et al., 2015).

The pea originates from the Middle East and was first cultivated approximately 10.000 years ago (Zeven & Jukovski, 1975). It is believed that *Pisum sativum* derived from *Pisum elatius* and spread from the Fertile Crescent along two distinct routes: eastward through South Asia and

westward through North Africa and the Mediterranean region (Blixt, 1972; Zohary, 1996; Mithen, 2003). Archaeological evidence suggests a rapid expansion throughout the Mediterranean region, followed by a significant delay before its further spread northward (Colledge *et al.*, 2005; Kreuz *et al*., 2005).

The rapid distribution of peas in Europe is evidenced by their early cultivation at Neolithic sites such as Kovačevo, Bulgaria (5790–5630 BCE) and their presence in Armenia, Germany, and France across various historical periods (Rösch, 1997; Hovsepyan & Willcox, 2007; Marinova & Popova, 2008). Peas were a staple in Celtic, Germanic, and Gallo-Roman diets, with finds in Austria (700–200 BCE) and Moselle, France (100–200 CE) (Swidrak, 1999; Preiss *et al*., 2005). Linguistic evidence suggests that peas were widespread in Europe before the formation of modern language families (Ljuština & Mikić, 2010).

In Romania, the earliest references to pea cultivation date back to Alexandrescu (1873), who described late-maturing varieties with purple flowers and garden varieties with white flowers. Subsequently, Alessiu (1894) classified peas into types consumed fresh and those harvested at full maturity, further differentiating between dwarf and climbing (staked) varieties. The first pea cultivars documented as being grown in Romania were described by I. Morlova (1926), and included the wrinkled-seed variety *Merveille*, the dwarf types *Mammuth*, *Daisy*, and *Folger*, as well as the tall varieties *Telephon*, *Remontant*, and *Clamart*". Later, Scurtu (1998) emphasized the development of novel pea cultivars in Romania, targeted for industrial processing, underlining the alignment of breeding programs with the needs of the processing industry. In a subsequent contribution, Ionescu *et al*., (1999) presented new genotypes within the assortment of leguminous vegetables, further evidencing the continuous enrichment of the national germplasm and its adaptation to the evolving demands of cultivation and processing.

Peas rank third among leguminous crops worldwide, following dry beans and chickpeas. They are primarily cultivated in temperate regions, both for fresh consumption and as a dry legume used in human and animal nutrition. In 2022, global green pea production was estimated at approximately 20 million tons (FAOSTAT, 2022). The main producers of dry peas India, Canada, Russia, China, and the United States, benefit from favorable climatic conditions and well-developed agricultural practices. (Rawal *et al*., 2019).

Pea cultivation has expanded significantly worldwide since 2010, with the global harvested area increasing from 6.58 to 7.15 million hectares, and total production rising from 10.44 to 14.16 million tons. In contrast, green pea cultivation in Romania has markedly declined, from 5,803 hectares and 23,313 tons in 2010 to a historic low of 670 hectares and 1,340 tons in 2021. Meanwhile, the area cultivated with dry peas initially increased between 2010 and 2016, but subsequently declined, reaching 68,060 hectares and 109,240 tons by 2022 (FAOSTAT, 2022 (FAOSTAT, 2022).

Climatic variability exerts a profound influence on agriculture, particularly in the context of global climate change, which is characterized by an increasing frequency of extreme phenomena such as heatwaves, droughts, and excessive precipitation. Pea cultivation, with its moderate requirements for temperature and humidity, is particularly susceptible to these alterations, especially during critical developmental phases, such as germination, flowering, and pod formation. Consequently, the climatic conditions experienced during each growing season play a significant role in determining both yield and the quality of the production. Within this framework, the present study undertakes an analysis of the phenology of 15 pea accessions from the germplasm collection of VRDS Buzău, spanning the period from 2020 to 2023, with a particular focus on elucidating the impact of climatic conditions on the primary phenophase.

MATERIALS AND METHODS

The experiment was conducted in the years 2020-2023 at the experimental plot of the Vegetable Research and Development Station Buzău (VRDS Buzău), located in Buzău County, Romania (latitude: 5.13711°N, longitude: 26.81711°E, and elevation: 95 m above sea level).

The plant material comprised fourteen pea accessions (L1, L2, L3, L4, L5, L6, L7, L8, L9, L10, L11, L12, L13, L14) originating from the germplasm collection of the Vegetable Research and Development Station (VRDS) Buzău, together with one registered pea cultivar, *Getica* (L15), which was developed through the breeding program of VRDS Buzău. All fifteen varieties were subjected to evaluation, and their main morphological and agronomic traits are briefly summarized below.

L1 – an early genotype with plant height ranging between 22.12 and 35.9 cm. The flowers are white, the pods are green, with lengths varying between 8.14 and 9.23 cm. Each pod contains 6–8 seeds, and the weight of 1,000 dry seeds is 247.95 g.

L2 – a semi-late genotype, 48.65–52.26 cm in height. The flowers are white, pods are green, with lengths between 10.21 and 12.56 cm. Each pod contains 7–9 seeds, with a 1,000-seed weight of 339.92 g.

L3 – a semi-early genotype with a height ranging from 41.25 to 60.13 cm. Flowers are white, pods are green, with lengths between 6.92 and 8.15 cm. Each pod contains 6–7 seeds, and the 1,000-seed weight is 312.45 g.

L4 – a semi-late genotype with plant height between 62.7 and 69.84 cm. Flowers are white, pods are green, with lengths between 7.47 and 8.69 cm. Pods contain 5–7 seeds, with a 1,000-seed weight of 265.85 g.

L5 – a semi-early genotype, 42.62–77.21 cm in height. Flowers are white, pods are green, with lengths ranging from 7.42 to 8.54 cm. Pods contain 5–7 seeds, and the 1,000-seed weight is 293.85 g.

L6 – a mangetout-type, late genotype, 60.45–78.46 cm in height. Flowers are violet-purple, pods are green, with lengths ranging between 8.14 and 14.85 cm. Pods contain 4–6 seeds, with a 1,000-seed weight of 153.87 g.

L7 – a late genotype with plant height varying between 32.85 and 43.78 cm. Flowers are purple, pods are also purple, with lengths between 6.1 and 7.22 cm. Each pod contains 5–7 seeds, and the 1,000-seed weight is 402.15 g.

L8 – a semi-late genotype with plant height ranging between 41.25 and 66.85 cm. Flowers are white, pods are green, with lengths between 7.15 and 8.56 cm. Pods contain 7–9 seeds, with a 1,000-seed weight of 392.55 g.

L9 – an extra-early genotype with plant height ranging between 46.55 and 51.25 cm. Flowers are white, pods are green, with lengths between 6.7 and 8.33 cm. Pods contain 7–9 seeds, and the 1,000-seed weight is 277.93 g.

L10 – a semi-early genotype with plant height ranging from 37.44 to 52.19 cm. Flowers are white, pods are green, with lengths between 8.14 and 9.25 cm. Pods contain 6–9 seeds, and the 1,000-seed weight is 456.85 g.

L11 – a semi-early genotype, 42.36–61.45 cm in height. Flowers are white, pods are green, with lengths between 8.5 and 9.2 cm. Pods contain 4–7 seeds, and the 1,000-seed weight is 418.45 g.

L12 – a late genotype, 38.2–64.12 cm in height. Flowers are white, pods are green, with lengths between 7.55 and 8.52 cm. Pods contain 6–7 seeds, with a 1,000-seed weight of 466.12 g.

L13 – a semi-early genotype, 42.14–49.9 cm in height. Flowers are white, pods are green, with lengths ranging from 8.6 to 10.16 cm. Pods contain 8–10 seeds, with a 1,000-seed weight of 286.96 g.

L14 – an early genotype with plant height between 54.2 and 81.69 cm. Flowers are white, pods are green, with lengths varying between 11.78 and 14.95 cm. Pods contain 7–9 seeds, and the 1,000-seed weight is 559.85 g.

L15 – an early genotype, 53–59.45 cm in height. Flowers are white, pods are green, with lengths ranging between 8.4 and 9.96 cm. Pods contain 5–7 seeds, and the 1,000-seed weight is 454.84 g.

The sowing was performed manually on March 3rd in each year of the study. The seeds were sown on raised beds, each 70 cm in width, with four plant rows arranged two on each side of the bed, maintaining a 3.5 cm inter-plant distance within each row (Fig.1), thus achieving a density of 114.28 plants per m². Irrigation was implemented via gravitational flow through furrows, while the soil was maintained free of weeds and aerated through mechanical tillage.

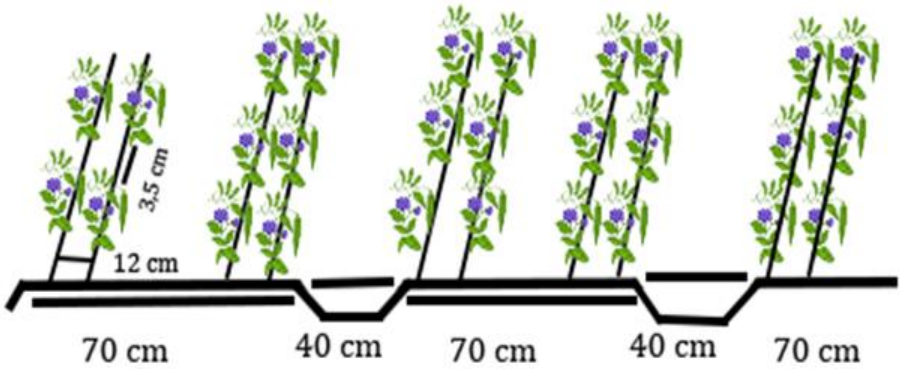

Figure 1. Sowing scheme of the studied accessions / Schema de semănat a liniilor studiate

The analysis of the meteorological conditions in the cultivation area, conducted over the period 2020-2023, aimed at evaluating the climatic factors relevant to plant development and crop performance. This included the monitoring of monthly average temperatures, precipitation, relative humidity, and other important meteorological parameters to understand how they influence the duration of the vegetative cycle of studied accessions. For the analysis of climatic effects, agro-meteorological data were recorded from the local weather station for the period 2020-2023, covering daily temperatures from March to July, which coincided with the vegetation period of the pea crop, from sowing to harvest. These data were compared with the values obtained at the VRDS Buzău to assess climatic deviations and their impact on the pea crop.

Phenological observations regarding the sowing date, emergence date, flowering onset date, first pod formation date, consumption maturity date, and physiological maturity date were recorded for each plot during all study years. Additionally, reproductive phenophases were observed and analyzed, along with their respective durations:

- **Emergence**: The number of days from sowing until half of the plants had emerged.
- **Flowering**: The number of days from emergence until half of the plants had at least one open flower.
- **Pod formation**: The number of days from flowering until half of the plants had set at least one pod.
- **Consumption maturity**: The number of days from emergence until half of the pods reached consumption maturity.
- **Physiological maturity**: The number of days from emergence until the physiological maturity of the pods (which corresponds to the beginning of seed harvesting).

Statistical analyses were performed using EXCEL STAT software.

RESULTS AND DISCUSSION

Phenology refers to the study of biological phenomena associated with specific biological rhythms or periodic vegetative phases (phenophases) of plants and their interactions with environmental factors (Mundarain et al., 2005). The investigation of phenological processes is crucial for optimizing performance in pea cultivation, as it facilitates the identification of critical developmental stages that exert a direct influence on crop productivity.

Meteorological conditions and phenology are closely related, with plant phenophases being highly dependent on weather. In 2020, emergence occurred after a period of 14 days (L5) and ended 26 days after sowing (L10), specifically from March 17, 2020, to March 29, 2020. The average temperature was 8.4°C, with a minimum temperature of -4.3°C on March 7, 2020, and a maximum temperature of 21.9°C on March 21, 2020. There was only one rainy day, on March 24, 2020, with precipitation of 0.2 l/m².

The first flowers appeared on May 2, 2020, and continued until May 28, 2020. The average temperature during this period was 16.4°C, with a minimum of 8.3°C on May 7, 2020, and a maximum of 31.1°C on May 15, 2020. The total precipitation was 105 l/m², accumulated over 9 rainy days.

On May 13, 2020, the first pods formed in L1, while L7, the latest accession, formed pods on June 12, 2020. The minimum temperature during this period was 7.4°C on June 3, 2020, with a maximum of 31.6°C on June 6, 2020. The average temperature was 17.9°C, and precipitation amounted to 100 l/m², recorded over 11 rainy days.

At 65 days after emergence, L1 reached consumption maturity on May 23, 2020. L7 was once again the last accession to reach consumption maturity, after 96 days from emergence. The average temperature during this period was 20.5°C, with a maximum of 33.3°C on June 28, 2020, and a minimum of 7.4°C on June 3, 2020. Precipitation amounted to 98 l/m², accumulated over 18 rainy days.

Physiological maturity was recorded between June 10, 2020, and July 27, 2020. The average temperature during this period was 23.5°C, with a minimum of 13.5°C on July 13, 2020, and a maximum of 34.1°C on July 3, 2020. Precipitation during the final phenophase delayed harvesting and reduced yield, especially for early accessions. A total of 231 l/m² of precipitation was recorded over 17 rainy days.

Accession L1 exhibited the shortest vegetative cycle, lasting 83 days, followed closely by accession L9, which had a cycle of 90 days. In contrast, accession L6 was the latest, with a duration of 121 days from emergence to physiological maturity. Accession L10 reached physiological maturity after 101 days, while accessions L15 and L11 reached maturity after 104 days. Accessions L3, L2, and L8 completed their cycles after 106 and 107 days, respectively. Accession L5 reached maturity in 109 days, accession L4 in 113 days, and accessions L13 and L14 in 115 days. Finally, accessions L7 and L12 reached physiological maturity 118 days after emergence.

The reproductive phenology of the pea accession in 2020 is shown in Fig.2.

In 2021, in contrast to the previous year, the first accession to emerge was L3, 18 days after sowing. Compared to 2020, the emergence was slower, with the germination period extending up to 34 days for accession L6, which emerged on April 2. Emergence occurred after 29 days for L1, 30 days for L2 and L4, 28 days for L5, 31 days for L7, 33 days for L8, 29 days for L9, 30 days for L10, 28 days for L11, 30 days for L12, 29 days for L13, 27 days for L14, and 27 days for L15. During the emergence period, the average temperature was 6.7°C, with a minimum of -1.4°C on March 24, 2021, and a maximum of 19.8°C on April 2, 2021. Precipitation was spread over five days, totaling 11 l/m².

Flowering lasted for 38 days, with an average temperature of 13.1°C. The minimum temperature during this period was 2.4°C on April 17, 2021, and the maximum temperature reached 28.1°C on May 2, 2021. The total precipitation was 40 l/m², accumulated over 9 rainy days.

Accession L11 was the first to flower, followed by accessions L1 and L9, both of which flowered 37 days after emergence. Accession L3 recorded the latest flowering, after 55 days, while accession L7 flowered after 52 days, and accessions L14 and L15 flowered 50 days after emergence.

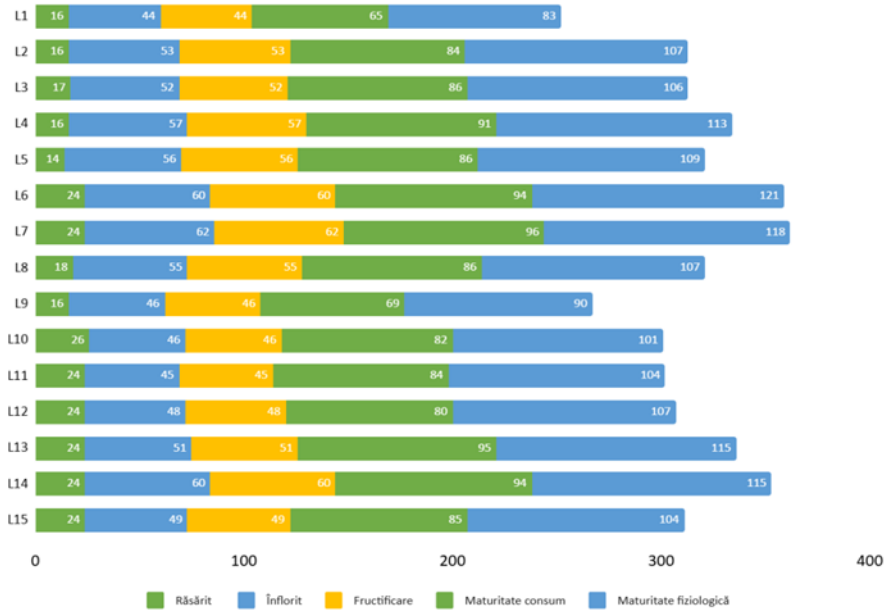

Fig. 2. The reproductive phenogram of pea accessions for the year 2020 / Fenograma reproductivă a liniilor de mazăre pentru anul 2020

The first pod formation occurred at accession L1, 12 days after flowering, while accession L7 was the last to form pods. Accession L2 began pod formation 13 days after flowering, L3 after 17 days, L4 and L5 after 16 and 17 days, respectively, L6 after 18 days, L8 after 14 days, L9 after 16 days, L10 after 15 days, L11 after 16 days, L12 after 14 days, L13 after 15 days, L14 after 17 days, and L15 formed pods 19 days after flowering. Pod formation took place over a period of 25 days, with an average temperature of 17.6°C. The minimum temperature during this period was 9.2°C on May 22, 2021, and the maximum temperature reached 26.7°C on May 23, 2021. Of the 25 days, 18 were rainy, accumulating 126 l/m² of precipitation.

On June 30, 2021, accession L11 was the first to reach consumption maturity, after 61 days from emergence, followed by accession L1, which reached maturity after 63 days, and accession L9, which took 68 days. Accession L15 was the last to reach physiological maturity, a month later, after 93 days. The other accessions reached consumption maturity after the following intervals: L2 after 77 days, L3 after 89 days, L4 after 78 days, L5 after 79 days, L6 after 88 days, L8 after 90 days, L10 after 79 days, L12 after 88 days, L13 after 91 days, and L14 after 92 days.

The average temperature during the maturation period was 20°C, with fluctuations ranging from a minimum of 10.7°C on June 1, 2021, to a maximum of 32°C on June 30, 2021. The abundant rainfall in June made it impossible to intervene in the crop (Fig. 3), leading to competition between weeds and plants, which negatively affected accession development. Of the 32 days, 22 were rainy, with a total precipitation of 235 l/m².

The first accession to reach physiological maturity was L11, 71 days after emergence, followed by accession L1, which reached maturity after 79 days, and accession L9, which required 83 days. The other accessions reached physiological maturity after the following periods: L2 after 97 days, L3 after 108 days, L4 and L5 after 100 days, L6 after 114 days, L7 after 115 days, L8 after 106 days, L10 after 98 days, L12 after 107 days, and L13 and L15 after 116 days.

The average temperature during this period was 23.3°C, with a minimum temperature of 10.9°C recorded on June 15, 2021, and a maximum temperature of 33.6°C on July 17, 2021.

Precipitation was consistent, hindering harvest activities and promoting the appearance and development of diseases, as seen in Fig.4. The number of days with precipitation was 20, accumulating a total of 206 l/m².

Fig. 3. The effects of heavy precipitation on pea plants / Efectele precipitatiilor abundente asupra liniilor de mazăre

Fig.4. The effects of heavy precipitation on pea accessions during the harvesting period / Efectele precipitatiilor abundente asupra plantelor în perioada recoltării

The year 2022 stood out as the most atypical within the study period, marked by exceptionally warm climatic conditions during December and January. The drought, coupled with strong and dry winds, hindered emergence under optimal parameters. On February 25 and 26, 2022, precipitation occurred in the form of snow, covering the soil with a layer of approximately 5 cm (Fig.5). However, this amount of snow was insufficient to compensate for the soil moisture deficit.

Fig. 5. Overview of the experimental field on February 26, 2022 / Imagine de ansamblu a câmpului experimental la data de 26.02.2022.

During emergence, the average temperature was 5.4°C, with a minimum temperature of -8.4°C and a maximum temperature of 24.8°C recorded on April 1, 2022. Precipitation occurred on only 14 days, totaling 55 l/m².

The pea accessions began to emerge after 35 days, and this process took place over a span of 11 days, representing the longest emergence period in the four years of the study. In some cases, the emergence period was longer than the vegetation period of certain accessions.

Accession L4 was the first to bloom, 33 days after emergence, followed by accessions L6, L12, and L15, which completed blooming 44 days after emergence.

The maximum temperature during this period, recorded on May 21, 2022, was 31.3°C, while the minimum was 8°C on May 15, 2022. The average temperature was 19.6°C. The total precipitation amounted to 47 l/m², collected over 6 rainy days.

The first accessions to form pods were L1 and L8, 12 days after flowering, while L12 was the last to develop pods, taking 20 days after flowering. The average temperature during this period was 22.1°C, with a maximum of 31.9°C recorded on June 2, 2022, and a minimum of 14.9°C observed on both June 8 and June 16, 2022. Precipitation occurred over 9 days, totaling 12 l/m².

Accession L1 reached maturity for consumption in 59 days, closely followed by accession L4, which reached maturity after 61 days, accession L2 at 62 days, and accession L5 at 66 days. Accession L15 was the last to reach maturity for consumption.

The average temperature during this period was 23.8°C, with a minimum of 14.2°C on June 22, 2022, and a maximum of 35.4°C on June 30, 2022. There were 16 rainy days, accumulating a total of 18 l/m² of precipitation.

Due to fluctuating temperatures and prolonged drought, the accessions reached physiological maturity in a shorter interval, but the yield was significantly impacted. Accession L1 reached physiological maturity in 74 days, followed by L4, which reached maturity in 73 days, and L2 at 79 days. The latest accession was L7, which took physiological maturity at 105 days from emergence.

The average temperature during this period was 25.1°C, with a minimum of 13°C recorded on July 13, 2022, and a maximum of 37.1°C on July 24, 2022. The total precipitation during this period was 19 l/m², collected over 15 rainy days.

The winter of 2022-2023 (December-January) was favorable, as December 2022 recorded precipitation of 31 l/m², and January 2023 saw 81 l/m² of rainfall. These precipitation levels contributed to the formation of a water reserve in the soil, which was essential for supporting the germination process of pea seeds.

The year 2023 was marked by the fastest emergence observed in all the study years. Accession L1 was the first to emerge, 12 days after sowing, while accession L6 was the last, emerging 18 days after sowing. Accession L2 emerged after 14 days, L3 and L9 after 13 days, and accessions L4, L11, and L15 after 17 days. Accessions L5, L7, and L12 emerged after 16 days, while L8, L10, and L13 emerged 15 days after sowing.

The average temperature recorded during this period was 8.5°C, with a minimum of -0.3°C on March 7, 2023, and a maximum of 19.1°C on March 9, 2023. There were no rainy days during this period.

L7 was the first accession to bloom, 31 days after emergence, while accessions L5 and L14 bloomed the latest, 49 days after emergence. Accessions L1 and L2 bloomed after 36 days, L3 after 45 days, L4 and L9 after 38 days, L6 after 39 days, and accessions L8, L11, and L15 bloomed after 33 days. Accession L10 bloomed after 42 days, L12 after 41 days, and L13 after 35 days following emergence. The average temperature during this period was 11.4°C, with a minimum of 2.8°C on April 3, 2023, and a maximum of 20.7°C on April 13, 2023. There were 9 rainy days, accumulating 41 l/m² of precipitation.

The accessions formed pods over a period of 24 days. The first pod developed 16 days after flowering in accession L3, while accession L11 formed pods 40 days after flowering. Accession L1 started forming pods 31 days after flowering, L2 after 22 days, L4 after 23 days, and accessions L5 and L14 after 21 days. Accession L6 formed pods after 34 days, L7 after 38 days, L8, L12, and L13 after 35 days, L9 after 27 days, L10 after 30 days, and L15 formed pods 28 days after flowering.

The maximum temperature was 26.5°C, recorded on May 19, 2023, and the minimum temperature was 2.8°C, observed on both April 6 and April 13, 2023. The average temperature was 12.8°C, and the total precipitation amounted to 127 l/m², accumulated over 20 rainy days.

The first accession reached consumption maturity on May 10, 2023, and the latest accession reached the same stage on June 14, 2023, after a period of 34 days. Accession L1 reached consumption maturity after 67 days from emergence, followed by accessions L5 and L8, which reached this stage after 83 days. Accession L2 reached maturity after 85 days, L3 and L7 after 87 days, L4 and L12 after 88 days, L14 after 91 days, L13 after 92 days, and accessions L6 and L15 reached maturity after 95 days. Accession L11 reached consumption maturity after 96 days, L10 after 99 days, and L9 was the last accession to reach consumption maturity, at 101 days from emergence. The maximum amount of precipitation recorded in a single day was 49 l/m², on May 21, 2023, and the total precipitation accumulated was 65 l/m², distributed over 9 rainy days.

The average temperature during this period was 18.9°C, with a minimum of 5.8°C recorded on May 12, 2023, and a maximum of 29.5°C on June 10, 2023.

The first accession to reach physiological maturity was L1, after 100 days from emergence, while the latest accession was L10, which took 119 days to reach the same stage. The physiological maturity of the accessions occurred over a period of 19 days. Accession L4 and L8 required 105 days, L5 required 106 days, L3 and L13 required 107 days, L6 required 108 days, and accessions L7, L12, and L14 reached maturity after 109 days. Accession L2 reached maturity after 112 days, while accessions L11 and L15 required 113 days, and L9 took 116 days to reach physiological maturity.

The average temperature during this period was 22.9°C, with a minimum of 12.7°C recorded on June 29, 2023, and a maximum of 34.2°C on June 24, 2023. There were 10 rainy days, totaling 83 l/m² of precipitation.

Therefore, considering that throughout the research we applied the same cultivation technology for all the studied accession and that they were grown under the same environmental conditions, the analysis of the data provided by the phenological observations revealed variation in the duration of different phenophases, depending on the cultivar. This variation emphasizes the importance of the genetic adaptability of each accession to the environmental conditions and the specific climatic factors of each studied year. Also, careful monitoring of climatic conditions is essential for the efficient management of pea crops, thereby optimizing agricultural production.

CONCLUSIONS

1. This study highlighted the significant influence of climate variability on pea phenology, affecting key phenophases such as emergence, flowering, pod formation, consumption maturity, and physiological maturity. Temperature and precipitation played a crucial role in determining the duration of the crop cycle and overall yield, underscoring the importance of climate monitoring for optimizing production.
2. Phenological differences were observed across the studied years, influenced by specific meteorological conditions. In 2020, emergence occurred between 14 and 26 days after sowing, while consumption maturity was reached between 65 and 96 days. Excessive precipitation during the final phenophase delayed harvest and reduced yield. In 2021, lower temperatures resulted in slower emergence (18–34 days), and physiological maturity extended to 71–116 days, with frequent rainfall favoring disease development. The year

2022 was marked by extreme drought and strong winds, leading to significant delays in emergence and a substantial decrease in yield. In contrast, 2023 presented more favorable meteorological conditions, facilitating rapid emergence and uniform maturation, demonstrating the crucial role of balanced climatic conditions in achieving optimal yields.

3. The analysis of the studied accessions revealed considerable variation in their responses to environmental conditions, emphasizing the importance of genetic adaptability to abiotic stress. Early-maturing accessions were more affected by excessive rainfall, whereas late-maturing accessions were more susceptible to drought stress. These findings highlight the necessity of selecting climate-resilient genotypes to ensure yield stability in the face of changing environmental conditions.
4. Genotypes with higher thousand-seed weights, such as L10, L11, L12, and L14, represent valuable genetic resources for improving for improving seed production potential in pea breeding programs.
5. The purple-flowered and purple-podded line L7 may be of particular interest for developing novel pea varieties that combine agronomic performance with ornamental value.
6. This study underscores the essential role of climate monitoring and adaptive strategies in pea crop management. Given the increasing challenges posed by climate change, a deeper understanding of the relationship between environmental factors and crop development is crucial for optimizing agricultural production and ensuring its long-term sustainability.

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THE IMPACT OF AGROTECHNICAL PRACTICES ON BIODIVERSITY AND BENEFICIAL ENTOMOFAUNA IN VINEYARDS

IMPACTUL PRACTICILOR AGROTEHNICE ASUPRA BIODIVERSITĂȚII ȘI ENTOMOFAUNEI UTILE ÎN PLANTAȚIILE VITICOLE

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Abstract
This review summarizes key management practices which promote biodiversity conservation in vineyards. As perennial agricultural landscapes, vineyards shape ecosystems, support biodiversity, and contribute to distinct cultural heritage. Traditionally, viticulture was part of a diversified agricultural system that fostered a high level of functional biodiversity. In recent decades, this balance has been disrupted by intensive vineyard management. A diverse range of species including plants, beneficial insects, and microorganisms enhance the vineyard resilience. Integrated Pest Management (IPM) and organic viticulture have emerged as effective strategies that help biodiversity to minimize reliance on chemical inputs and enhance natural ecosystem functions. The Târnave Vineyard in Transylvania, known for its rich entomofauna and diverse agroecological infrastructure, highlights the importance of maintaining landscape complexity to enhance biodiversity and ecosystem resilience. However, significant knowledge gaps remain, particularly in understanding the long-term impacts of large-scale vineyard landscape configuration and the integration of biodiversity-driven conservation strategies. Future research should prioritize region specific conservation strategies and long-term biodiversity monitoring frameworks to support the development of sustainable viticultural practices that balance productivity, environmental health, and climate resilience.

Keywords: vineyards, biodiversity, agrotechnical practices, useful entomofauna

Rezumat
Acest review prezintă tehnicile esențiale de management care susțin conservarea biodiversității în plantațiile viticole. Fiind ecosisteme agricole perene, plantațiile viticole influențează mediul, susțin biodiversitatea și contribuie la păstrarea unor tradiții culturale. Istoric, viticultura tradițională a făcut parte dintr-un sistem agricol divers, care a favorizat o biodiversitate funcțională ridicată. Cu toate acestea, în ultimele decenii, acest echilibru a fost perturbat de practicile viticole intensive. Reziliența ecologică este susținută de prezența unei game variate de specii, inclusiv plante, insecte benefice și microorganisme. Viticultura ecologică și gestionarea integrată a dăunătorilor (IPM) reprezintă instrumente eficiente pentru susținerea biodiversității, reducerea dependenței de substanțele chimice și îmbunătățirea funcțiilor naturale ale ecosistemelor. Podgoria Târnave din Transilvania, care se remarcă prin bogata sa entomofaună și infrastructura agroecologică diversă, reprezintă un exemplu în care menținerea complexității peisagistice a contribuit la abundența biodiversității și creșterea rezilienței ecosistemelor. Cu toate acestea, există încă numeroase lacune, în special în ceea ce privește efectele pe termen lung ale plantațiilor de mari dimensiuni și integrarea abordărilor de conservare bazate pe biodiversitate. Pentru a sprijini dezvoltarea unor practici viticole sustenabile, care echilibrează productivitatea, sănătatea mediului și reziliența climatică, viitoarele cercetări ar trebui să acorde prioritate strategiilor de conservare specifice fiecărei regiuni, precum și sistemelor de monitorizare a biodiversității pe termen lung.

Cuvinte cheie: plantațiile viticole, biodiversitate, practici agrotehnice, entomofauna utilă

INTRODUCTION

Vineyards represent cultural, economic, and ecological systems of great importance in many regions with a temperate climate. As perennial agricultural systems, they shape the entire landscape,

creating unique ecosystems and contributing to the formation of distinct cultural traditions (Daniel *et al*., 2012).

Over the centuries, traditional viticulture was integrated into a diversified agricultural system, which included meadows and orchards, fostering an ecosystem with a high level of functional biodiversity. This balanced approach has gradually been replaced in recent decades by an intensive vineyard management model. The new model is characterized by heavy mechanization and extensive use of plant protection products, impacting the ecosystem. Consequences include erosion, soil structure degradation, loss of soil fertility, groundwater contamination, and the use of high levels of agricultural inputs (Zaller *et al*., 2015).

The conversion of regional land from natural habitats to intensive grape production leads to the loss of both agrobiodiversity and the surrounding natural habitats, which can result in the reduction of essential ecosystem services, such as biological control (Miles *et al*., 2012).

In response to these challenges, plant protection specialists have developed more sustainable strategies, such as integrated pest management (IPM), aimed at reducing pesticide use and minimizing environmental impacts, while preserving vineyard biodiversity (Pretty & Bharucha, 2015).

Despite these advances, significant gaps remain in the full understanding of how specific agrotechnical practices influence biodiversity at different trophic levels in vineyards. Recent studies indicate that, globally, over 70% of the vineyards are being managed conventionally with minimal concern for the environment (Nicholson *et al*., 2020). Though it only accounts for roughly 3% of total agricultural land, recent studies estimate that viticulture contributes nearly 10% of the pesticide use in European agriculture, so its relatively environmental effect cannot be ignored (Eurostat, 2020; Gómez-Rodríguez *et al*., 2022). Intensive vineyard management has been connected to decreases of up to 30–40% in beneficial arthropod diversity in comparison to more extensively managed or organically certified vineyards, therefore highlighting the need for biodiversity-friendly practices.

Furthermore, studies have shown that even though organic and integrated management techniques promote greater degrees of biodiversity than traditional systems, the level and consistency of these advantages vary considerably depending on regional climatic conditions, landscape structure, and vineyard lifespans (Winter *et al*., 2018; Geldenhuys *et al*., 2021).

Current research efforts increasingly emphasize the need for long-term biodiversity monitoring programs, with a focus on linking specific agrotechnical measures—such as inter-row vegetation management, ecological infrastructure enhancement, and pesticide reduction—with measurable biodiversity outcomes and ecosystem service provision (Möth *et al*., 2023). Developing region-specific, resilient viticulture systems able to sustain high productivity while protecting environmental health in the face of continuous climate and land-use changes depends on addressing these gaps.

HISTORICAL OVERVIEW OF VINEYARD MANAGEMENT PRACTICES

Historically, grape cultivation was largely dependent on manual labor and organic inputs, including natural fertilizers and traditional pest control methods. These early techniques, while rudimentary by today's standards, aligned closely with the rhythms of nature and emphasized sustainable resource use within localized environments.

A significant transformation began to unfold in the early 20th century with the expansion of industrial agriculture. In an effort to meet rising production demands and reduce labor intensity, vineyard management saw a marked increase in the use of synthetic fertilizers, herbicides, and pesticides. While these innovations significantly boosted grape yields, they also disrupted natural ecosystems, leading to a notable decline in biodiversity and long-term soil health (Hurajová *et al*., 2024; Stefanucci *et al*., 2018).

Since concerns over the ecological consequences of these intensive methods began to intensify, a growing body of scientific literature started to reveal the harmful effects of synthetic inputs, not only on soil microbiota and water systems but also on beneficial insect populations. This shift in perspective laid the groundwork for **Integrated Pest Management** (IPM), a more nuanced approach that blends biological control strategies with targeted chemical use, emphasizing monitoring and ecological balance over broad-spectrum pesticide applications (O'Brien *et al*., 2025; Winter *et al*., 2018).

In more recent decades, there has been a notable revival of interest in sustainable and organic vineyard management. These modern approaches prioritize soil vitality, biodiversity preservation, and the minimization of synthetic inputs. Organic vineyards, for instance, forego synthetic chemicals in favor of compost applications, cover cropping, and biological pest controls. Studies consistently demonstrate that organic practices foster improved soil biodiversity and deliver essential ecosystem services, such as nutrient cycling and natural pest suppression (Hendgen *et al*., 2018; Paiola *et al*.,).

A more specialized chapter of this trend is **biodynamic viticulture**, developed in the 1920s by Rudolf Steiner. Biodynamic viticulture builds upon organic principles but introduces additional spiritual and ecological dimensions, treating the vineyard as a self-sustaining organism. It emphasizes biodiversity, soil vitality, lunar and cosmic rhythms, and the use of specific preparations made from fermented herbs, minerals, and

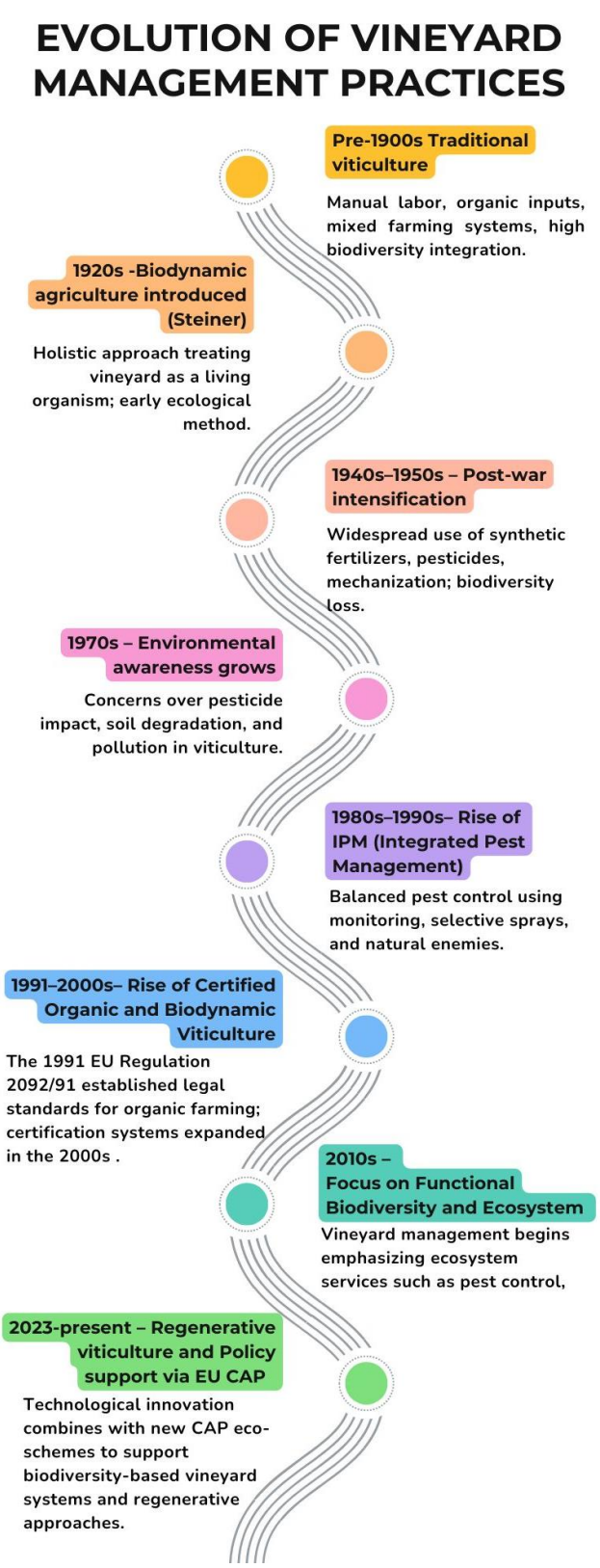

Figure 1. Timeline of milestones in the evolution of vineyard management practices (O'Brien *et al*., 2025; Turinek *et al*., 2009; Winter *et al*., 2018; Geldenhuys *et al*., 2021).

animal manure (Turinek *et al*., 2009). Drawing on both agricultural and philosophical principles, biodynamic methods incorporate natural preparations and follow lunar and celestial cycles to guide farming activities. Though often met with skepticism, empirical findings suggest that these practices may further enrich microbial life in soil and enhance the resilience of vineyard ecosystems (Ghiglieno *et al*., 2020; Karimi *et al*., 2021).

Today, the concept of **functional biodiversity** has emerged as a key focus in sustainable vineyard management. Rather than focusing solely on species numbers, this perspective emphasizes the roles that organisms play in vital processes like pollination, pest control, and nutrient cycling. Measures such as planting cover crops, maintaining hedgerows, and reducing soil tillage are increasingly adopted to support functional biodiversity (Beaumelle *et al*., 2023; Fiera, Ulrich, Popescu, Buchholz *et al*., 2020).

Recent studies have explored the delicate balance between maximizing wine production and fostering ecosystem services. Reducing the intensity of vegetation management can yield multiple benefits, from mitigating soil erosion to enhancing natural pest control, all without compromising grape yields (Winter *et al*., 2018; O'Brien *et al*., 2025).

In summary, the evolution of vineyard management reflects a complex interplay between tradition, industrialization, and a modern push toward sustainability. The growing emphasis on biodiversity-friendly practices highlights an increasing recognition that healthy vineyard ecosystems are essential—not only for the environment but for the long-term success of viticulture itself.

BIODIVERSITY AND ECOLOGICAL INFRASTRUCTURES IN THE VINEYARD

A variety of populations are included in the **viticultural biocoenosis**, including fungi, bacteria, viruses, insects, plants (grapevines, intercrops, and spontaneous weeds), and other plant and animal species. Despite their territorial interdependence, these population groups function somewhat independently. Predominant natural environmental factors determine the biocoenosis's composition and borders (Oprea, 2001).

Abiotic factors like soil characteristics, water availability, and climate variability have a significant impact on the overall functioning of the vineyard ecosystem, which is the outcome of the intricate interactions between all living things (Bernard *et al*., 2006).

Ecological infrastructures are defined as semi-natural elements either on the farm or within a 150 meters buffer zone that offer significant ecological value and enhance functional biodiversity in agricultural systems (Böller, Häni, & Poehling, 2004). These features, such as hedgerows, wildflower strips, unmanaged field margins, meadows, stone piles, and woodland patches, create habitats and resources that support beneficial arthropods and other organisms. They are essential for maintaining ecological equilibrium, helping in natural pest control and pollination, while lowering dependence on synthetic inputs.

In order to sustain proper biodiversity, it is estimated that ecological infrastructures has to be around 15% of the land used for agriculture, however the International Organization for Biological and Integrated Control (IOBC) proposes a minimum of 5% (Böller *et al*., 2004). These zones offer essential ecological services, including overwintering habitats, alternative prey, nectar and pollen for pollinators and natural enemies, as well as microclimates beneficial to predator development and reproduction.

Böller *et al*. (2004) state that three essential components are necessary to sustain functional biodiversity in vineyard systems:

- A vineyard-specific list of key pests, diseases, and weeds requiring regular monitoring and management.
- A minimum of two identified key natural antagonists (e.g., parasitoids or predatory insects).
- The allocation and proper management of at least 5% of total farm area as ecological infrastructure.

Landis, Wratten, and Gurr (2000), underline the need of habitat management in preserving natural enemies of agricultural pests, suggesting that increased biological control is linked with structurally complex settings. Pywell *et al*. (2015) similarly shows that ecological intensification allows wildlife-friendly farming practices, including the preservation of field edges and flowering habitats, to increase both biodiversity and crop production.

While green borders offer clear ecological benefits, they can also present certain drawbacks. Several studies have shown that unmanaged vegetation strips and field margins can become reservoirs for pest species, especially those with broad host ranges. For instance, Tirello, Pozzebon, and Duso (2013) found that vineyards with substantial soil cover and adjacent neglected vegetation were more susceptible to leafhoppers and planthoppers, which are vectors of grapevine diseases such as flavescence dorée. In addition to the fact that it encourages predators, Trivellone, Barbagallo, and Pavan (2012) suggested that insufficiently managed natural vegetation may serve as a host to insect pest populations. Ecological infrastructures may induce a trade-off by augmenting the population of both beneficial and detrimental insects.

To minimize these risks, an integrated approach is essential — one that combines species-specific pest management, regular monitoring, and thoughtful selection of vegetation, particularly avoiding plants that serve as hosts for key vineyard pests.

Vineyard biodiversity spans multiple taxonomic groups, including soil microbes, arthropods, birds, and mammals (Paiola *et al*., 2019). Research has frequently shown that vineyards with greater habitat heterogeneity tend to sustain richer and more functionally varied populations of species (Winter *et al*., 2018). Maintaining native vegetation within vineyard landscapes enhances bird diversity and ecological interactions, such as seed dispersal and insect control (Muñoz-Sáez, 2017). Furthermore, arthropod communities, particularly beneficial predatory insects and pollinators, show increased richness and abundance in vineyards with complex vegetative structures, including hedgerows and flower-rich ground cover (Thomson & Hoffmann, 2009; Costello & Daane, 2003).

Practices like as reduced tillage, organic amendments, and permanent cover crops also help to promote soil biodiversity, which includes microbial communities and detritivores like springtails and mites (Burns et al., 2016; Zaller et al., 2015). Vineyards set within mosaics of semi-natural habitats—such as grasslands, woodlots, and riparian buffers—tend to show more resilience at the landscape level, thereby encouraging the spreading and recolonization of beneficial species (Geldenhuys *et al*., 2021; Möth *et al*., 2023). Given these ecological dynamics, it's essential to have clear, reliable ways to measure biodiversity in vineyard ecosystems.

QUANTIFYING METHODS FOR SPECIES DIVERSITY IN VITICULTURAL ECOSYSTEMS

Understanding biodiversity in vineyard systems requires more than casual observation, it calls for reliable, standardized methods that can capture ecological complexity. While most studies are carried out at the local scale and often focus on a single crop, they typically examine functional groups that both support agricultural productivity and respond sensitively to management intensity (Cohen *et al*., 2015).

Various biodiversity parameters are used to quantify species diversity in biocenoses, the most used being:

- **Shannon–Wiener Index (H'):** One of the most used metrics in vineyard biodiversity research, this index accounts for both abundance and evenness of species in a community (Štrbac *et al*., 2023).
- **Simpson's Diversity Index (D):** Emphasizes the dominance of particular species; useful when pest outbreaks or predator-prey imbalances occur (Trivellone *et al*., 2012).
- **Species Richness (S):** A count of the number of different species in a given area, often used alongside other indices for a fuller picture (Tomoiagă *et al*., 2022).

- **Evenness (E):** Measures how evenly individuals are distributed across species, which can be a key indicator of functional stability in vineyard agroecosystems.

Sampling techniques in vineyards:

Biodiversity assessments in vineyards often require a multimethod approach, tailored to target both above and below the ground organisms:

- Pitfall Traps: Widely used for ground-dwelling arthropods (e.g., beetles, spiders). This method is non-selective and effective in vineyards with permanent vegetation (Thomson & Hoffmann, 2009);
- Yellow Sticky Traps: Common for monitoring flying insects, including pollinators, parasitoids, and leafhoppers (Hogg *et al*., 2013). Placement height can be adjusted to focus on canopy or ground-level fauna;
- Sweep Netting: Especially effective in inter-row vegetation to assess plant-dwelling insects like predatory bugs, aphids, and hoverflies (Štrbac *et al*., 2023);
- Vacuum Sampling (D-Vac): Sometimes used in vineyards to standardize suction-based collection of small arthropods across a defined area, reducing observer bias (Winter *et al*., 2018);
- Visual Surveys and Transects: Useful for larger taxa or where identification requires in-field observation (e.g., butterflies, certain beetles, birds).

Soil biodiversity monitoring:

With increasing focus on below the ground health, studies have also incorporated soil biodiversity assessments:

- Soil core sampling and Berlese funnels: For extracting micro-arthropods, nematodes, and springtails (Zaller *et al*., 2015).
- DNA Metabarcoding: A cutting-edge method now being applied in some viticultural studies to assess total microbial or invertebrate diversity in soil samples (Möth *et al*., 2023).
- Enzyme Activity Assays: Used to measure microbial functional diversity and soil health under different vegetation or tillage regimes (Burns *et al*., 2016).

Functional diversity and ecosystem services:

Increasingly, researchers are moving beyond taxonomic diversity to evaluate functional diversity, which considers the roles species play in ecosystems. This can include:

- Trophic guild analysis: examines how species are grouped based on their feeding roles, providing insights into how energy and matter flow through vineyard ecosystems (e.g., predators, herbivores, detritivores) (Costello & Daane, 2003)
- Pollination or parasitism potential focuses on assessing how different species contribute to critical ecosystem services, like enhancing fruit set through pollination or naturally controlling pest populations via parasitism (Rodriguez-Saona *et al*., 2012),
- Functional trait approaches, which link species ecological traits to habitat features and agrotechnical inputs (Geldenhuys *et al*., 2021).

Landscape-scale biodiversity monitoring

Some studies combine vineyard-scale sampling with broader landscape metrics, such as:

- Habitat complexity indices (e.g., % of natural land within 500m radius);
- Connectivity models to assess movement of beneficial species between patches (Möth *et al*., 2023).

These methods help isolate the effects of local vs. landscape-level biodiversity drivers, which is especially useful in fragmented vineyard regions like those in central and eastern Europe. Particularly for implementing sustainable or organic techniques, biodiversity monitoring in vineyard ecosystems has to be thorough and pay close attention to the small impacts of agrotechnical management. While traditional biodiversity measurements like Shannon or Simpson offer a good beginning point, determining the ecological effect of vineyard management needs a combination of functional and landscape-level evaluations. As technology evolves, genomic and

trait-based approaches are expected to play more important roles in biodiversity monitoring since they offer high-resolution insights on the sustainability of vineyard systems.

INFLUENCE OF AGROTECHNICAL METHODS AND THE PEST–NATURAL ENEMY EQUILIBRIUM

One of the main goals of biodiversity-based vineyard management is to achieve and maintain an ecological balance between pest species and beneficial entomofauna. Agrotechnical techniques including mowing frequency, tillage intensity, pesticide use, and cover crop plants species selection can notably impact this equilibrium state. When managed well, diverse agroecosystems create a regulatory environment in which natural enemies can effectively suppress pest populations beforethey reach damaging populations (Landis *et al*., 2000; Rodriguez-Saona, Blaauw & Isaacs, 2012).

Reduced mowing or postponed mowing of inter-row vegetation, can preserve floral supplies and refuge habitats for beneficial insects during vital times, therefore sustaining their populations. On the other hand, excessive mechanical disruption or the use of broad-spectrum insecticides can disturb this equilibrium, leading to reducing populations of natural antagonists and potentially contributing to secondary pest outbreaks or pest resurgence (Wilson & Daane, 2017). Lowering intervention levels in pest control, keep the flowering species, and selective planting of insectary plants help to maintain pest species under control and increase the presence and activity of beneficial fauna.

A functionally diversified and well-managed vineyard seeks not the total eradication of pests, but a stable equilibrium where natural predators maintain pest populations below levels that cause financial harm. This ecological strategy, focused on biodiversity, enhances the resilience of vineyard systems, especially in the cool-climate areas, where the growing season shorter and pest dynamics may rapidly change due to climatic instability.

Below are **key agrotechnical practices** commonly applied in viticulture, along with their influence on biodiversity:

A. Inter-row vegetation management

Establishment of cover crops or maintenance of spontaneous vegetation between vine rows.

- **Positive impact**: Enhances habitat complexity, provides floral resources (nectar and pollen), overwintering shelters, and alternative prey for beneficial arthropods such as predatory beetles, spiders, parasitoids, and pollinators (Brambilla & Gatti, 2022; Thomson & Hoffmann, 2009).
- **Risk**: Poorly managed cover crops may also host pest species like leafhoppers if plant species selection is not carefully considered (Tirello, Pozzebon, & Duso, 2013).

B. Mowing frequency and timing

Mechanical cutting of vegetation in vineyard inter-rows or margins.

- **Reduced or delayed mowing** preserves flowering plants and structural habitat for beneficial insects, leading to greater predator and parasitoid abundance (Geldenhuys *et al*., 2021).
- **Frequent, intensive mowing** reduces habitat availability, decreases floral resources, and disrupts the life cycles of ground-dwelling arthropods and pollinators (Winter *et al*., 2018).

C. Soil tillage practices

Mechanical soil disturbance for weed control or soil aeration.

- **Reduced tillage or no-till systems** preserve soil structure, maintain microbial diversity, and protect ground-dwelling beneficials like predatory mites, beetles, and springtails (Zaller *et al.*, 2015).
- **Frequent deep tillage** destroys ground nests of solitary bees and exposes natural enemies to predation and environmental stress (Burns *et al*., 2016).

D. Pesticide use

Application of chemical products to control pests and diseases.

- **Selective, low-toxicity pesticides** (such as biopesticides or pheromone-based methods) minimize non-target effects and allow beneficial species to persist (Wilson & Daane, 2017).
- **Broad-spectrum insecticides** severely reduce populations of beneficial arthropods, leading to pest resurgence and secondary pest outbreaks due to the collapse of natural enemy communities (Rodriguez-Saona, Blaauw & Isaacs, 2012).

E. Use of ecological infrastructures

Implementation of features like hedgerows, wildflower strips, stone piles, and unmanaged field margins around or within vineyards.

- Provides permanent refuges and resources for beneficial insects (e.g., spiders, predatory bugs, parasitoids, pollinators).
- Increases species richness and functional diversity, enhancing pest suppression and pollination services (Böller, Häni & Poehling, 2004; Möth *et al*., 2023).

F. Irrigation practices

Regional meteorological and edaphic conditions have a major impact on the need of irrigation in vineyards, though. In cooler, precipitation-favored regions like northern Romania, including the Târnave Vineyard, irrigation is usually not required because of sufficient natural moisture availability during the vegetative period. Classified as "Dfb" in the Köppen-Geiger Climate Classification, this area has a fully humid continental climate with warm summers and a moderate yearly rainfall of about 544.6 mm. These factors, combined with appropriate soil qualities, offer sufficient water supply for grapevines without requiring artificial irrigation. Not having to add irrigation not only lowers environmental input costs but also helps to maintain soil microbial populations by preventing too much soil moisture and compaction (Fiera *et al*., 2020).

- **Drip irrigation** tends to favor beneficial soil organisms by maintaining moderate soil moisture without over-saturating the environment (Gómez *et al*., 2018).
- **Over-irrigation** can negatively impact soil structure and microbial health, leading to a decline in soil biodiversity.

G. Pruning and canopy management

Training and thinning of grapevines to optimize fruit exposure and air circulation.

- **Moderate canopy thinning** reduces humidity and fungal disease pressure, minimizing pesticide needs and indirectly benefiting beneficial arthropods.
- **Over-pruning** can eliminate microhabitats used by predatory mites, spiders, and parasitoid insects (Geldenhuys *et al*., 2021).

Table 1. Comparison of agrotechnical practices in conventional and biodiversity-based vineyard management and their impacts on biodiversity / Compararea practicilor agrotehnice în managementul convențional al plantațiilor viticole și în cel bazat pe biodiversitate și impactul acestora asupra biodiversității

AGROTECHNICAL PRACTICE	CONVENTIONAL APPROACH	BIODIVERSITY-BASED APPROACH	BIODIVERSITY IMPACT	ADVANTAGES OF BIODIVERSITY-BASED PRACTICE
Soil Tillage	Frequent, deep tillage for weed control and aeration	Reduced or no-till systems, with mulching and cover crops	conventional → soil biota loss; low disturbance → enhanced soil life	Improved soil structure, microbial diversity, and erosion control
Inter-row Management	Bare soil or frequent mowing of spontaneous vegetation	Diverse cover crops or spontaneous vegetation	Low structural complexity vs. high	Natural pest control, pollination support, and weed suppression
Pesticide Use	Broad-spectrum chemical pesticides used prophylactically	Targeted application, selective biopesticides, or pheromone traps	High non-target impact vs. selective impact → preservation of natural enemies	Reduced pest resistance, natural pest suppression, lower residue risk

Fertilization	Synthetic fertilizers focused on rapid nutrient delivery	Organic compost, green manures, and microbial inoculants	Reduced microbial diversity vs. enhanced belowground biodiversity	Soil fertility, organic matter retention, balanced nutrient cycling
Ecological Infrastructures	Often absent or minimal	Hedges, flower strips, stone piles, uncultivated borders	Low habitat heterogeneity vs. high → more beneficial and ecological niches	Greater functional diversity and landscape-level resilience
Canopy and Pruning Management	Standardized pruning for productivity	Site-specific pruning optimizing beneficial insect habitat	Can affect arthropod shelter and predator habitat quality	Reduces fungal pressure, supports natural enemies
Irrigation	Uniform or excessive watering	Precision irrigation or dry farming depending on climate	Overwatering reduces microbial diversity; precision protects habitat quality	Maintains microbial activity and avoids water stress or excess
Cover Crops	Rarely used or replaced by herbicides	Flowering forbs, legumes, or species mixes	No floral resources vs. extended blooming and prey habitat for beneficials	Attracts pollinators, improves soil health, suppresses pests
Pest Monitoring	Reactive: spraying after symptoms appear	Preventive: pest thresholds, monitoring traps, biological indicators used	Conventional: fewer feedback loops; sustainable: promotes ecological balance	Reduces unnecessary sprays, supports early interventions
Livestock Integration *(optional)*	Not practiced	Sheep grazing under vines in dormancy periods	Adds nutrient cycling and reduces mowing; minimal in conventional	Enhances soil biology, reduces compaction and herbicide needs

Note. This table summarizes how different agrotechnical practices affect biodiversity in vineyards, contrasting conventional viticulture with biodiversity-based approaches. Practices such as reduced tillage, inter-row cover cropping, and the use of ecological infrastructures have been associated with greater functional biodiversity and improved ecosystem services (Geldenhuys *et al*., 2021; Burns *et al*., 2016; Thomson & Hoffmann, 2009).

Biodiversity-friendly vineyard management hinges on thoughtful application of agrotechnical practices that support natural enemies and maintain ecological balance. From reduced tillage and selective pesticide use to strategic vegetation and habitat enhancement, each decision plays a role in shaping the vineyard's biological community. Among these strategies, the use of cover crops stands out as a particularly versatile tool - capable of influencing both above and below ground biodiversity. The next chapter explores how different types of cover crops affect beneficial entomofauna, offering insight into their role as a cornerstone of sustainable viticulture.

COVER CROP TYPES AND THEIR IMPACT ON BENEFICIAL ENTOMOFAUNA IN VINEYARDS

Cover crops are a central component of biodiversity-friendly vineyard management. When selected and managed properly, they provide floral resources, shelter, and microclimates that enhance beneficial entomofauna, especially predators, parasitoids, pollinators, and soil invertebrates.

The most frequently used cover crops in viticulture include:

- **Legumes** (e.g., *Trifolium spp.*, *Vicia spp.*): These species fix atmospheric nitrogen, improve soil structure, and attract pollinators. A greenhouse study by Sharifi et al. (2024) demonstrated that legume species like Ladino white clover and Dutch white clover exhibited superior biomass production and nitrogen content, making them effective for enhancing soil fertility in vineyards
- **Grasses** (e.g., *Festuca spp.*, *Lolium spp.*): Provide ground cover and erosion control but offer limited nectar/pollen.
- **Flowering forbs / Wildflower mixes** (e.g., *Phacelia tanacetifolia*, *Melilotus officinalis*, *Sinapis alba, etc*): Rich in nectar and pollen, highly attractive to beneficial insects.

Mixtures combining legumes and forbs are increasingly recommended to maximize both soil and ecological benefits. Recent experimental work has highlighted the differential effects of various cover crop regimes on vineyard insect communities. For instance, in a multi-year field study conducted in UK, the use of a wild bee-optimized floral seed mix significantly increased the abundance and richness of beneficial insects when compared to traditional mowed grass inter-rows. Notably, solitary wasps and pollinators showed heightened diversity in plots where spontaneous vegetation or tailored seed mixes were allowed to flourish, while the lowest diversity was observed in areas with frequent mowing and vegetation suppression (Griffiths-Lee *et al*., 2022). A comparable trend was observed over the course of a long-term study in Spanish vineyards, where insect abundance and species richness rose notably in intensively managed plots after flowering cover crops were introduced. Interestingly, the most significant ecological improvements emerged during the second and third years of implementation, highlighting the importance of maintaining stable, continuous floral resources over time to fully realize biodiversity benefits (Peris-Felipo *et al.*, 2021).

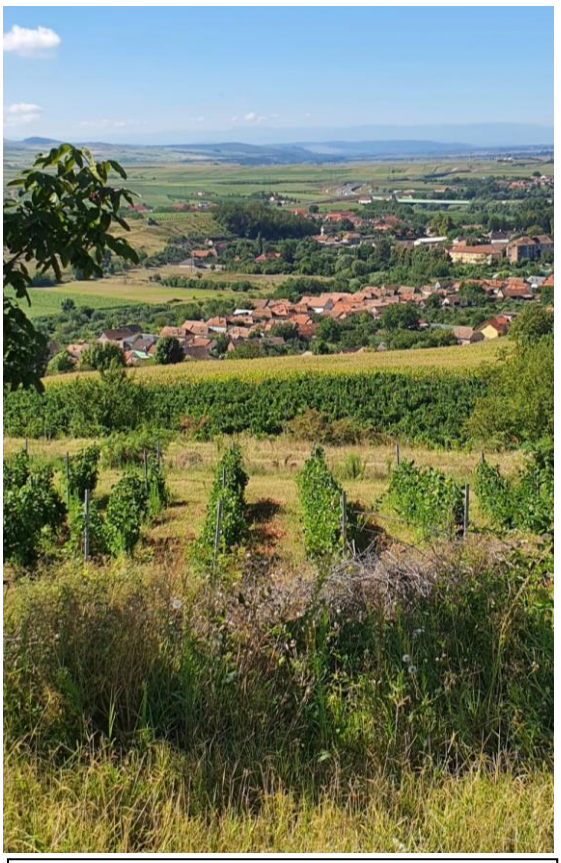

Figure 2. Vineyard landscape in Ciumbrud, Transylvania, an example of biodiversity-friendly viticulture, with diverse inter-row vegetation supporting ecosystem stability.

In Central and Eastern Europe, recent cover cropping trials are beginning to reveal how these practices perform under regional conditions. A study from Romania investigated the impact of artificially established cover crops, specifically *Trifolium repens* and *Melilotus officinalis*, on both soil-dwelling and surface-active insect communities from two vineyard sites. The results showed a marked increase in both the abundance and diversity of beneficial arthropods, particularly species from the orders *Coleoptera* and *Araneae*, when compared to conventionally managed black tilled systems. In addition, soil microbial activity was notably higher in the mulched plots, suggesting that cover cropping can deliver complementary gains for both soil health and biodiversity (Șerdinescu *et al*., 2023).

Cover crops support individual beneficial species, also they help shape entire vineyard food chains. Species like phacelia, buckwheat, and mustard, which bloom in succession, extend nectar and pollen availability into late spring and early summer—a critical period when natural enemies often face prey shortages. This overlap has been linked to better control of pests such as leafhoppers and mites (Altieri & Nicholls, 2005).

Overall, when thoughtfully selected and timed, cover crops can serve as a powerful tool in sustainable viticulture. As vineyards confront increasing climate and pest pressures, integrating diverse cover species may enhance both ecological stability and crop resilience.

ANTAGONISM AND THE ROLE OF BENEFICIAL ENTOMOFAUNA IN THE BIOLOGICAL CONTROL OF VINEYARD PESTS

Beneficial entomofauna refers specifically to insect species that contribute to maintaining ecological balance and the natural control of pest populations in agricultural ecosystems. Biological control through **antagonism** refers to organisms that can suppress, control, or reduce populations. These antagonists can include predators, parasitoids, pathogens (such as fungi or bacteria) and other organisms with a direct effect on pests. Predators like spiders, green lacewings, predatory mites, and birds (such as falcons) play significant roles in reducing pest damage in vineyards (Kross *et al*., 2012).

Notable examples of potential antagonists include spiders, raptors, egg parasitoids, and entomopathogenic fungi such as *Beauveria bassiana*. The effectiveness of these biological agents depends largely on the specific ecological conditions of the vineyard environment.

Vegetative ground cover, especially when composed of native or diverse plant species, serves as a multifunctional component of landscape management—providing shelter, alternative food sources, and microhabitats for insect predators and parasitoids (Wang, 2018).

Figure 3. *Coccinellidae* (lady beetle) on grapevine, a key beneficial predator, contributing to natural pest control.

Figure 4. *Argiope bruennichi* (wasp spider) on grapevine foliage, helps regulate insect populations by capturing pests in its orb web.

Predators

Among arthropods, the most significant predators involved in biological control in vineyards include spiders, lacewings (*Chrysopidae*), predatory mites (*Phytoseiidae*), predatory bugs (*Anthocoridae*, *Miridae, and Nabidae*), hoverflies, and lady beetles. However, other taxa such as birds, bats, and reptiles may also play key roles depending on the structure of the agroecosystem. A particularly compelling example is the introduction of the New Zealand falcon (*Falco novaeseelandiae*) into vineyards, which led to a 95% reduction in grape losses due to pest birds, compared with vineyards lacking falcon presence (Kross *et al*., 2012).

Parasitoids

Parasitoids, typically target and parasitize the immature phases of pest insects, such as eggs, larvae, or pupae of moths and leafhoppers, thereby greatly lowering their numbers. Parasitoids from the *Trichogrammatidae* and *Braconidae* families have shown especially good results. *Trichogramma* spp., for instance, are well known for parasitizing moth eggs, so preventing larval emergence and so reducing vineyard infestation (Hogg *et al*., 2013).

Another important parasitoid in vineyard is *Coccophagus scutellaris* and *Coccophagus lycimnia*, both of which are endoparasitoid wasps that contribute to the control of grapevine soft scales. *Coccophagus scutellaris*, for example, parasitizes the larval stages of these pests, which damage

grapevines by sucking plant sap and transmitting diseases. By parasitizing these pests, these wasps help reduce their populations and minimize their negative impact on the crop. The incorporation of such parasitoids into biological control programs offers vineyard managers a practical strategy to reduce chemical insecticide use, contributing to sustainable viticulture practices (Guerrieri & Noyes, 2009).

Examples such as *Trichogramma* spp., *Anagrus epos*, and *Coccophagus scutellaris* highlight the important role of parasitoids in Integrated Pest Management (IPM) strategies. (Hogg *et al*., 2013; Guerrieri & Noyes, 2009).

Other biological contorol agents

In addition to arthropod predators and parasitoids, several microbial biological control agents are used in vineyard pest management. These include fungi such as *Beauveria bassiana*, *Ampelomyces* sp., *Trichoderma* sp., and bacteria such as *Bacillus thuringiensis*. *Beauveria bassiana* is an ascomycete fungus that can establish itself as an endophyte in grapevine tissues and retains its antagonistic potential against insect pests and even microbial pathogens, such as the downy mildew pathogen (*Plasmopara viticola*). This dual functionality makes *B. bassiana* a promising tool in biological control strategies that target both insect pests and grapevine diseases (Rondot & Reineke, 2013). Similarly, *Metarhizium anisopliae* has been identified as a promising candidate for future vineyard applications, offering pest suppression potential comparable to *B. bassiana* (Ownley *et al*., 2017).

Figure 5. *Coccophagus* spp. – endoparasitoid wasps that attack the larval stages of scale insects such as *Pulvinaria vitis*, reducing pest populations.

Some newer IPM models suggest combining predators + parasitoids + fungi show better pest suppression than using a single group alone (Gurr *et al*., 2017; Rondot & Reineke, 2013).

Sustainable vineyard management increasingly recognizes the necessity of a holistic, multi-trophic approach that not only suppresses pest populations but also reinforces the ecological integrity of viticultural landscapes. Maintaining an appropriate biological balance between beneficial and harmful species, as well as protecting and increasing the number of beneficial species, especially through non-polluting methods, significantly contributes to the health of the grapevine, the conservation of natural biodiversity, and the reduction of environmental pollution (Ostandie *et al*., 2021).

CASE STUDY - BENEFICIAL ENTOMOFAUNA AND BIODIVERSITY BALANCE IN TÂRNAVE VINEYARD

Situated in the heart of Transylvania, the Târnave Vineyard is distinguished by its rich diversity of beneficial insects, which play a vital role in ecological balance and pest suppression. Field studies have revealed that predatory mite species such as *Typhlodromus pyri* and *Amblyseius andersoni* are notably more diverse in this region compared to other European wine-growing areas (Möth *et al*., 2023). These mites are not only effective natural enemies but also serve as indicators of ecological health within vineyard systems.

The biodiversity of vine plantations in Transylvania, and particularly in Târnave Vineyard, reflects a complex semi-artificial viticultural ecosystem, encompassing the vineyard plantation and a diverse beneficial entomofauna. This plays an essential role in maintaining ecological balance and natural pest control, thus contributing to the sustainability of viticultural ecosystems. Studies conducted in 2015 in Târnave vineyards revealed a high percentage of agro-ecological infrastructure (IAE) of 28.46%, indicating a significant functional biodiversity compared to other

viticultural regions in Romania, where this percentage is considerably lower (Tomoiagă *et al*., 2016). The beneficial entomofauna identified in these vineyards includes a diversity of *Coleoptera* species, representing 64% of the total beneficial entomofauna, followed by species from the *Chrysopidae* family and other groups of natural predators. These beneficial insects help maintain pest populations below the economic damage threshold (EDT), thereby reducing the need for chemical inputs (Tomoiagă *et al*., 2013).

Evaluations also show that the variety of climatic and agronomic conditions in these regions significantly impacts the density and distribution of beneficial and harmful species, suggesting that careful management of these factors can enhance biodiversity and ecosystem resilience. In the Târnave area, the implementation of agroecological technologies in vineyards has led to an increase in the diversity of beneficial entomofauna, especially among *Heteroptera* and *Hymenoptera* species (Tomoiagă *et al*., 2022). The diversity and equity of beneficial species were superior in agroecological systems, highlighting the importance of these practices for biodiversity conservation and maintaining a healthy viticultural ecosystem (Tomoiagă *et al*., 2022). Furthermore, the surrounding landscape composition played a significant role in shaping entomofauna diversity. A higher proportion of vineyards in the local landscape was positively correlated with increased densities of predatory mites—demonstrating that landscape-level planning is as crucial as in-field practices (Möth *et al*., 2023).

Adopting viticultural practices that support entomofauna is essential for maintaining the health and sustainability of vineyards. Implementing ground cover, reducing pesticide use, creating ecological corridors, and cultivating plant diversity are effective strategies for conserving beneficial insects and improving ecosystem services in viticulture. The diversity of beneficial entomofauna in Târnave Vineyard is an indicator of the ecological health of this viticultural region. Local management of vegetation and conservation of natural habitats play a crucial role in maintaining and increasing populations of predatory mites, thus contributing to ecological sustainability and reducing the need for chemical pesticides.

Regional agricultural authorities should consider developing clear guidelines identifying plant species to avoid, particularly those known to harbor pests or serve as vectors for plant diseases and viruses. To promote faunal diversity and landscape resilience, farm planning should integrate both linear elements (such as flower strips, hedgerows, ditches, and stone walls) and non-linear features (like tree clusters, ponds, and natural clearings). Combining these elements strategically ensures spatial and temporal continuity, which is essential for supporting diverse and stable insect and wildlife populations.

FUTURE DIRECTIONS IN AGROTECHNICAL PRACTICES FOR BIODIVERSITY-FRIENDLY VITICULTURE

As viticulture adapts to growing environmental and economic challenges, future agrotechnical practices are increasingly oriented toward regenerative, nature-based, and multifunctional systems. These approaches move beyond input reduction, emphasizing ecosystem function, biodiversity enhancement, and resilience to climate pressures. Among the most promising directions are regenerative viticulture, the integration of livestock, and digital innovations for ecological monitoring and precision management.

Regenerative viticulture is an emerging paradigm that blends principles from agroecology, functional biodiversity, and permaculture to support soil regeneration, carbon sequestration, and ecosystem resilience. A comprehensive review by O'Brien *et al.* (2025) illustrates that regenerative practices such as cover cropping, compost application, no-till soil management, and reduced synthetic inputs, can substantially improve vineyard ecological performance, including enhanced microbial diversity, reduced erosion, and improved water retention. However, the review also identifies a gap in long-term empirical studies assessing the impact of regenerative practices on grape yield and wine quality across diverse viticultural regions (O'Brien *et al*., 2025).

A noteworthy innovation within regenerative viticulture is the reintroduction of livestock, particularly sheep, into vineyard ecosystems. Research in New Zealand has shown that integrating sheep during the vine dormancy period can significantly reduce herbicide applications and mowing frequency, resulting in both environmental and economic benefits. Sheep provide a biological solution to under-vine weed management, reduce tractor passes, and contribute to nutrient cycling through manure deposition. Participating winegrowers reported savings of up to US$120 per hectare annually due to fewer herbicide treatments and mowing operations (Niles *et al*., 2018). Recent studies suggest that grazing can stimulate soil microbial enzyme activity, even if it does not directly increase microbial diversity, showing a decoupling between soil biological function and composition (Bansal *et al*., 2024).

Technological innovation will also shape future agrotechnical practices. Robotic systems designed to perform selective weeding, pruning, or pest scouting are becoming increasingly feasible and cost-effective. Precision viticulture tools, including drones, soil sensors, and remote imaging, enable fine-scale monitoring of ecological variables, facilitating targeted interventions that minimize environmental disturbance. Moreover, mobile apps and biodiversity databases are being trialed to support real-time species identification and ecosystem assessments, making ecological monitoring more accessible to growers (Luglio *et al*., 2024).

Policy instruments, such as the European Union's Common Agricultural Policy (CAP) reform (2023–2027), are now aligning with these trends, supporting the adoption of eco-schemes, biodiversity infrastructure, and nature-based solutions in viticulture. Integrating traditional ecological knowledge with innovations like regenerative livestock integration and precision agroecology offers a powerful path forward for sustainable vineyard systems.

In summary, the future of agrotechnical practices in viticulture is poised to shift toward multifunctional, ecosystem-based strategies. Combining regenerative principles with targeted innovations and supportive policy frameworks will be crucial to ensuring both vineyard productivity and ecological integrity in the coming decades.

CONCLUSIONS

1. Conventional vineyard management, still practiced on over 70% of global vineyard land, poses a substantial threat to biodiversity and long-term ecosystem health, with studies linking it to 30–40% declines in beneficial arthropod populations compared to organic or integrated systems.
2. Biodiversity is essential to maintaining ecosystem balance in vineyards, supporting natural pest control, pollination, and soil health. Maintaining a functional balance between pest species and natural enemies (e.g., parasitoids, predators) is more effective than total pest eradication and contributes to stable vineyard ecosystems.
3. Inter-row vegetation and ecological infrastructures (e.g., hedgerows, flower strips) increase species richness and functional diversity. Also, using cover crops, compost, and no-till management boost soil microbial activity, sequester carbon, and help reduce erosion. Reduced tillage preserves soil structure, maintains microbial activity, and supports beneficial entomofauna such as predatory mites and beetles.
4. Selective pesticide use and fewer chemical interventions support a stable pest–natural enemy equilibrium and reduce ecological disruption. The selective application of low-toxicity pesticides and integrated pest management help maintain a stable pest–natural enemy equilibrium.
5. Organic and integrated vineyard systems consistently show higher biodiversity than conventional ones, though the benefits vary depending on local climate, vineyard age, and landscape context. These findings highlight the importance of long-term, region-specific biodiversity monitoring.
6. Policy frameworks, particularly the European Union's CAP 2023–2027, are beginning to align with ecological goals, promoting the adoption of eco-schemes and biodiversity infrastructure. These

policies, when combined with traditional knowledge and modern technologies, support the transition to resilient, regenerative vineyard systems.
7. Technological advancements, including drones, soil sensors, remote imaging, and AI-based pest recognition systems, enable precision ecological monitoring, reducing unnecessary interventions and improving biodiversity outcomes; additionally, emerging tools like DNA metabarcoding and genetic identification allow high-resolution tracking of species composition and functional diversity, offering deeper insights into ecosystem health and pest–natural enemy dynamics.
8. The Târnave Vineyard stands out as a strong model for biodiversity preservation in viticulture, with 28.46% of its area dedicated to agroecological infrastructure and supporting a rich community of beneficial insects such as predatory mites, *Coleoptera*, and *Heteroptera*; these results highlight the effectiveness of regionally adapted, biodiversity-friendly practices in enhancing ecological balance and reducing the need for chemical inputs.
9. Future research should prioritize long-term, region-specific studies to better evaluate the ecological and agronomic impacts of agrotechnical practices, aiming to optimize both vineyard productivity and ecosystem stability through evidence-based, biodiversity-focused management.

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DISTRIBUTION OF THE VIRAL COMPLEX *GRAPEVINE PINOT GRIS VIRUS* AND *GRAPEVINE FLECK VIRUS* IN GRAPEVINE ON PHENOPHASES OF VEGETATION

DISTRIBUȚIA COMPLEXULUI VIRAL *GRAPEVINE PINOT GRIS VIRUS* ȘI *GRAPEVINE FLECK VIRUS* LA VIȚA-DE-VIE PE FENOFAZE DE VEGETAȚIE

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Abstract

Grapevine Pinot gris virus and Grapevine fleck virus are widely distributed in most vine growing countries of the world. In general, the viruses have an uneven and different distribution in the plant. The existence of viral complexes formed by two or more viruses raises problems regarding the choice of the most suitable testing period during the year, and the plant material collected for analysis. The sample should have a high virus concentration, so that the diagnosis has a high degree of reliability. When it is necessary to obtain virus-free plants, it is desirable to initiate the virus elimination process with tissues with a low virus concentration. The study of the distribution of the viruses that make up the viral complex in different plant organs, on vegetation phenophases, recommends for ELISA diagnosis the leaf blade and petiole in the phenophase before flowering, while the regeneration of healthy plants by applying viral elimination methods can be initiated from intensely regenerative apices during the active growth period of the plant.

Keywords: *Vitis, GPGV, GFkV, ELISA, detection*

Rezumat

Virusul Pinot gris și virusul fleck au o largă răspândire în majoritatea țărilor viticole ale lumii. În general, virusurile au o distribuție neuniformă și diferită în plantă. Existența complexelor virale alcătuite din două sau mai multe virusuri ridică probleme privind alegerea celei mai potrivite perioade de testare din timpul anului și a materialului vegetal prelevat pentru efectuarea analizelor. Proba ar trebui să aibă o concentrație mare de virus, astfel încât diagnosticul să aibă un grad mare de siguranță. Atunci când este necesară obținerea de plante libere de virusuri, devirozarea este de dorit să fie inițiată cu țesuturi cu o concentrație redusă de virusuri. Studiul distribuției virusurilor ce alcătuiesc complexul viral în diferite organe ale plantei, pe fenofaze de vegetație, recomandă pentru diagnosticul prin ELISA limbul foliar și pețiolul, în fenofaza înainte de înflorit, în timp ce regenerarea de plante sănătoase prin aplicarea metodelor de eliminare virală poate fi inițiată din apexuri intens regenerative în perioada de creștere activă a plantei.

Cuvinte cheie: *Vitis, GPGV, GFkV, ELISA, detecție*

INTRODUCTION

Molecular sequencing techniques NGS (Next Generation Sequencing) have led to the continuous identification of new viruses in grapevine. To date, 102 viruses infecting grapevine are known, classified in 44 genera and 21 families or taxonomically unclassified, identified in different *Vitis* germplasm collections worldwide (Fuchs, 2023; 2025). Viral infection produces physiological and structural changes, affecting: photosynthesis, gas exchange, chlorophyll content, carbon translocation, phloem transport, cellular metabolism (González *et al*., 1997; Bertamini et al., 2004; Endeshaw et al., 2014, Martínez-Lüscher, 2019; Kappagantu et al., 2020).

One of these viruses discovered by NGS is *Grapevine Pinot gris virus* (GPGV). The disease expressed by mottling, stunting and leaf deformation was first reported in Italy, Trento region, where it infected Pinot gris and also plants of Traminer and Pinot noir genotypes (Martelli, 2012).

At this time, the presence of GPGV has been reported in grapevine fruiting cultivars and rootstocks in many growing countries from Asia, Europe, North America, South America, as well as in three states from Australia (Saldarelli *et al*., 2017a); Constable *et al*., 2019; EPPO, 2022). In the presence of GPGV, losses in production quantity and quality may occur, affecting commercial production (Tarquini *et al*., 2021).

In Australia, GPGV has been found in a wide range of wine grapevine varieties, table grapevines and rootstocks, but the characteristic symptoms of grapevine leaf mottling and deformation (GLMD) caused by GPGV infection have not been reported (Giampetruzzi *et al*., 2012; Constable *et al.*, 2019). There has been some speculation that GPGV is associated with a growth symptom observed in spring in Australian table grapevine varieties, which includes delayed bud break, short internodes, stunting, and zig-zag shoots (Constable *et al*., 2019). The hybrid Tamnara (*V. vinifera* × *V. labrusca*) grown in South Korea has a reduced number of bunches and heavily necrotic grapes (Cho *et al*., 2013). Recently, GPGV has been shown to be present in plants grown from infected grapevine seeds (Zhang *et al*., 2022).

Furthermore, GPGV was detected in non-*Vitis* host plant around vineyards: *Silene latifolia*, *Chenopodium album*, *Asclepias syriaca*, *Rosa sp.*, *Rubus sp.*, *Fraxinus sp*. (Gualandri *et al.*, 2017; Demián *et al*., 2018). However, mechanical transmission to herbaceous plants was not observed (Malagni *et al*., 2016).

GPGV is currently considered a major pathogen of grapevines in Europe (Cieniewicz *et al*., 2020).

One study realized in Romania showed a GPGV infection incidence of 53.76% (107 positive samples out of 199, analysed by ELISA (Enzyme-Linked Immunosorbent Assay). Of these, the largest incidence was recorded in old varieties from germplasm collections (37.38%). As expected, the clones followed with a close percentage (32.71%). The GPGV presence in clonal selections (9.26%) confirmed once again the necessity to use a virus-free grapevine material in breeding programs. A lower percentage (9.35%) of GPGV-infected plants belonging to cultivars created after 1994 year is probably due to the fact that grapevine viruses are generally not seed-borne. The tests revealed the association of GPGV with GFkV (5 samples) and GLRaV-1+3 (2 samples). Three of the 5 plants diagnosed with GFkV + GPGV mixed infection had no symptoms. Also, in this study were analyzed 12 samples collected from 4 rootstock genotypes that were ELISA positive for GPGV (Guță and Buciumeanu, 2021a).

Grapevine fleck virus (GFkV) is another virus widespread in all grapevine growing countries Due to the latent symptoms in European cultivars and most American rootstocks, and often detection in viral complexes with viruses having an economic impact, it must be eliminated by sanitation procedures (Buciumeanu and Vișoiu, 2000; Martelli and Boudon-Padieu, 2006; *Bota et al.*, 2014; Guță *et al*., 2014; Crnogorac *et al*., 2021). Although many grapevine varieties and rootstocks infected with only GFkV are asymptomatic, infection may be associated with graft incompatibility (https://www.wineaustralia.com/getmedia/b1e5038d-cc30-48d8-b3c2-25844af8e795/201107-Grapevine-fleck-and-associated-viruses?ext=.pdf).

The evaluation of the phytosanitary status in five production vineyards with Romanian grapevine varieties in the Muntenia and Oltenia Hills Viticultural Region proved the presence of simple and mixed viral infections in different percentages, without a positive correlation with the symptoms presence. The identification of GFkV was noted in all plantations and, also, in all studied varieties, recording maximum values of 16.67% GFKV in simple infections, 4.76% GFkV + GLRaV-1+3, 6.98% GFkV + (GFLV +ArMV), and 2.32% GFkV + GRLaV-1+3 + (GFLV+ArMV) (Buciumeanu *et al*., 2015).

The presence of GFkV reduces rhizogenesis and graft take; the effects depend on the virulence of the viral isolate. GFkV alone or in the leafroll presence induces a reduction in graft take and affects plant vigor in nurseries. GFkV infection associated with vein necrosis and vein mosaic reduced the amount of cut wood in rootstocks 420 A and Kober 5BB by 52% and 37%,

respectively, but it did not affect growth in Teleki 5A. There is no direct evidence of the GFkV influence on the quantity and quality of grape production (Walter and Martelli, 1996).

Although certification regulations require the absence of GFkV only in rootstocks, the virus can easily pass from scion to rootstock through the phloem and can negatively affect grape yield and quality, usually in combination with other viruses. So, a GFkV-infected scion, clone or variety, can not enter in a certification program (Teray, 1990; Kovács *et al*., 2001; Komar et al., 2007; Cretazzo *et al*., 2010). Simultaneous infection with GFkV and leafroll produces ajinashika disease in the Japanese grapevine variety Koshu, characterized by a reduction in the amount of sugar in the fruit (Terai and Yano, 1980; Walter and Martelli, 1996). Following field experiments, it is considered that, in general, grapevines on their own roots are more tolerant to viral infections (Garau *et al*., 1997).

The widespread of GPGV poses a challenge to the scientific community and grapevine growers, requiring increased viral disease management measures, given that this virus is not a regulated pest (Guță and Buciumeanu, 2021b). The widespread presence of GPGV both in simple and mixed infections has determined the need to study viral elimination, in order to launch into culture healthy propagation material.

Assessment of viral concentration in the grapevine plant is necessary because virus distribution in young shoot tissues is uneven and viral concentration decreases significantly in apical meristems, where cells are continuously dividing (Lai and Lai, 2019). These plant fragments constitute the source of biological material used in the application of methods of viral elimination and new healthy plants regeneration through tissue cultures.

The paper deals with the distribution of GPGV and GFkV in grapevine plants having mixed infection with these viruses, in different phenophases, with the aim to identify the tissues with high virus concentration (most suitable tissue for virus detection), and, also, to identify the tissues with the minimum viral concentration, to be used in virus elimination procedures.

MATERIALS AND MEHODS

Different organs and tissues of *Vitis vinifera* L., **Negru mare** cv. double infected with GPGV + GFkV were analysed by ELISA (Clark and Adams, 1977) with commercial reagents (Bioreba, Switzerland), following the manufacturer's instructions, during the phenophases of grapevine growth and development. The grafted plants showing GPGV infection symptoms were grown in *ex situ* grapevine collection belonging to INCDBH Ștefănești-Argeș. Two grapevine plants were analysed before flowering (05/16/2023), after flowering (06/24/2023), fall (09/12/2023), and dormancy phenophases. From each plant, 2 shoots were analysed, from which were collected: leaf (leaf blade and petiole) located at the base, middle and top of the shoot; lateral shoot; apex; rachis; berry skin; seed; cane after leave fall. The plant material was grinded and homogenised with extraction buffer at 1/10 ratio (m/v). At completion of reactions, optical densities (OD) were measured with double filter at 405/492 nm, using a Chromate microplate reader (Awareness Technology, Inc., Florida, USA). The sample was considered positive when its OD 405/492 nm value was at least three times the negative control value.

RESULTS AND DISCUSSION

The most important strategy to prevent the viruses spread is the use of healthy propagation material, selected through periodic monitoring followed by the elimination of diseased plants, or obtained by virus elimination methods. It is known that the virus titre is variable during the vegetation period in different organs and tissues of the plant. Such studies are useful both to establish the optimal period of virus diagnosis and to identify the biological material suitable for virus elimination processes initiation.

One study regarding *Grapevine fanleaf virus* (GFLV) distribution on vegetation phenophases, in different organs of the plant showed that young leaves had a high virus concentration during the vegetation period, while mature leaves, tendrils and inflorescences at the beginning of the vegetation period only. The rachis, mature leaves and tendrils had a significantly decreased titre during the vegetation period. ELISA results were confirmed by molecular analyses. In addition, the viral titre was influenced by the genotype and the presence of other viruses in mixed infections (Krebelj *et al*., 2015).

Similar studies performed for *Grapevine leafroll-associated viruses 1, 2, 3, 4, 2RG* (GLRaV -1, GLRaV-2, GLRaV-3, GLRaV-4, GLRaV-2RG) revealed that virus titre was the lowest early in the growing season (April/May) and the viral load was different between GLRaVs serotypes (Osman *et al*., 2018).

The study of the GFLV, GLRaV-3 and GFkV distribution showed that during the growing season, all samples were positive and no significant differences were observed between ELISA values (OD 405 nm) of leaves along a shoot (bottom, middle, and top). However, some differences were recorded between shoots of the same plant. During the warmest period of the year, the viral concentration was fluctuating along the shoots in the case of GFLV or GFkV, but the GLRaV-3 concentration at the base of the plant was higher for each of the three analysed shoots. Also, ELISA values were lower in the warm period as compared to the previous one. The viruses were detected during dormancy both in the canes and in the buds, without significant differences between ELISA values along the shoots (Buciumeanu and Guță, 2012).

The discovery of a new virus, with a wide spread in the grapevine -growing countries of the world (Saldarelli *et al.*, 2015), GPGV, determined the initiation of several studies including the efficiency of diagnosis by ELISA. As a result, several analyses were carried out during the entire vegetation period, in different grapevine organs, on Negru mare cv. maintained in vegetation pots in greenhouse, naturally infected with the viral complex GFkV + GPGV. Considering the apex as a possible regeneration organ of new virus-free plants, in the pre-flowering phenophase the titre of both viruses was below the detection limit of ELISA method. After flowering, GPGV concentration decreased, such that in 3 out of 5 ELISA values were below the detection limit, and for GFkV only 1 out of 5 samples was ELISA negative. The transport of assimilates in the plant determined a higher viral concentration of both viruses in apices in the ripening phenophase (Guță *et al*., 2022).

In order to deepen these observations, two grapevine plants of the same **Negru mare** cv. infected with viral complex GPGV + GFkV stored in the *ex situ* collection were analysed along of the year, both in the vegetation period and in dormancy.

GPGV+GFkV have been detected before flowering period in all analysed tissues, but registering some differences between plants, lower leaf blade of plant 2 giving negative results for GPGV at that time. The positive results were represented by high OD values (Table 1).

Table 1. ELISA results on 2 grapevine plants belonging to Negru mare cv. infected with GFkV + GPGV viral complex, in the phenophase of vegetation before flowering / Tabelul 1. Rezultate ELISA la 2 plante aparținând genotipului **Negru mare** infectat cu complexul viral GFkV + GPGV, în fenofaza înainte de înflorit

Sampling location and biological material type		**Shoot 1**				**Shoot 2**			
		GFKV		**GPGV**		**GFKV**		**GPGV**	
		OD	**Result**	**OD**	**Result**	**OD**	**Result**	**OD**	**Result**
Plant 1									
Lower leaf	Leaf blade	2.777	+	0.298	+	3.303	+	0.352	+
	Petiole	1.948	+	3.285	+	2.276	+	2.965	+
Leaf, node 5 below inflorescence	Leaf blade	3.238	+	3.062	+	2.995	+	3.573	+
	Petiole	1.017	+	3.549	+	1.676	+	3.555	+
Apex		0.454	+	2.022	+	nt.	nt.	nt.	nt.
Plant 2									
Lower leaf	Leaf blade	1.936	+	0.123	-	3.549	+	0.236	-

	Petiole	1.318	+	3.572	+	2.185	+	3.495	+
Leaf, node 5 above inflorescence	Leaf blade	3.403	+	3.528	+	3.011	+	3.260	+
	Petiole	0.439	+	3.381	+	0.621	+	3.5723	+
Apex		1.614	+	3.393	+	nt.	nt.	nt.	nt.
Tendril		nt.	nt.	nt.	nt.	0.821	+	3.570	+

Note: Plants cultivated *ex situ*, from different organs located in different zones of the trunk, in the phenophase of vegetation before flowering (05/16/2023). Temperature at the sampling time = 30°C. OD = optical density at 405/492 nm. The test result was expressed as positive (+) = presence of virus, negative (-) = not identified by ELISA, and nt. = not tested / Notă: Plante cultivate *ex situ*, din diferite organe situate în zone diferite ale butucului, în fenofaza de vegetație înainte de înflorit (05/16/2023). Temperatura în momentul prelevării = 30°C. DO = densitatea optică la 405/492 nm. Rezultatul testului a fost exprimat în pozitiv (+) = prezența virusului, negativ (-) = neidentificat prin ELISA și nt. = netestat

After flowering, all tissue were positive for GFkV, while the GPGV have been detected in the petiole of the leaf located to the upper part of the shoot, rachis and berry, while the result of the analysis from apex was inconclusive (Table 2).

In all analysed tissues, ELISA-positive results have been obtained in the ripening phenophase for GFkV. In the case of GPGV, rachis, berry skin in shoot 1 of plant 1 were positive, and the result of the analysis from apex was still uncertain (Table 3).

GPGV+GFkV viral complex have been detected in the dormancy when samples taken from three zones of the cane, registering an exception on cane 2 of plant 2, at which at the base and the top, GPGV has not been detected (Table 4).

Analyses performed from different tissues during the vegetation phenophases showed that GPGV poses problems regarding the sampling period and the type of tissue suitable for ELISA detection. It seems that before flowering the leaf blade, but especially the petiole showed a high viral concentration which decreased in the next phenophases.

Also, Saldarelli *et al.* (2017b) showed that GPGV concentration in cv Glera decreased in both symptomatic and symptomless vines with the progress of the vegetative season.

The most suitable sampling period of biological material necessary for culture initiation for GPGV-elimination proved to be the post- flowering phenophase, when ELISA values were lower in most zones of plant, as compared with pre-flowering and ripening phenophases. In the same time, higher ELISA values for GFkV have been registered in all phenophases.

Table 2. ELISA results on 2 plants belonging to Negru mare cv. infected with GFkV + GPGV viral complex, in the vegetation phenophase after flowering / Tabelul 2. Rezultate ELISA la 2 plante aparținând genotipului **Negru mare** infectat cu complexul viral GFkV + GPGV, în fenofaza de vegetație după înflorit

Sampling location and biological material type		**Shoot 1**				**Shoot 2**			
		GFKV		**GPGV**		**GFKV**		**GPGV**	
		OD	**Result**	**OD**	**Result**	**OD**	**Result**	**OD**	**Result**
Plant 1									
Lower leaf	Leaf blade	3.031	+	0.148	-	2.636	+	0.166	-
	Petiole	2.569	+	0.225	-	1.798	+	0.290	-
Leaf, node 11	Leaf blade	2.980	+	0.128	-	2.360	+	0.129	-
	Petiole	2.439	+	2.787	+	2.111	+	2.283	+
Lateral shoot, node 15 (whole)		0.660	+	0.625	+	nt.	nt.	nt.	nt.
Tendril, node 10		2.337	+	0.817	+	nt.	nt.	nt.	nt.
Apex		nt.	nt.	nt.	nt.	1.516	+	0.212	-
Rachis		nt.	nt.	nt.	nt.	0.782	+	2.396	+
Berry		nt.	nt.	nt.	nt.	2.297	+	1.717	+
Plant 2									
Lower leaf	Leaf blade	2.286	+	0.152	-	2.494	+	0.136	-
	Petiole	3.536	+	0.298	-	3.327	+	0.273	-
Leaf, node 9	Leaf blade	1.567	+	0.133	-	nt.	nt.	nt.	nt.
	Petiole	1.774	+	1.225	+	nt.	nt.	nt.	nt.

Apex		1.816	+	0.875	+	nt.	nt.	nt.	nt.
Tendril, node 8		3.493	+	1.214	+	nt.	nt.	nt.	nt.
Rachis		nt.	nt.	nt.	nt.	0.854	+	2.604	+
Berry		nt.	nt.	nt.	nt.	3.048	+	1.030	+

Note: Plants cultivated *ex situ*, from different organs located in different zones of the trunk, in the vegetation phenophase after flowering (06/24/2023). Temperature at the sampling time = 25°C. OD = optical density at 405/492 nm. The test result was expressed as positive (+) = presence of virus, negative (-) = not identified by ELISA, and nt. = not tested / Notă: Plante cultivate *ex situ*, din diferite organe situate în zone diferite ale butucului, în fenofaza de vegetație după înflorit (06/24/2023). Temperatura în momentul prelevării = 25°C. DO = densitatea optică la 405/492 nm. Rezultatul testului a fost exprimat în pozitiv (+) = prezența virusului, negativ (-) = neidentificat prin ELISA și nt. = netestat.

*Table 3. ELISA results on 2 plants belonging to **Negru mare** cv. infected with GFkV + GPGV viral complex, in the ripening phenophase / Tabelul 3. Rezultate ELISA la 2 plante aparținând genotipului **Negru mare** infectat cu complexul viral GFkV + GPGV, în fenofaza de pârgă*

Sampling location and biological material type		**Shoot 1**				**Shoot 2**			
		GFKV		**GPGV**		**GFKV**		**GPGV**	
		OD	**Result**	**OD**	**Result**	**OD**	**Result**	**OD**	**Result**
Plant 1									
Lower leaf	Leaf blade	1.298	+	0.098	-	1.455	+	0.101	-
	Petiole	0.770	+	0.114	-	0.616	+	0.105	-
Leaf, node 7	Leaf blade	1.885	+	0.104	-	3.196	+	0.100	-
	Petiole	1.088	+	0.102	-	0.545	+	0.163	-
Apex		2.398	+	1.082	+	2.761	+	3.480	+
Rachis		nt.	nt.	nt.	nt.	3.490	+	1.020	+
Skin		nt.	nt.	nt.	nt.	3.559	+	3.562	+
Seed		nt.	nt.	nt.	nt.	0.275	+	3.569	+
Plant 2									
Lower leaf	Leaf blade	2.769	+	0.125	-	2.394	+	0.100	-
	Petiole	0.703	+	0.094	-	2.106	+	0.097	-
Leaf, node 7	Leaf blade	1.396	+	0.103	-	2.657	+	0.099	-
	Petiole	0.519	+	0.099	-	0.935	+	0.097	-
Apex		2.068	+	0.139	-	3.082	+	0.189	-

Note: Plants cultivated *ex situ*, from different organs located in different zones of the trunk, in the ripening phenophase (09/12/2023). Temperature at the sampling time = 25°C. OD = optical density at 405/492 nm. The test result was expressed as positive (+) = presence of virus, negative (-) = not identified by ELISA, and nt. = not tested / Notă: Plante cultivate *ex situ*, din diferite organe situate în zone diferite ale butucului, în fenofaza de pârgă (09/12/2023). Temperatura în momentul prelevării = 25°C. DO = densitatea optică la 405/492 nm. Rezultatul testului a fost exprimat în pozitiv (+) = prezența virusului, negativ (-) = neidentificat prin ELISA și nt. = netestat.

Table 4. ELISA results in 2 plants belonging to Negru mare cv.infected with GFkV + GPGV viral complex, during dormancy / Tabelul 4. Rezultate ELISA la 2 plante aparținând genotipului **Negru mare** infectat cu complexul viral GFkV + GPGV, în perioada de repaus vegetativ

Sampling location and biological material type	**Cane 1**				**Cane 2**			
	GFKV		**GPGV**		**GFKV**		**GPGV**	
	OD	**Result**	**OD**	**Result**	**OD**	**Result**	**OD**	**Result**
Plant 1								
Base	2.470	+	0.469	+	3.547	+	1.779	+
Middle	3.567	+	1.369	+	3.560	+	1.986	+
Top	3.540	+	1.778	+	3.572	+	2.727	+
Plant 2								
Base	3.540	+	0.453	+	3.567	+	0.113	-
Middle	3.554	+	0.648	+	3.537	+	0.393	+
Top	3.539	+	0.796	+	3.538	+	0.257	-

Note: Plants cultivated *ex situ*, from different zones of the canes 1 and 2 (base, middle, top), during dormancy. The length of the analysed canes was 14 internodes on plant 1, and 12 internodes on plant 2. OD = optical density at 405/492 nm. The test result was expressed as positive (+) = presence of virus, and negative (-) = not identified by ELISA. / Notă: Plante cultivate ex situ, din diferite zone ale coardelor 1 și 2 (bază, mijloc, vârf) în perioada de repaus vegetativ. Lungimea coardelor analizate a fost de 14 internodii la planta 1 și 12 internodii la planta 2. DO = densitatea optică la 405/492 nm. Rezultatul testului a fost exprimat în pozitiv (+) = prezența virusului și negativ (-) = neidentificat prin ELISA.

The intensively regenerative apex is considered the appropriate organ for the initiation of tissue cultures both from the point of view of the tissue's juvenility and from the perspective of the fact that, depending on the type of virus and its ascent in the plant during the phenophases of vegetation, the viral concentration is at a minimum level.

For GPGV infection, the results led to the hypothesis that the collecting period of biological material necessary for the initiation of virus-elimination cultures can be from the middle of August (ripening phenophase), when the most ELISA values were below the detection limit in all zones of the plant, including the apices.

Comparison the ELISA values obtained from grapevine plants infected with GPGV + GFkV viral complex, located in the G0 depository greenhouse in vegetation pots (Guță *et al*., 2022), with those from plants belonging to the same genotype grown on soil in the *ex situ* collection regarding the viral concentration of GPGV in the intensively regenerative apices, highlighted the following:

- in the pre-flowering phenophase (April - in protected space, May - in the field) the apices are ELISA negative in the greenhouse, while in the field the ELISA values were high (2.022; 3.393);

- in the post-flowering/berry growth phenophase (June in both locations), in greenhouse plants, three out of five apex samples were ELISA negative, but even in the positive ones the viral concentration was low (0.118; 0.113), while in field all samples were ELISA negative;

- in the ripening phenophase (August in the greenhouse, September in the field) the apices registered a high viral concentration in both locations (1.357; 0.721; 1.082; 3.480), except for 2 values on one plant, that were ELISA negative.

The diagnosis by ELISA also showed that GFkV concentration in the intensely regenerative apices was high in all vegetation phenophases.

These results reinforce the necessity to use virus elimination methods (*in vitro* chemotherapy, electrotherapy, thermotherapy and/or combined methods) to create a gap between virus multiplication and plant growth that allow the regeneration of new virus-free plants.

CONCLUSIONS

1. The results confirmed the uneven distribution of viruses in the grapevine plant throughout the year. In order to obtain reliable results, it is recommended to choose the most appropriate tissue type and sampling period according to the virus/viral complex and the purpose pursued (ELISA diagnosis or virus elimination).
2. The detection of GPGV+GFkV viral complex can be realized by ELISA both in the vegetation and dormancy. The highest ELISA values have been registered before flowering phenophase for both viruses in leaf blade and petiole.
3. Since GPGV and GFkV concentrations are fluctuating in apex, the initiation of tissue cultures to eliminate the viral complex can be done in different grapevine during the active growth period of the plant.

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THE ASSESSMENT OF THE DAMAGE CAUSED BY HAIL TO SOME PLUM CULTIVARS IN BISTRITA AREA

EVALUAREA PAGUBELOR PRODUSE DE GRINDINĂ ASUPRA UNOR SOIURI DE PRUN IN ZONA BISTRIȚA

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Abstract
*The European plum (Prunus domestica L.) remains the leader of the fruit tree species cultivated in Romania in terms of cultivated area and total fruit production. In order to obtain profitable yields, however, the impact of abiotic and biotic stress factors, which can cause significant damage to the trees, must also be considered. The climate change experienced globally, and in Romania as well, contributes to the increasingly frequent occurrence of extreme weather events that can irreparably affect crops. Hail is one of the meteorological phenomena with negative effects in fruit growing, sometimes causing significant economic losses. Such an unfortunate event occurred on June 4, 2024, at the FRDS Bistrița, with a torrential rain accompanied by strong hail that caused considerable damage to the plum crop. To assess the impact of the hail, fruit samples from 26 plum cultivars grwun in a field trial were collected and analyzed in terms of the mechanical effects on the fruits. Thus, four damage classes were established: (1) no lesions, (2) superficial lesions, (3) open lesions, and (4) 31% to 60% of pulp destroyed. The cultivars **Elena**, **Gras ameliorat** and **Agent** are noteworthy, which had fruit without lesions in a proportion of 28.1%, 21.3% and 16.9%, respectively. In contrast, in the cultivars **Diana**, **Joganta**, **Jofela**, **Topend plus**, **Anna Spath**, **Delia**, **Zamfira**, **Iulia**, **Flora**, **Jubileu 50**, **Stanley** and **Minerva**, no intact fruits were identified. The results highlight important differences in the behavior of the varieties both in terms of tolerance to mechanical hail damage and susceptibility to Monilinia spp infections, underlining the importance of genetic selection in the context of intensifying climatic stress.*

Keywords: *climatic events, damages, hail, plum, abiotic stress resistance*

Rezumat
*Prunul european (Prunus domestica L.) rămâne principala specie pomicolă cultivată în România, atât din perspectiva suprafeței ocupate, cât și a producției totale de fructe. Pentru asigurarea unei producții profitabile, este esențială luarea în considerare a impactului factorilor de stres abiotic și biotic, care pot genera pierderi semnificative în plantațiile pomicole. În contextul schimbărilor climatice resimțite la nivel global și național, se observă o frecvență crescută a fenomenelor meteorologice extreme, cu efecte potențial devastatoare asupra culturilor. Grindina reprezintă unul dintre fenomenele meteorologice cu efecte negative în pomicultură, uneori cauzând pierderi economice semnificative. Un astfel de eveniment a avut loc în data de 4 iunie 2024 la SCDP Bistrița, respectiv o ploaie torențială însoțită de grindină puternică care a provocat pagube însemnate la specia prun. Pentru a evalua impactul grindinei, probe de fructe de la 26 de soiuri de prun dintr-o cultură compatrativă au fost prelevate și analizate sub raportul efectelor mecanice asupra fructelor. Astfel, s-au stabilit patru clase de dăunare: (1) fără leziuni, (2) cu leziuni superficiale, (3) cu leziuni deschise și (4) cu pulpa distrusă în proporție de 31-60%. Rezultatele au evidențiat soiurile **Elena**, **Gras ameliorat** și **Agent**, care au avut fructe fără leziuni în proporție de 28,1%, 21,3%, respectiv 16,9%. La polul opus s-au situat soiurile **Diana**, **Joganta**, **Jofela**, **Topend plus**, **Anna Spath**, **Delia**, **Zamfira**, **Iulia**, **Flora**, **Jubileu 50**, **Stanley** și **Minerva**, la care nu s-au identificat fructe intacte. Rezultatele relevă diferențe importante în comportamentul soiurilor atât în ceea ce privește toleranța la loviturile mecanice ale grindinei, cât și susceptibilitatea la infecțiile cu Monilinia spp,. subliniind importanța selecției genetice în contextul intensificării stresului climatic.*

Cuvinte cheie: *accident climatic, daune, grindină, prun, rezistența la stres abiotic*

INTRODUCTION

Prunus domestica L., commonly known as the European plum, is globally valued for its delicious and nutritious fruits. Rich in essential vitamins and antioxidants, plums are recommended

for a healthy diet, with a suggested daily consumption of 2-4 fruits (Wills *et al*., 1983; Gil *et al*., 2002). According to FAOSTAT data (2025), China leads the world in plum production, with an impressive harvest of 6,888,894 tons. Romania holds a significant position in plum cultivation, ranking second globally with a production of 645,090 tons. The popularity of this fruit species is increasing, supported by the global presence of over 2,000 plum cultivars (Sottile *et al*., 2022).

Hail represents an extreme weather phenomenon with a significant impact on fruit crops. This is not only affects the physical integrity of the trees but also has a considerable impact on the quality and quantity of the fruits in the current and the following year (Bal *et al*., 2014). Considering the significant potential for damage and the considerable economic losses it can cause, hail is a particular concern in regions frequently exposed to this phenomenon (Changnon, 1999). Furthermore, climate change, along with the increased intensity and frequency of extreme weather events, has increased the susceptibility of plum crops to such incidents, highlighting the importance of an in-depth analysis of the effects of hail on them (Milošević & Milošević, 2023).

After a hailstorm, plum trees can experience a wide range of damage, from superficial injuries to leaves and fruits to severe breakage of branches and significant harm to the fruit. Mechanical damage, such as broken branches and defoliation, reduces the trees' ability to carry out photosynthesis, which negatively affects their growth and development (Gardiner, et al., 2016). Hail also induces bruising and puncturing of the fruits, increasing their susceptibility to infections and pest attacks. Moreover, the incidence of fungal and bacterial infections may rise, further contributing to a reduction in overall yield (Prusky, 2011).

Comparative studies evaluating the impact of hail on plum trees highlight that the degree of damage depends on several factors, including the size and intensity of the hailstones, the development stage of the fruit at the time of impact, and the age of the trees (Khan, 2022). Young trees are generally more vulnerable to hail damage than mature ones, and fruits at an advanced stage of development may suffer more severe damage than those at an earlier stage. These variables determine the variability in the intensity and nature of the damage observed under different climatic and geographical conditions (Zwiers *et al*., 2013).

Another important aspect of studying the impact of hail on plum orchards is the analysis of the resilience of different plum cultivars to this phenomenon (Abasi *et al*., 2025). Studies have demonstrated that certain plum cultivars are more resistant to hail damage due to their physiological and structural characteristics, such as the skin thickness, the type of branches, and the crown architecture (Seethapathy *et al*., 2022). This variable resilience provide valuable information for farmers and researchers, enabling them to select varieties that are better suited to the climatic conditions of regions frequently exposed to hail (Benkeblia *et al*., 2018).

Hail also plays a significant role in promoting infections caused by *Monilinia* spp. on the European plum (*Prunus domestica* L.) fruits by causing mechanical injuries that promote pathogen entry. *Monilinia laxa*, *M. fructigena*, and *M. fructicola* are the major fungal pathogens of stone fruits, including of plums (Landi *et al*., 2016), and are responsible for brown rot, a disease that can lead to considerable yield losses both in the field and during storage.

Studies have shown that the virulence of *Monilinia* species is influenced by both the host and environmental conditions. However, the presence of wounds remains a decisive factor for successful infection. For instance, a research conducted in Serbia demonstrated that three *Monilinia* species can infect plum fruits when wounds are present, with *M. laxa* identified as particularly virulent on this host (Vasić & Vico, 2013).

Infections by *Monilinia* species are closely associated with the presence of wounds on fruit surfaces, which may result from mechanical damage such as hail, insect activity, or improper handling during harvest (Rungjindamai *et al*., 2014). These lesions serves as entry points for fungal conidia, which under favorable humidity and temperature conditions, can rapidly colonize the fruit tissues, leading to the typical symptoms of brown rot (Holb *et al*., 2016).

A thorough understanding of the effects of hail on plum trees and the identification of

appropriate prevention and management strategies are essential for protecting crops and ensuring sustainable agricultural production (Chattopadhyay *et al*., 2017). Future research should focus on developing innovative tree protection technologies and identifying the most resistant plum varieties to reduce vulnerability to extreme weather events (Sattar *et al*., 2021).

This study aimed to assess the impact of a severe hailstorm on a large assortment of plum cultivars grown within a young field trial, focusing on the physical damage caused by hailstones, as well as the cultivars' response to *Monilinia* spp. infections on immature fruits.

MATERIALS AND METHODS

The experiment was conducted in a young comparative plum orchard established on 2020 at the Fruit Research and Development Station (FRDS) Bistrița, by planting 26 plum cultivars (**Diana**, **French Improved**, **Joganta**, **Jofela**, **Tophit**, **Topper**, **Topend Plus**, **Top Five**, **Jojo**, **Anna Späth**, **Matilda**, **Elena**, **Delia**, **Gras ameliorat, Zamfira**, **Iulia**, **Agent**, **Flora**, **Andreea**, **Jubileu 50**, **Stanley**, **Ivan**, **Minerva**, **Doina**, **Centenar**, and **Carpatin**). The experimental plot is located in the hilly region of Bistrița, Romania, under a climate area that is classified as moderately temperate-continental, with warm summers and cold winters, and an uneven distribution of precipitation throughout the year, often exceeding the national average (Minoiu & Bilegan, 1990). Weather data are recorded through an Adcon Telemetry weather station, which is part of the FRDS Bistrița equipment. The monitored parameters include: temperature, wind speed, atmospheric humidity, and precipitation.

In early of June 2024, a significant portion of Bistrița-Năsăud County (Figure 1) was severely impacted by an extreme weather event. Torrential rainfall, accompanied by hailstones measuring approximately 1.5–2 cm in diameter, resulted in extensive damage, particularly to fruit orchards, but also affected other key economic sectors across the region. For about 30 minutes, the trees were struck by ice crystals from the north-south direction, from which the fruit samples were also collected.

Fig 1. Aspects of hailstones at FRDS Bistrița (June, 2024) / Fig. 1. Aspecte ale grindinei de la SCDP Bistrița (iunie, 2024)

In order to quantify the mechanical impact of hail on fruits, a few days after the hailstorm, samples were collected and analyzed from the 26 plum cultivars. A total of 100 fruits were randomly sampled from each cultivar and subjected to evaluation. At that time, the development stage of the fruits for the majority of cultivars was 75, according to the BBCH scale for stone fruits. For a detailed and standardized analysis of the mechanical injuries caused by hail on plum fruits, a scale structured into four damage classes was used (Table 1). The intensity of the damage was

calculated according to the fromula:

$$I= (n_1 \times i_1 + \ldots\ldots + n_4 \times i_4) / N$$

Where: n_{1-4} is the number of organs affected in a class; $I_{1\ldots\ldots4}$ is the upper limit of the intensity percentage assigned to the respective class and N is the total number of affected organs.

Table 1. Types of lesions and damage classes on plum fruits due to hail / Tipuri de leziuni și clase de daune la fructele de prun cauzate de grindină

Damage classes	Types of injuries on fruits
1	No lesions
2	Superficial lesions (less than 5% of the fruit was damaged)
3	Open lesions (less than 30% of the fruit was damaged)
4	31-60% of the pulp destroyed

The damage degree (DD) was calculated using the formula:

$$DD=I \times F/100$$

Where: DD= damage degree; I=intensity; F=frecquency of the sympthoms

As a consequence of the injuries caused by hail, despite additional phytosanitary treatments, *Monilinia* spp. infections were subsequently reported on the immature fruits of the plum cultivars in 2024. Therefore, the investigation was extended to evaluate the progression of the disease under natural conditions and identify cultivar-specific responses. Thus, the frequency of infections was determined on the fruits as a percentage of the total fruits. The observations were made 14 days after the meteorological event that occurred on early June of 2024. It shoul noted that the experimental plot was treated against brown rot two times in the period between the hail and the time of the observations. The first treatment was carried out 24 hours after the hailstorm using a solution of copper oxychloride at a dose of 500g/ha (in 1000L of water). The second treatment was carried out 7 days after the first and the substances used were copper from copper hydroxide 200g/ha and cypronidil 225g/ha.

RESULTS AND DISCUSSION

Effects of the hailstorm: direct damage

The classification of 26 plum cultivars based on the severity of hail-induced lesions, revealed the existence of differences in varietal susceptibility to this type of abiotic stress (Table 2). This variability reflects the diversity of phenotypic responses to mechanical lesions, suggesting the presence of distinct genetic traits associated with resistance to the physical impact of hailstones.

The first damage class comprises fruits with no visible lesion, with four cultivars demonstrating a notable proportion of undamaged fruits: **Elena** (28.1%), **Gras ameliorat** (21.3%), **Agent** (16.9%), and **Centenar** (13.3%). These results indicate a high degree of mechanical tolerance, likely attributable to the firmness of the pulp as well as the thickness or elasticity of the epidermis. These cultivars may serve as valuable genetic resources in breeding programs aimed at developing valuable genotypes in terms of some environmental resilience, such as resistance to hailstorms. In contrast, a considerable number of cultivars (**Diana**, **Joganta**, **Jofela**, **Topend Plus**, **Anna Spath**, **Delia**, **Zamfira**, **Iulia**, **Flora**, **Jubileu 50**, **Stanley**, and **Minerva**) exhibited complete fruit damage following the hailstorm, with a 100% frequency of damaged fruits, thereby reflecting a pronounced susceptibility to this type of mechanical stress.

The second damage class includes fruits exhibiting minor superficial lesions, on less than 5% of the fruit, predominantly affecting the epidermis without penetrating into the pulp. In this category, the degree of fruits damage ranged from 2.8% (**Minerva**) to 42.6% (**Gras ameliorat**). Based on the percentage of affected fruits, the cultivars were grouped as follows: lesions under

10%: **Jofela**, **Topend Plus**, **Anna Spath**, **Elena**, **Delia**, **Iulia**, **Jubileu 50**, and **Carpatin**; lesions between 11-20%: **Diana**, **Joganta**, **Topper**, **Jojo**, **Zamfira**, **Flora**, and **Stanley**; lesions between 21-30%: **Tophit**, **Topfive**, **Matilda**, **Agent**, **Andreea**, and **Doina**; lesions more then 30%: **French Improved** and **Centenar**. This distribution indicates that, although some cultivars sustained limited deep tissue damage, superficial injuries were still prevalent, reflecting widespread exposure to the adverse meteorological event.

The third damage class includes fruits exhibiting open lesions that often extend beyond the epidermis and into the pulp tissue. Frequency of damage in this category of severity ranged from 36.1% (**Gras ameliorat**) to 98.2% (**Iulia**). The distribution of these values highlights substantial inter-varietal differences in fruit resistance to hailstones.

The final and most severe damage class includes fruits that had 31% to 60% of the pulp damaged**.** Only 8 of the 26 cultivars had lesions of this severity, and the frequency of fruits presenting such a high damage was ranging from 1.5% (**Doina**) to 12.9% (**Carpatin**).

Table 2. The distribution of diffrent lession type for each cultivar and hail damage frequency on fruits / Distribuția diferitelor tipuri de leziuni pentru fiecare soi și frecvența daunelor provocate de grindină la fructe

Cultivar	Hail Damage Classes				Total frequency of damaged fruits (%)
	Class 1 No Damage	Class 2 Superficial lesions	Class 3 Open lesions	Class 4 31-60% of the flesh destroyed	
Diana	0	15.3	78.4	6.3	100
French Improved	11.2	37.0	51.8	0	88.8
Joganta	0	18.4	78.3	3.3	100
Jofela	0	6.2	86.1	7.7	100
Tophit	12.0	22.0	66.0	0	88
Topper	9.7	17.7	72.6	0	90.3
Topend plus	0	5.0	95.0	0	100
Top five	1.8	23.2	75.0	0	98.2
Jojo	1.6	14.5	83.9	0	98.4
Anna Spath	0	3.2	96.8	0	100
Matilda	1.6	21.6	76.8	0	98.4
Elena	28.1	9.4	62.5	0	**71.9**
Delia	0	7.5	92.5	0	100
Gras ameliorat	21.3	42.6	36.1	0	**78.7**
Zamfira	0	18.3	81.7	0	100
Iulia	0	1.8	98.2	0	100
Agent	16.9	27.7	55.4	0	**83.1**
Flora	0	12.1	86.2	1.7	100
Andreea	9.3	21.5	61.5	7.7	90.7
Jubileu 50	0	3.4	91.6	5.0	100
Stanley	0	15.3	84.7	0	100
Ivan	1.7	8.3	90.0	0	98.3
Minerva	0	2.8	97.2	0	100
Doina	1.5	21.5	75.5	1.5	98.5
Centenar	13.3	36.7	50.0	0	86.7
Carpatin	1.6	4.8	80.7	12.9	98.4

The damage degree calculated based on the intensity and frequency of the damage reflect the differences between the cultivars in regard to the resistance to hail wounds (Fig. 2). The least affected cultivar was **Gras ameliorat** with 12.92%DD, while at the opposite pole the most affected cultivars were: **Carpatin**, **Jofela** and **Jubileu 50** with more than 30%DD. Centenar and

French improved were cultivars that recorded a relatively low degree of damage, these being 16.84% and 17.39% respectively followed by **Agent** (18.01%) and **Elena** (19.22%). All the other cultivars had a DD between 20% and 30%, which meant a significant deterioration in the quality of the production due to mechanical lesion.

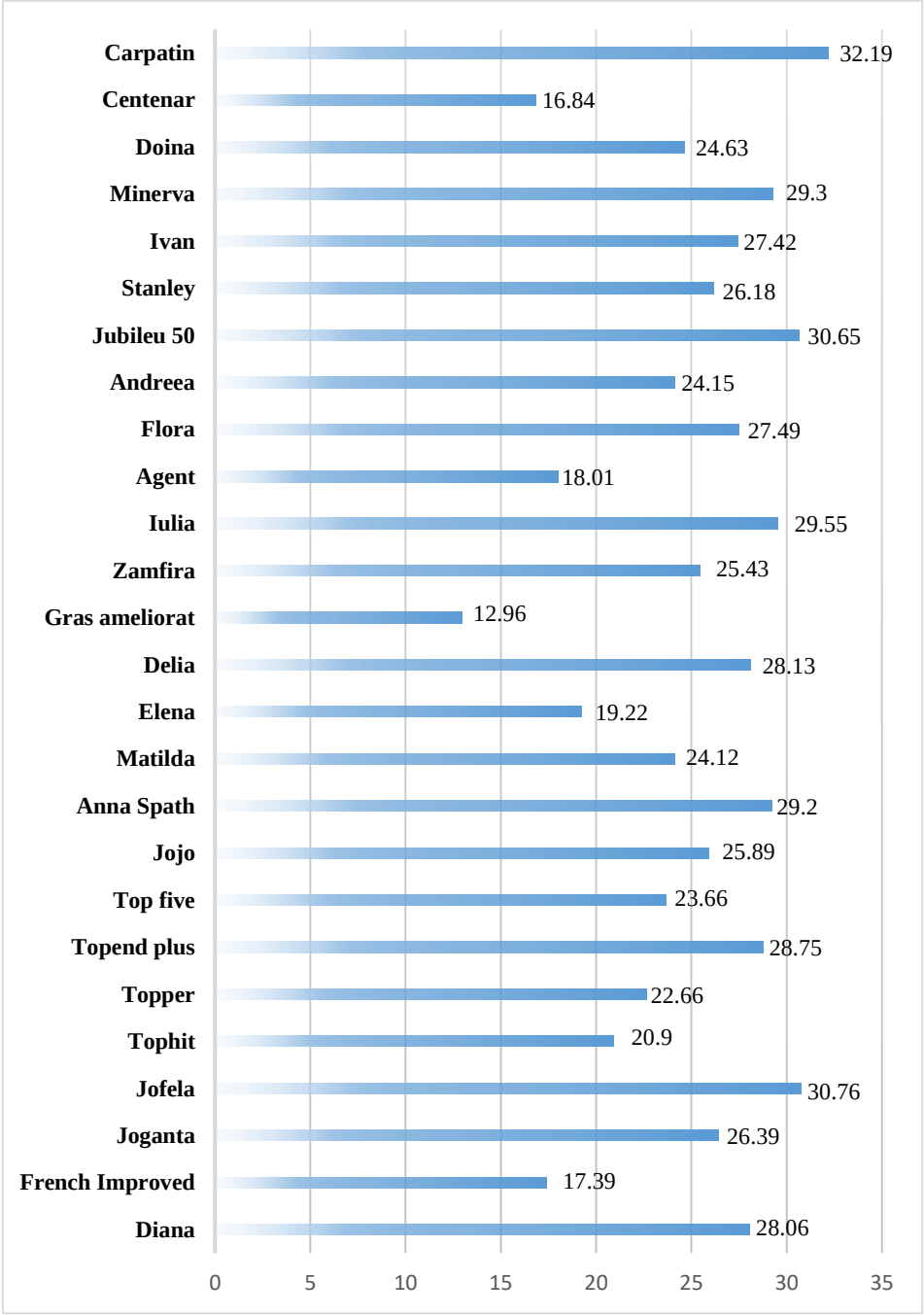

Fig. 2. The damage degree (%) caused by hail on 26 plum cultivars in 2024 / Fig. 2. Gradul de deteriorare (%) cauzat de grindină la 26 de soiuri de prun în 2024

Indirect effects of Monilinia spp. infection

Pathogens of the *Monilinia spp.* colonized the hail-induced injuries, leading to a further deterioration of the yield, ultimately compromising the entire production.The results showed that infection occured in all 26 plum cultivars that were affected by the disease, despite the antifungal treatments (Fig. 3). The percentage of fruits affected by brown rot waried widely amoung the

cultivars, ranging between 15 to100%, similar to the observed variability in lesion severity.

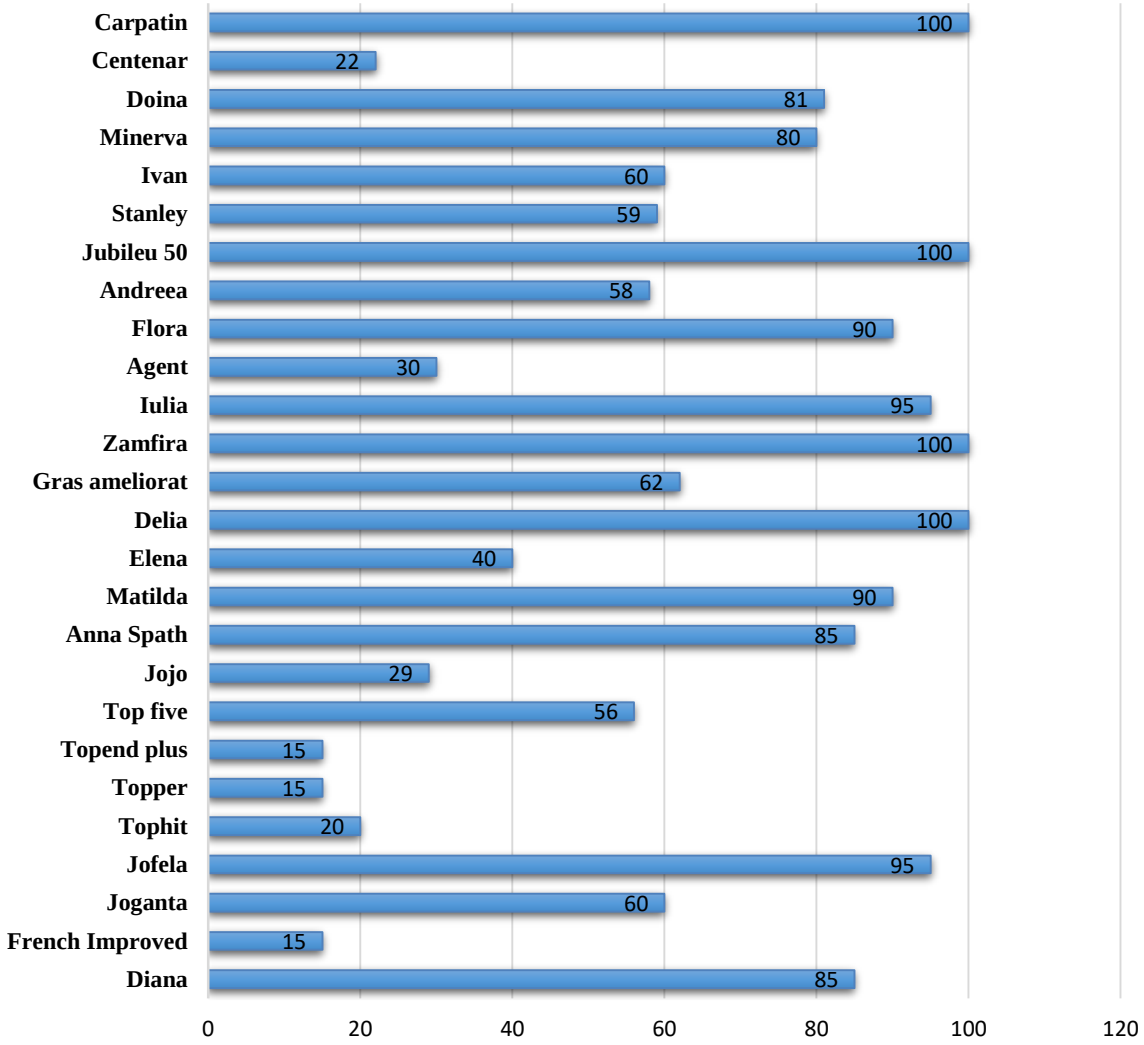

Fig. 3. The frequency of infections with *Monilinia* spp. on the fruits of the plum cultivars / Fig. 3. Frecvența infecțiilor cu *Monilinia* spp. pe fructele soiurilor de prun

The plum cultivars least affected by brown rot were **Topend plus**, **Topper** and **French Improved** each showing 15% infected fruit. In contrast , the most severely affected plum cultivars were: **Delia**, **Zamfira**, **Jubileu 50** and **Carpatin**, all fruits of these fruits exhibiting symptoms of infection. Other cultivars with a high incidence of disease, exceeding 80% infected fruits by *Monilinia* spp., were **Jofela**, **Iulia**, **Matilda**, **Flora**, **Diana**, **Anna Spath**, **Doina**, **Minerva** and **Gras ameliorat**. These results underline the considerable variability in susceptibility to *Monilinia* spp. among the studied plum cultivars, under the hailstorm condition mentioned.

CONCLUSIONS

The assessment of damage caused by hail revealed significant differences in the susceptibility of the 26 cultivars to this type of abiotic stress. This variability highlights the diversity of phenotypic responses to mechanical injuries, suggesting the presence of distinct genetic traits associated with resistance to the physical impact of hail. The most favorable responses to hail damage were observed in three indigenous cultivars (**Elena**, **Gras ameliorat**, and **Agent**), which demonstrated notable resilience. In terms of susceptibility to *Monilinia* spp. infections following severe hail events, the most tolerant genotypes were **Topend Plus**, **Topper**,

and **French Improved**. These findings emphasize the importance of genetic selection in breeding programs aimed at improving resilience to both abiotic stress and pathogen infection in plum cultivation.

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REGENERATION POTENTIAL AND QUALITY OF REGENERANTS THROUGH ORGANOGENESIS OF PLUM AND CHERRY VARIETIES

POTENȚIALUL DE REGENERARE PRIN ORGANOGENEZA ȘI CALITATEA REGENERANȚILOR LA SOIURI DE PRUN ȘI CIREȘ

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Abstract

Screening for adventitious regeneration ability remains an important step when in vitro techniques are considered useful for clonal propagation or genetic improvement of fruit species. Studies conducted highlight the difficulties in regenerating shoots in vitro and emphasize the importance of selecting appropriate strategies for identifying and isolating transgenic cells. The investigation targeted the evaluation of direct or indirect regeneration ability from cotyledon explants of some plum and cherry cultivars and the assessment of the quality of regenerated plants (growth and vigor). Significant differences in the genetic potential for organogenesis from in vitro cultured immature cotyledons were found in both species. The overall results showed that the plum cotyledons, irrespective of culture medium and pretreatment are generally more responsive than the cherry cotyledons. The frequency of cotyledons forming shoots on the most adequate culture medium exceeded 50% in seven out of nine investigated plum cultivars. At cherry cultivars, frequency of shoot regeneration ranged from 8.3% at Van to 38.4% at Rivan. In case of cultivars which prove a low ability of shoot regeneration from their in vitro cultured immature cotyledons, wounding of cotyledons and the use of TDZ could allow higher frequencies of explants regenerating shoots, as well as a higher number of shoots formed per responsive cotyledons.

Keywords: organogenesis, in vitro, cotyledon, plum, cherry

Rezumat

Evaluarea capacității de regenerare adventivă rămâne un pas important atunci când tehnicile in vitro sunt luate în considerare pentru propagarea clonală sau îmbunătățirea genetică a speciilor de pomi fructiferi. Studiile realizate evidențiază dificultățile în regenerarea lăstarilor in vitro și subliniază importanța selectării unor strategii adecvate pentru identificarea și izolarea celulelor transgenice. Investigația a vizat evaluarea capacității de regenerare directă sau indirectă a explantelor de cotiledoane la câteva soiuri de prun și cireș, precum și evaluarea calității plantelor regenerate (creștere și vigoare). Au fost găsite diferențe semnificative în potențialul genetic pentru organogeneză din cotiledoanele imature cultivate in vitro, atât la prun, cât și la cireș. Rezultatele generale au arătat că, cotiledoanele de prun, indiferent de mediul de cultură și de pretratamente, au raspuns în general mai bine decât cotiledoanele de cireș. Frecvența cotiledoanelor care formează lăstari pe cel mai adecvat mediu de cultură a depășit 50% în șase din cele nouă soiuri de prun investigate. La soiurile de cireș, frecvență de regenerare a lăstarilor a variat de la 8,3% la soiul Van la 38, 4% la soiul Rivan. La soiurile cu o capacitate scăzută de regenerare a lăstarilor din cotiledoanele imature cultivate in vitro, rănirea cotiledoanelor și utilizarea TDZ ar putea permite frecvențe mai mari de explante care regenerează lăstari, precum și un număr mai mare de lăstari formați pe cotiledoanele sensibilizate.

Cuvinte cheie: organogeneză, in vitro, cotiledon, prun, cireș

INTRODUCTION

Biotechnologies represent the future of pomology and a key pillar of national food security. Fruit species biotechnologies can be essential for several reason. Through the use of biotechnologies, more productive fruit tree varieties can be obtained, with higher yields and better adaptation to local climatic conditions and genetic engineering and other biotechnological techniques can contribute to developing varieties resistant to diseases and pests, thereby reducing the need for pesticides and minimizing production losses. Varieties with higher levels of vitamins,

antioxidants, and other essential nutrients for public health can be created (Awais and Schuyler, 2022; Silvestri *et al*., 2024; Shubham and Shikha, 2024).

Biotechnologies such as in vitro culture allow the conservation and regeneration of rare or traditional species, contributing to genetic diversity and the preservation of the national pomological heritage (FAO, 2004; Pence *et al*., 2022). Therefore, the implementation of biotechnologies in pomology can bring significant benefits for both farmers and consumers, reinforcing long-term food security (Sarasan *et al*., 2022).

The in vitro techniques and gene transfer technology were not only considered but even used as complementary tools for genetic improvement of *Prunus* cultivars (Gribaudo and Franks, 2011). If indirect organogenesis is considered to be much more successful for the practical application aiming at the enhancement of genetic variation and regeneration of somaclonal variants, direct organogenesis is highly desirable for obtaining transgenic plants from transformed tissues, avoiding the interference with additional and unwanted genetic modifications (Hammerschlag, 2002).

As long as plant regeneration from leaf explants remains inefficient in most plum cultivars (Escalettes and Dosba, 1993), the induction of shoot regeneration from cotyledons by organogenesis is the main chance for the application of in vitro techniques in plum improvement (Mante et al., 1989).

It is generally accepted in the genus *Prunus* that genotype and culture medium are critical factors for regeneration capacity (Popescu and Isac, 1998; Popescu and Militaru, 2002; Tang *et al*., 2002). Cotyledons excised from immature or mature embryos were suggested as an alternative which would allow the genetic manipulation of plum by somaclonal variation and transformation (Mante *et al*. 1991; Pooler and Scorza, 1995). Plant regeneration from cotyledons could be also an efficient way for the rescue of hybrid genotypes in early ripening plum varieties, in which the embryos have a very low germination ability or this is rather absent (Bini and Bellini, 1972). Moreover, the rescue of hybrid genotypes by shoot regeneration from cotyledons was already proposed for cherry varieties (Schmidt and Kardel-Meisner, 1992; Schmidt and Ketzel, 1993; Canli and Tian, 2008).

The addition of PGRs is very important since this decides whether direct or indirect organogenesis occurs from the explant. In most cases, 6-benzyl-aminopurine (BA) with or without kinetin (Kin) or indole acetic acid (IAA) and thidiazuron (TDZ) are used for the direct organogenesis and 2,4-dichlorophenoxyacetic acid (2,4-D) with or without Kin or BA is preferred for indirect organogenesis (Adya and Dennis Thomas, 2024).

In this paper, we report the results of our investigations on establishing the influence of genotype and hormones routinely used for regeneration from somatic tissues and, especially, on the possibility to control the pattern of organogenesis in some of plum and sweet cherry cultivars, offering the chance to adapt the regeneration system to the requirements of the specific application.

MATERIALS AND METHODS

Nine plum cultivars ('**Alina', 'Anna Spath', 'Blue Free', 'Gras ameliorat', 'Record', 'Silvia', 'Stanley', 'Tuleu gras'** and '**Valor**') and three cherry cultivars ('**Rivan', 'Bigarreau Moreau'** and '**Van**') were investigated for their direct or indirect organogenesis potential from in vitro cultured cotyledons.

The biological material used was represented by cotyledons excised from immature embryos (extracted from still green fruits) or in the phase preceding morphological and physiological maturity.

The basal medium, Murashige & Skoog (MS,1962), was used, supplemented with BA 0.3 and 0.5 mg/l or TDZ 1.0 and 1.5 mg/l in combination with either 3-indolylbutyric acid (IBA 0.1 and 0.5 mg/l) or 2,4-D 0.5 and 1.0 mg/l to induce organogenesis. The carbon source was sucrose at a concentration of 3%, and the medium was solidified with 8g/l agar.

Because previous results indicated the favorable influence of explant wounding prior in vitro culture on the expression of organogenesis ability, for each treatment, half of the cotyledons were superficially wounded by longitudinal or transversal cuttings on the adaxial surface.

Although the orientation of the explants is considered not to affect the organogenetic response, all cotyledons were placed with the adaxial surface in complete contact with the culture medium. After an initial two-week treatment in the dark, an equal number of intact and wounded cotyledons, respectively, were transferred to the light in the growth chamber, at 23±1°C, under a 16/8 h photoperiod. The experimental design included at least 24 cotyledons in 6 replicates for each treatment.

The observations regarding the formation of shoots through direct or indirect organogenesis were carried out weekly, starting from the moment of completion of the two weeks of dark pretreatment and the transfer of cotyledon explant cultures to the growth chamber under photoperiodic conditions. For each experimental variant, the average percentage of cotyledons that formed shoots and the average number of regenerated shoots per explant (cotyledon) were calculated. The quality of the regenerated plants, namely growth and vigor, was evaluated.

The differences in genetic potential, reflected in the values of the frequency of regenerative explants recorded in the different experimental variants, were analyzed by the Duncan test.

RESULTS AND DISCUSSION

The observations made at regular time intervals showed that, regardless of the in vitro culture medium, the direct or indirect organogenesis potential reflected in the percentage of cotyledons from which adventitious shoots were formed and in the number of regenerated shoots from the same cotyledon was strongly influenced by the genotype. Shoot formation was achieved in all the investigated plum and cherry cultivars, but significant differences were found in the regeneration potential and the quality of the regenerants for some genotypes. In the same genotype, the organogenesis potential was expressed at a higher level by the immature cotyledons.

In all the cultivars we chose for the evaluation of organogenesis potential, callus formation and shoot regeneration from it was observed only under cultivation conditions on media supplemented with auxin 2,4-D. The process is dependent on both the 2, 4-D concentration and the auxin (2, 4-D) / cytokinin (BA) ratio.

The overall results showed that, regardless of the culture medium and pretreatment, plum cotyledons are generally more sensitive than cherry cotyledons, a fact highlighted by the higher regeneration percentages recorded in indirect organogenesis in plum compared to those recorded in cherry.

In the plum varieties '**Alina', 'Anna Spath', 'Blue Free', 'Silvia', 'Stanley**' and '**Valor**' indirect organogenesis was recorded in percentages of over 50% on the experimental variants tested (Figure 1), while none of the investigated cherry cultivars were found to have such regeneration potential.

The hormonal combination 1.0 mg/l 2, 4-D + 5.0 mg/l BA had the best influence on indirect organogenesis in 6 of the plum varieties tested, with the best result recorded (93.7%), in the '**Blue Free**' variety (Figure 1). It should be mentioned that most of the cotyledons with organogenetic capacity formed shoots before the appearance of the callus, respectively in the first 4 weeks after the initiation of the culture.

On the culture medium containing 2, 4-D and BAP, the regeneration process initially occurred through direct organogenesis, with shoot formation being triggered shortly after the introduction of the cotyledon explants into the culture. Such an evolution of the organogenesis process constitutes an advantage for the application of this regeneration system in genetic transformation works or even in clonal propagation.

In all 9 plum varieties investigated, adventitious bud differentiation and shoot formation occurred after the dark treatment period and transfer of explant cultures to light.

A first indication of the major influence of the cultivar on the organogenesis process was provided by the different reaction of the cotyledons after the pretreatment periods in the dark and transfer to light.

In some varieties (e.g. '**Tuleu gras**' and '**Gras ameliorat**') the cotyledons did not change their volume and color, and in others ('**Alina**', '**Anna Spath**', '**Stanley**' and '**Valor**') they increased significantly in volume and turned green quickly, as a result of a process of chlorophyll pigment synthesis carried out in parallel with the shoot formation process. However, it should be mentioned that, in the same genotype, the pigmentation process of the cotyledons was influenced by their degree of morphological and physiological maturity, being slower in immature cotyledons.

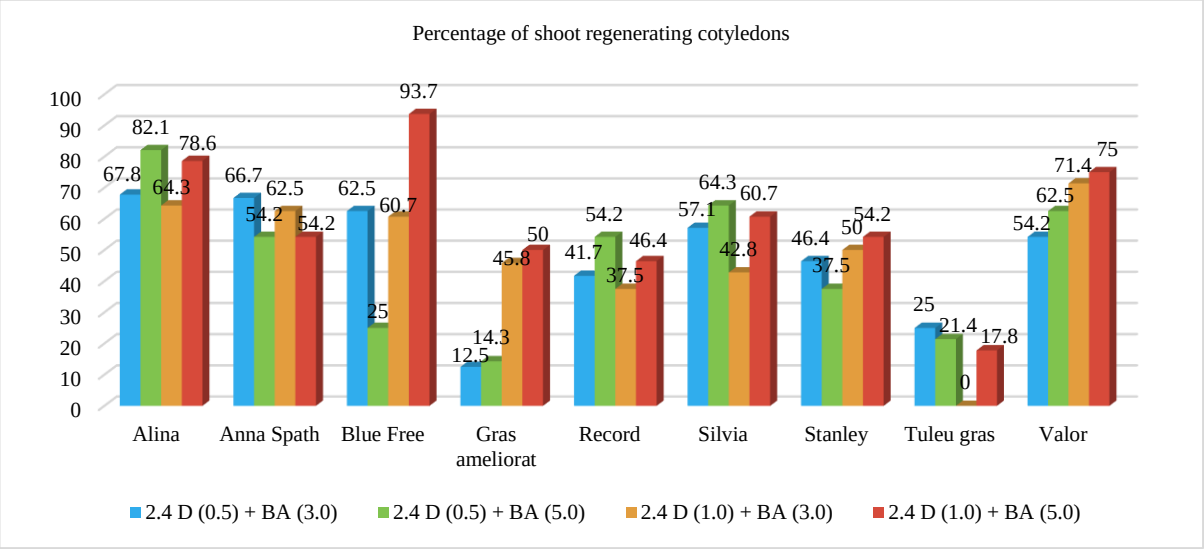

Figure 1. The potential of indirect organogenesis from cotyledons in plum varieties / Potențialul organogenetic al cotiledoanelor de prun în organogeneza indirectă

In cherry, the process of indirect organogenesis was much reduced compared to the results recorded with plum varieties. Frequency of shoot regeneration ranged from 8.3% in cv, **Van** to 38.4% in cv. **Rivan** on different culture media (Figure 2).

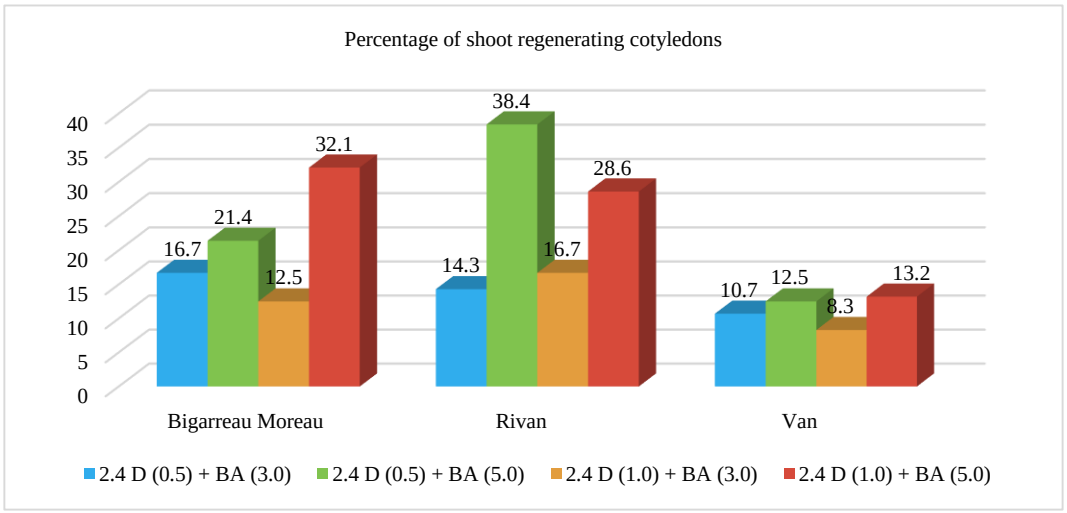

Figure 2. The potential of indirect organogenesis from cotyledons in cherry varieties / Potențialul organogenetic al cotiledoanelor de cireș în organogeneza indirectă

It was found that, regardless of the type of explant, in all three cherry cultivars used in this experiment the highest frequency of regenerated cotyledons (between 13.2% and 32.1%) was recorded on the culture medium containing the highest concentration of 2,4-D (1.0 mg/l) as in the case of plum varieties (Figure 2).

Subsequent observations showed that after 4 weeks a callus process of the explants is triggered, which determines the conversion of the regeneration mode from direct organogenesis to indirect organogenesis. An interesting finding is that in all cultivars investigated, in the case of cultivation on media containing 2,4-D in low concentration (0.5 mg/l), callus formation not only started later by over a week, but also ended the earliest, compared to media containing 1.0 mg/l 2,4-D. The inability to sustain the callus process for a longer period is probably the consequence of the faster triggering of adventitious bud differentiation on these media, favored by a higher BA/2, 4-D ratio (6:1 or 10:1, compared to 3:1 or 5:1). Therefore, the stop of callus proliferation is rather the result of competition for auxin with the process of differentiation of organogenic structures (shoots), than of its metabolism in a very short time.

After 12 weeks from the initiation of *in vitro* cultures in plum, the observations made showed the existence of considerable differences in organogenic potential. Regardless of the culture medium variant, the 'Alina' and 'Valor' varieties stand out by having 100% direct regeneration for both intact and injured cotyledons (Table 1). Even if the level of expression of the shoot regeneration potential was obviously dependent on the genotype, it should be mentioned that the frequency of shoot formation from wounded cotyledons was higher compared to the one recorded in explants of intact cotyledons (Table 1).

Table 1. The influence of genotype on organogenesis potential, as shown by the frequency of shoot formation from cotyledon explants cultivated on media containing 0.5 mg/l IBA and 3.0 mg/l BAP / Influența genotipului asupra potențialului de organogeneză, așa cum arată frecvența formării lăstarilor din explante de cotiledon cultivate pe medii care conțin 0,5 mg/l AIB și 3,0 mg/l BAP

Genotype	Type of explant			
	Intact cotyledons		Wounded cotyledons	
	Regeneration frequency	Duncan*	Regeneration frequency	Duncan*
Alina	100	a	100	a
Valor	100	a	100	a
Record	54.0	b	82.5	ab
Anna Spath	37.5	bc	56.2	b
Blue free	36.0	bc	62.5	b
Stanley	33.0	bc	50.0	bc
Silvia	31.2	bc	56.2	b
Gras ameliorat	24.0	c	37.5	c
Tuleu gras	0	d	18.7	d

**Values on the same column followed by the same letter are not significantly different for P= 5%*

It was noted that, as highlighted by the Duncan test, the difference in regenerative potential between these two cultivars and the rest of the cultivars is significant both in the case of regeneration by direct organogenesis (Figure 3) and regeneration by indirect organogenesis (Figure 1). The cultivars '**Blue Free'**, '**Anna Spath'** and '**Record**', have a moderate regeneration, with values between 36% and 54% for intact cotyledons and between 62.5% and 82.5% for injured cotyledons. The cultivars '**Stanley'** and '**Silvia**' have a lower regeneration, with regeneration values between 31.2% and 33% for intact cotyledons and between 50% and 56.2% for injured cotyledons (Table 1).

The cultivars **'Gras ameliorat**' and **'Tuleu gras'** can be considered having a very low potential for shoot regeneration from somatic tissues cultured in vitro. **'Tuleu gras'** is at the lower end of the scale with 0% regeneration for intact cotyledons and 18.7% for injured ones (Table 1). In fact, the last of these two cultivars is also the only one of the nine investigated, in which shoot regeneration could not be induced from cotyledon explants except on three of the four culture medium variants supplemented with 2,4-D. On the medium considered optimal for expressing at a

high level the organogenesis potential of plum cultivars, shoots regenerated only in a percentage of 16.2%, (Figure 3).

The fact that on any of the culture medium variants tested for the **'Tuleu gras'** variety the lowest values of the frequency of cotyledon explants with direct organogenesis capacity were recorded, constitutes conclusive evidence of the determining influence of the cultivars in the expression of the regenerative potential.

It was found that, regardless of the variety, the callus process was triggered 2-3 weeks earlier in the injured cotyledons, compared to the intact ones, respectively after 6-8 weeks from the initiation of the cultures. The superior genetic potential of plant regeneration from somatic tissues in the 'Alina' and 'Valor' cultivars is evident, these being the only cultivars in which the direct organogenesis capacity was expressed at a high level (50-100%) both on the culture medium variants supplemented with auxin 2,4-D (Figure 1) and on those supplemented with IBA (Figure 3). On the culture media containing IBA and BA, the formation of the first shoots was initiated 1-2 weeks later compared to those containing 2,4D and BA. The organogenesis process was most often triggered at the site of detachment of the cotyledon from the embryonic axis, respectively the site of injury, confirming the observations made by de Cossio and Lane (1986) in other *Prunus* varieties.

Since in all nine investigated cultivars the interval between the moment of initiation of the callus process and the cessation of the regeneration process was relatively short (about 2 weeks), it is possible that most of the shoots were formed directly from the explant cells (direct organogenesis). Such an evolution of the regeneration process has very important practical implications.

It is known that, while for the selection of somaclonal variants with modified characteristics, callus regeneration (indirect organogenesis) is an essential condition, for works aimed at gene transfer, direct organogenesis is preferable.

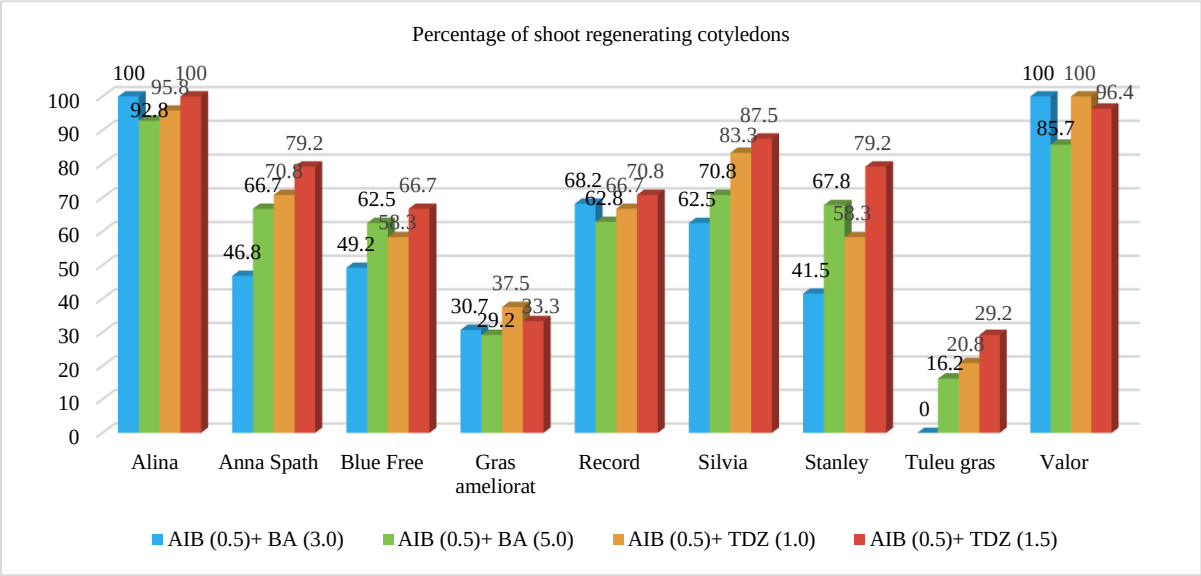

Figure 3. The potential for direct organogenesis from cotyledons in plum cultivars / Potențialul organogenetic al cotiledoanelor de prun în organogeneza directă

Observations showed that in all plum cultivars in which shoot regeneration was induced, the duration of maintaining the organogenesis capacity is shorter in cotyledon explants cultivated on the culture medium containing growth regulators in a ratio that induces the early onset of the regenerative process and a high frequency of regeneration.

Counting of shoots formed by each regenerating cotyledon showed that the number of formed shoots is in almost all investigated plum and cherry cultivars higher on culture media promoting callusing and subsequently indirect organogenesis.

Even though, in some varieties, the increase in the percentage of regenerative explants was still very low, the number of regenerated shoots per explant increased significantly within a period of about 6-10 weeks. An interesting finding was that the influence of genotype on the organogenesis process was conclusively reflected in the frequency of adventitious shoot formation from injured cotyledons. While in the varieties **'Silvia', 'Alina', 'Valor', 'Blue Free', 'Stanley', 'Anna Spath'** they differentiated from these on most of the culture medium variants, over 5 shoots/cotyledon in most cases, in the varieties '**Record**', **'Gras ameliorat'** and **'Tuleu gras'** the frequency of differentiated shoots did not significantly exceed that recorded in the case of intact cotyledons, which confirms the limited organogenesis potential of these varieties (Figure 4).

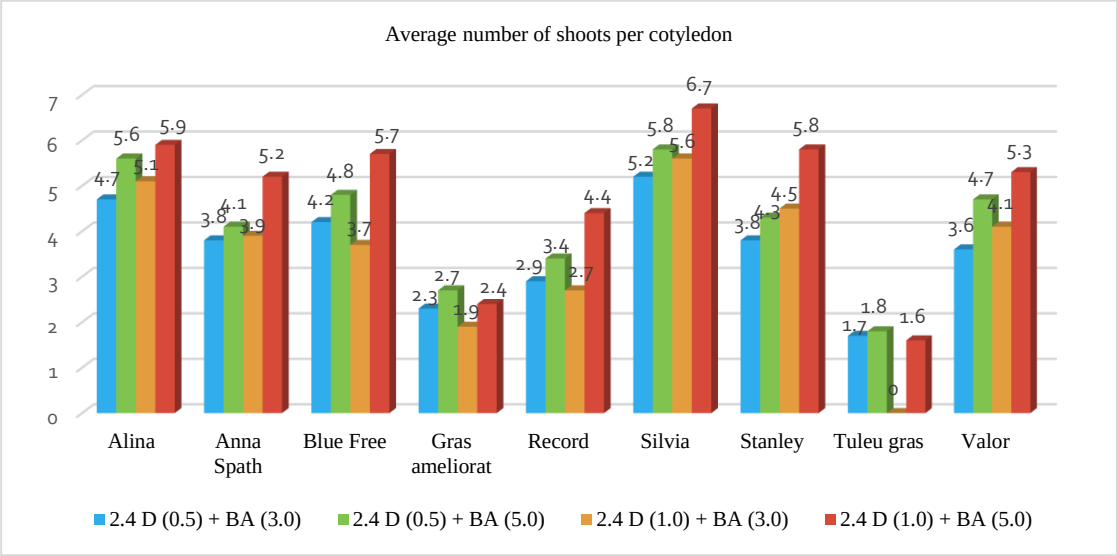

Figure 4. The average number of shoots regenerated per cotyledon by indirect organogenesis in plum cultivars / Numărul mediu de lăstari regenerați pe cotiledon prin organogeneză indirectă la soiurile de prun

Obvious differences were observed in terms of vigor and growth rate of shoots regenerated from cotyledon explants belonging to different varieties, in the sense of high vigor in the varieties '**Alina', 'Blue Free', 'Stanley', 'Anna Spath', 'Record**', and '**Valor**', respectively of low vigor and slow growth rate in the varieties 'Gras ameliorat' and 'Tuleu gras'.

In cherry, under the same environmental conditions, a relatively large variation was recorded in the average number of shoots regenerated per cotyledons, from 1.3 in '**Van**' to 4.2 in '**Rivan**'. Regardless of the growing environment, no cherry variety recorded the threshold of 5 shoots/cotyledon, but the 'Rivan' variety stands out with 3.6 shoots/cotyledon (Figure 5).

If the regeneration capacity was increased in some of the plum varieties investigated by using TDZ instead of BA, the growth and vigor of the regenerants were influenced rather negatively. The analysis of the ensemble of experimental results confirmed that the varieties '**Alina**', **'Valor', 'Silvia'**, have the highest potential for direct organogenesis. The average frequency of shoot regeneration exceeds 70% (Figure 3).

The observations also showed that these varieties were distinguished not only by the higher frequency of cotyledons that formed shoots, but also by the higher number of shoots formed from a single explant (the average number of shoots per explant was 6.4 in '**Silvia**', 6.2 in '**Alina**' and 5.2 in '**Valor**'), compared to the other varieties, which is a conclusive confirmation of their high capacity for organogenesis and implicitly of the very important role of the genotype (Figure 6).

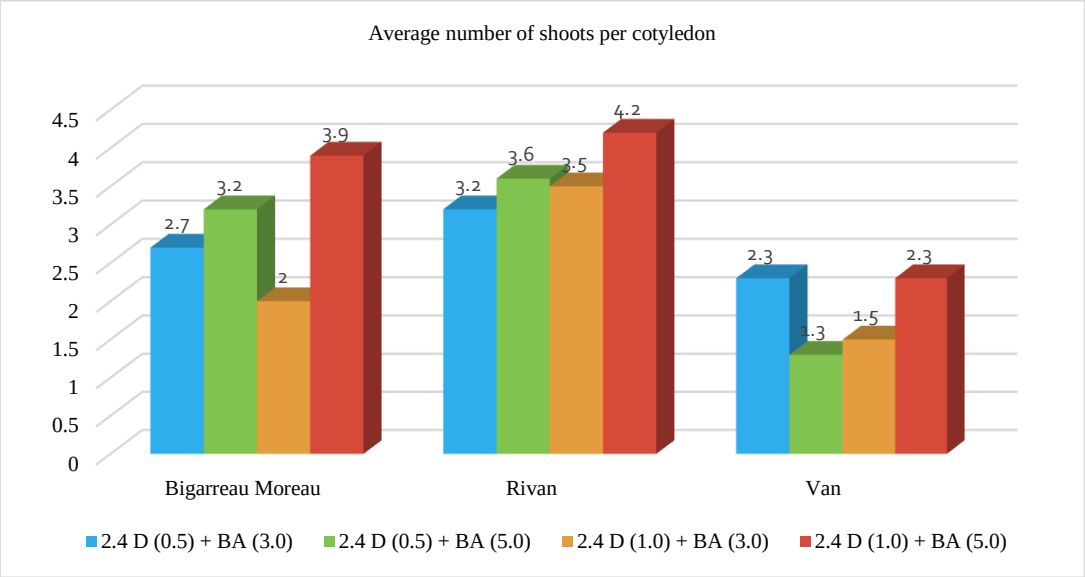

Figure 5. The average number of shoots regenerated per cotyledon by indirect organogenesis in sweet cherry cultivars / Numărul mediu de lăstari regenerați pe cotiledon prin organogeneză indirectă la soiurile de cireș

However, in these varieties, as expected, the poorest quality of regenerated shoots was found with the highest concentration of TDZ in the culture media. If the multiplication rate of plum and cherry shoots regenerated by organogenesis was significantly lower with poor shoots, with one exception (plum cv. Tuleu gras), both the rooting ability and acclimatization of plants regenerated from cotyledons were affected by the weakness of initial regenerated shoots.

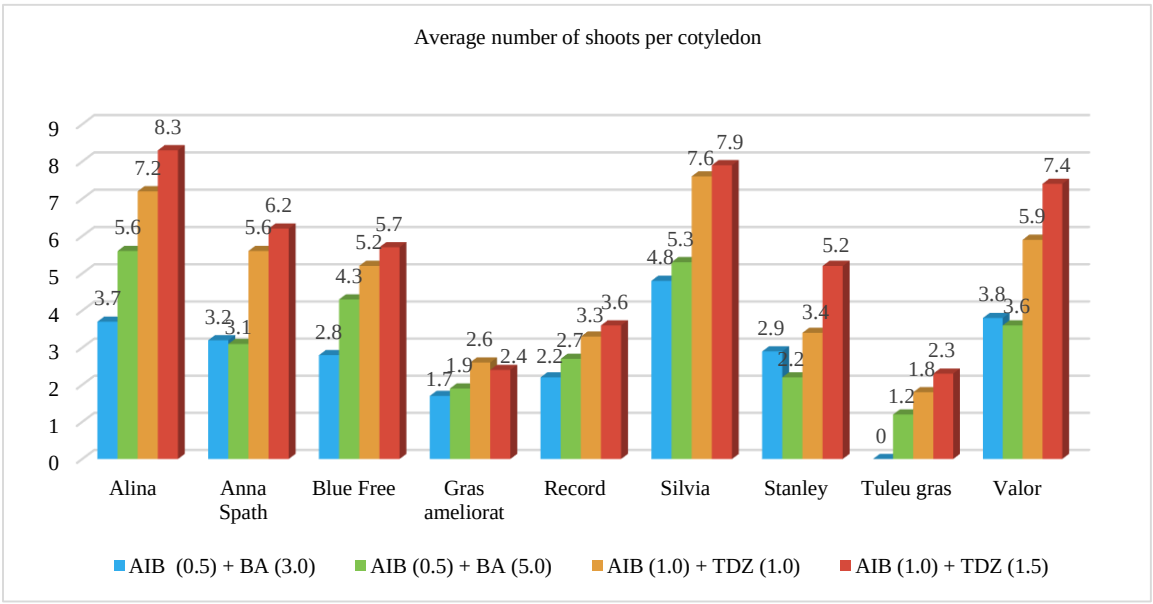

Figure 6. The average number of shoots regenerates per cotyledon by direct organogenesis in plum cultivars / Numărul mediu de lăstari regenerați pe cotiledon prin organogeneză directă la soiurile de prun

The organogenesis process was most often triggered at the site of detachment of the cotyledon from the embryonic axis, respectively the site of injury (Figure 7), confirming the observations made by de Cossio and Lane (1986) in other *Prunus* varieties.

Figure 7. Aspects regarding the organogenesis process in plum cotyledons/ Aspecte privind procesul de organogeneza la cotiledoane de prun

CONCLUSIONS

1. The regeneration potential of somatic tissues cultured in vitro is influenced to a greater extent by genotype than by the culture medium.
2. The plum cultivars 'Alina', 'Blue Free', 'Valor' and 'Silvia', have, in the order above, the highest potential for indirect organogenesis. These varieties were distinguished not only by the higher frequency of explants that formed shoots (over 50%), but also by the large number of shoots formed from a single explant (in most cases over 5 shoots/cotyledon).
3. The hormonal combination 1.0 mg/l 2, 4-D + 5.0 mg/l BA had the best influence on indirect organogenesis in 6 of the plum cultivars tested, with the best result recorded by the 'Blue Free' cultivar 2.(93.7%), while in direct organogenesis regardless of the culture medium variant, the 'Alina' and 'Valor' varieties stand out by having 100% direct regeneration for both intact and injured cotyledons.
4. The cultivars 'Stanley' and 'Silvia' can be included in the group of cultivars with low regeneration potential, and the varieties 'Gras ameliorat' and 'Tuleu gras' can be considered as having a very low potential for shoot regeneration from in vitro somatic tissues. 'Tuleu gras' is at the lower end of the scale with 0% regeneration for intact cotyledons and 18.7% for injured ones.
5. The very low frequency of plant regeneration from cotyledons taken from embryos belonging to the 'Tuleu gras' and 'Gras ameliorat' cultivars constitutes an indisputable confirmation of the genetic determinism of the organogenesis capacity, given that these cultivars are closely related, since the 'Tuleu gras' variety is one of the genitors of the 'Gras ameliorat' cultivar.
6. Although a few cherry cultivars were investigated for their organogenesis potential, we can conclude that the cotyledons of cherry have a very low ability to regenerate shoots, as compared to those of plum.
7. Significant differences in the genetic potential for organogenesis from *in vitro* culture of immature cotyledons was found in both plum and cherry cultivars which must be considered when attempting the use of somaclonal selection or transformation for their genetic improvement.
8. In cultivars having low ability of shoot regeneration from their *in vitro* culture immature cotyledons, wounding of cotyledons and the use of TDZ could allow higher frequencies of explants regenerating shoots, as well as higher number of shoots formed per responsive cotyledon.
9. Although the use of the auxin 2,4-D should promote callus formation and therefore indirect organogenesis when combined with BA, shoot formation occurred in some

cultivars by direct organogenesis, as in case of cotyledons cultured on medium containing IBA and either BA or TDZ.

10. In cultivars having low ability of shoot regeneration from their in vitro cultured immature cotyledons, wounding of cotyledons and the use of TDZ could allow higher frequencies of explants regenerating shoots, as well as higher number of shoots formed per responsive cotyledon.

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APPLIED BIOTECHNOLOGIES. *SOLANUM LYCOPERSICUM* ANTHER CULTURE

BIOTEHNOLOGII APLICATE. CULTURA DE ANTERE LA *SOLANUM LYCOPERSICUM*

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Abstract
Anther culture is an efficient biotechnological method for obtaining haploid and double haploid plants by in vitro *androgenesis. The technique involves the isolation of microspores from anthers and their regeneration under controlled laboratory conditions, followed by* ex vitro *acclimatization of the plant. This method significantly contributes to plant improvement by reducing the time required to develop new genetically stable genotypes. Evaluation of morphological traits and genetic analysis using molecular markers confirmed the haploid origin of the regenerated plants and their genetic diversity. The objectives of this study were to test the* in vitro *regeneration capacity of anthers in five tomato varieties obtained at INCDBH Ștefanesti, evaluate the phenotypic characteristics of the regenerated plants, and perform genetic analysis using Simple Sequence Repeat (SSR) markers to identify possible somaclonal variations resulting from the indirect organogenesis process. Successfully regenerated plants were obtained from only two of the five tested varieties, respectively 'Costate 21' and 'Argeș 20', and analysis with SSR markers revealed genetic differences between the regenerants and the donor plants, confirming that the regeneration process can generate genetic variability.*

Keywords: *androgenesis, morphological evaluation, genetic variability, Solanum lycopersicum.*

Rezumat
Cultura anterelor este o metodă biotehnologică eficientă de obținere a plantelor haploide și dublu haploide prin androgeneză in vitro*. Tehnica implică izolarea microsporilor din antere și regenerarea acestora în condiții controlate de laborator, urmată de aclimatizarea* ex vitro *a plantei. Această metodă contribuie semnificativ la ameliorarea plantelor prin reducerea timpului necesar dezvoltării de noi genotipuri stabile genetic. Evaluarea trăsăturilor morfologice, împreună cu analiza genetică folosind markeri moleculari, a confirmat originea haploidă a plantelor regenerate și diversitatea lor genetică. Obiectivul acestui studiu a fost testarea capacității de regenerare in vitro a anterelor la cinci soiuri de tomate obținute la INCDBH Ștefănești, evaluarea caracteristicilor fenotipice ale plantelor regenerate și analiza genetică prin markeri Simple Sequence Repeat (SSR), în scopul identificării posibilelor variații somaclonale rezultate din procesul de organogeneză indirectă. Plante regenerate cu succes au fost obținute doar din două dintre cele cinci soiuri testate, respectiv 'Costate 21' și 'Argeș 20', iar analiza cu markeri SSR a evidențiat diferențe genetice între regeneranți și plantele donatoare, confirmând că procesul de regenerare poate genera variabilitate genetică.*

Cuvinte cheie: *androgeneză, evaluare morfologică, variabilitate genetică, Solanum lycopersicum*.

INTRODUCTION

Solanum lycopersicum L. is the second most consumed vegetable in the world, after the potato (Ibitoye *et al*., 2009; Kambale *et al*., 2023), and is native to South America (Sumedrea *et al*., 2024). Tomatoes are being researched as a crop of global interest due to their high production

qualities in different crop and climate areas (Gerszberg and Hnatuszko-Konka, 2017) and their organoleptic qualities.

In vitro androgenesis, as a biotechnological approach for obtaining haploid and double-haploid plants, among the procedures for regenerating new genotypes in a single generation, has aroused great interest among researchers (Sánchez et al., 2020; Badulescu *et al*., 2022).

Tomatoes are still a recalcitrant species to this regeneration process and require improvements to be applied in breeding processes (Seguí-Simarro et al. 2011) compared to other types of crops (*Triticum aestivum* L., *Hordeum vulgare* L., × *Triticosecale Wittm*., *Oryza sativa* L., *Brassica* sp., *Solanum melongena* L., *Capsicum annuum* L. and *Nicotiana tabacum* L.) for these species this methodology of obtaining double haploids has been efficient for obtaining new genotypes (Saeed *et al*., 2019).

Tomato accessions and available germplasm constitute valuable resources in genetic breeding programs, used for morphological characterization and the evaluation of genetic diversity. By applying molecular markers, researchers can identify genotypes with traits and genes of interest, essential for improving tomato culture (Al-Shammari & Hamdi, 2021; Sumedrea *et al*., 2024). Under favorable *in vitro* culture conditions, plant regeneration from anthers can occur through direct or indirect embryogenesis, or indirect organogenesis, resulting in haploid or double-haploid plants (Seguí-Simarro and Nuez, 2007; Zhao *et al*., 2014; Méndez-Hernández *et al*., 2019). In the case of indirect regeneration, whether through organogenesis or embryogenesis, it is essential to analyze the ploidy level of calli to understand the origin of the regenerants and confirm their provenance from microspores (Julião *et al*., 2015). *In vitro* regeneration is often a laborious and complex process, so selecting the obtained plants requires precise methods for determining ploidy, such as chromosome counting in root cells or analysis of DNA content by flow cytometry (Murovec and Bohanec, 2012).

Existing studies have shown that *Solanum lycopersicum* is a difficult species in this type of regeneration, which highlights the need to improve regeneration techniques, as well as careful characterization of the newly obtained plant material (Tradeu De Faria *et al*., 2002; Prihatna *et al*., 2019).

Regardless of the regeneration method used, regenerants are selected based on their characterization using morphological, biochemical, and molecular markers (Cebolla-Cornejo *et al*., 2013; Prihatna et al., 2019).

Plants obtained from complex plant organs by *in vitro* regeneration may present morphological traits different from the donor genotype. In the case of androgenesis, the use of molecular markers such as SSR and RFLP allows confirming the genetic uniformity of regenerants or highlighting somaclonal variations induced during culture (Krishna *et al*., 2016; Cao and Deng, 2020; Bădulescu *et al*., 2022).

Considering the potential and challenges of plant regeneration through anther culture, this work set the following objectives: a) testing the *in vitro* regeneration capacity of anthers in five tomato cultivars; b) evaluating the phenotypic characteristics of the regenerants, compared to the original cultivar; c) the use of SSR markers to verify the genetic uniformity of plants derived from anthers and to detect possible somaclonal variations occurring in the case of regeneration through indirect organogenesis.

MATERIALS AND METHODS

Plant material

The biological material used for the initiation of *in vitro* anther cultures was represented by five tomato varieties (*Solanum lycopersicum*) obtained within the National Institute for Research and Development for Horticulture and Biotechnology (INCDBH): **Argeș 11** and **Argeș 20**

determinate growth varieties, and **Costate 21**, **Ștefănești 22** and **Ștefănești 24,** indeterminate growth varieties.

Microspore analysis and preparation of floral buds for in vitro culture initiation

Unopened floral buds, measuring between 3–7 mm in length, were collected in the early morning from vigorous, greenhouse-grown plants and stored at 4°C for approximately 48 hours to preserve viability. Subsequently, bud and anther lengths were measured, and the developmental stages of microspores were assessed microscopically using an OPTIKA digital binocular microscope and the LUNA-II automated cell counter. Before use, the buds were surface-sterilized according to a protocol involving treatment with 70% ethanol for 15–20 seconds, immersion in a 10% sodium hypochlorite solution for 20 minutes, and several rinses with sterile distilled water

Culture media optimization and conditions for anther culture

Anthers, under sterile conditions, in a laminar flow hood, were excised from the flower buds and inoculated onto Murashige and Skoog (MS, 1962) basal medium supplemented with 20 g/L sucrose and 7 g/L agar, adjusted to pH 5.8–6.0, and autoclaved at 121°C for 20 minutes.

Four variants of initiation medium, each containing MS macro- and micronutrient elements, with specific supplements were tested at this stage: (1) 0.5 mg/L kinetin (Kin) and 0.5 mg/L indole-3-acetic acid (IAA); (2) 2.0 mg/L 6-benzylaminopurine (BAP) and 1.0 mg/L IAA; (3) 1.0 mg/L BAP and 1.0 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D); (4) 2.0 mg/L BAP and 2.0 mg/L 2,4-D.

The cultures were incubated under intumescent conditions at 24±2°C for 14 days, followed by transfer to a growth chamber with a photoperiod of 16 hours light and 8 hours dark (light: 24°C, dark: 22°C).

Viable anthers and those with callus formation or organogenetic structures were transferred to MS induction media supplemented with: (5) 0.5 mg/L IAA and 0.25 mg/L zeatin (Z); (6) 2.0 mg/L BAP and 1.0 mg/L IAA. Shoots, 3–4 cm long, were transferred to MS medium without hormones for rooting.

Observations included the assessment of anther viability, callus formation, and differentiation of organogenetic structures. The data obtained were analyzed using ANOVA and Tukey and Duncan statistical tests.

Evaluation of morphological features

The characterization of the phenotypes of plants obtained from anther culture and transferred to soil in a greenhouse, as well as those grown in the second generation, was conducted according to international standards for tomato (*Lycopersicon* spp.) descriptors, as established by the International Plant Genetic Resources Institute (IPGRI, 1996). The assessments were performed at optimal developmental stages, as specified by IPGRI guidelines, to ensure accuracy and consistency in the assessment of morphological traits of the regenerants compared to the donor plant.

DNA extraction and SSR marker analysis

Total genomic DNA was extracted from young leaf tissues of field-grown plants using the Qiagen DNeasy Plant Mini Kit protocol. For genetic analysis, nine SSR markers were employed: SSR47, SSR62, SSR63, SSR70, SSR107, SSR110, SSR111, SLM6-7, and SLM6-12. PCR amplification and the analysis of the resulting products were conducted following the procedures described by Bădulescu *et al.* (2022).

RESULTS AND DISCUSSION

Flower bud selection and culture conditions for anther culture in tomatoes

The success of anther culture in tomatoes depends largely on the correct identification of the optimal stage of flower bud development and the careful selection of culture conditions.

In this study, five tomato genotypes, **Argeș 20**, **Argeș 11**, **Ștefănești 22**, **Ștefănești 24**, and **Costate 21**, were analyzed for morphological and physiological parameters that influence the efficiency of anther culture and haploid plant regeneration.

Morphological traits and optimal harvest time

The results demonstrated that the optimal time for harvesting flower buds for anther culture varied between genotypes, with a clear correlation between the size of flower components (sepals, petals, pistils) and the potential for callus formation (Popescu *et al*., 2022).

Larger flower buds generally indicated a higher probability of containing undifferentiated cells suitable for *in vitro* dedifferentiation, supporting the findings of Zhao *et al.* (2014). The genotypes differed in their floral morphology: **Argeș 20** and **Argeș 11** were distinguished by their longer perianth elements and light green bracts and sepals, suggesting increased protection of the reproductive organs. In contrast, **Ștefănești 22**, **Ștefănești 24** and **Costate 21** had shorter sepals, but the bud surfaces with denser hairs suggested alternative protection (Fig.1).

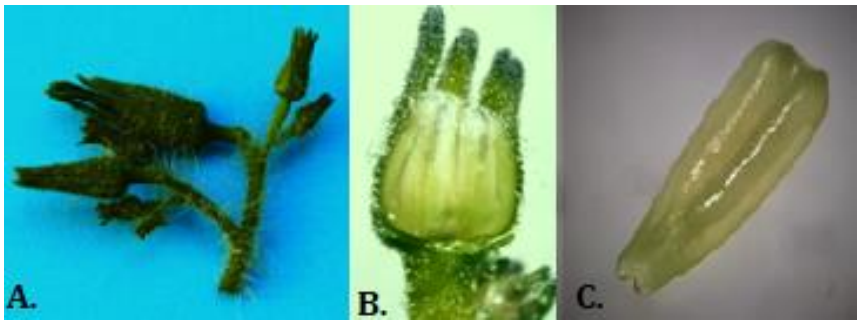

Fig. 1. Developmental stages of tomato floral buds for efficient anther culture: optimal bud selection, carpel elimination, and anther isolation - A. Buds at the optimal harvest stage; B. Removal of carpels; C. Excised anthers / *Fig. 1. Stadiile de dezvoltare ale mugurilor florali de tomate pentru cultura eficientă a anterelor: selecția optimă a mugurilor, eliminarea carpelului și izolarea anterelor - A. Muguri la stadiul optim de recoltare; B. Îndepărtarea carpelelor; C. Anterele excizate.*

Measurements showed that bud sizes ranged from 4.7 to 8.7 mm in length, with corresponding anther sizes ranging from 2.1 to 6.8 mm, reinforcing that anther length is typically 2.1–3.4 mm shorter than the floral bud itself. This consistent relationship provides a reliable morphological marker for identifying buds containing microspores at the appropriate developmental stage.

This is in agreement with Summers *et al*. (1992) and Seguí-Simarro & Nuez (2005), who highlighted the importance of anther length in selecting microspores during meiotic division or the tetrad stage, crucial phases for gynogenesis. In addition to size, anther color and texture, especially a bright yellow and translucent appearance, were important indicators of readiness for culture.

Culture initiation and cold pretreatment

A cold pretreatment at 4°C was applied before culture initiation to increase the success rate of anther culture. This approach, supported by Shariatpanahi et al. (2006), induced dedifferentiation and improved viability. When floral buds were inoculated immediately after harvest without this treatment, cultures failed due to necrosis. However, in the case of cold-treated anthers, viability ranged from 33.9% **Argeș 20** to 47.9% **Argeș 11** after two weeks in the dark. Some viable anthers showed wall ruptures and early callus formation, and upon first transfer to fresh media, differentiation processes accelerated.

The culture media had varied compositions, with some variants containing 2,4-D, while others used balanced combinations of IAA, BAP, and Z. Although 2,4-D media induced more callus formation, media 1 and 2 facilitated both callus and organogenic structure development (15.3% in **Ștefănești 24** to 30.5% in **Argeș 20**).

Anther culture and in vitro plant regeneration

To initiate anther culture, flower buds selected based on the size and stage of development of the anthers were used. Before inoculation, the biological material was maintained at 4°C, which led to a significant increase in the viability of the explants. In the absence of this treatment, the cultures showed early necrosis and did not generate a morphogenetic response.

Following the treatment, the viability of the anthers after two weeks of cultivation in the dark ranged between 33.9% (**Argeș 20**) and 47.9% (**Argeș 11**). During the cultivation, cases of rupture of the anther wall were observed, followed by the initiation of callus formation. After the first transfer to fresh media, the differentiation processes were accelerated (Fig.2.C).

Fig.2 Processes of differentiation and regeneration in callus derived from tomato anthers over 5 months from culture initiation – A. Differentiation of callus into embryogenic tissue (months 2–3), B. Callus induction from anthers, C. Plant regeneration after 5 months from culture initiation / *Fig. 2 Procese de diferențiere și regenerare în calus derivat din antere de tomate peste 5 luni de la inițierea culturii – A. Diferențierea calusului în țesut embriogen (lunile 2–3), B. Inducerea calusului din antere, C. Regenerarea plantelor după 5 luni de la inițierea culturii*

The composition of the culture media varied: some variants contained 2,4-D as the main regulator, while others included combinations of IAA, BAP, and zeatin. The 2,4-D media predominantly induced callus formation, without subsequent differentiation, while the media designated 1 and 2 allowed both callus formation and the initiation of organogenesis, with frequencies ranging from 15.3% (**Ștefănești 24**) to 30.5% (**Argeș 20**).

The explants were subsequently transferred to differentiation media (variants 5 and 6), where two distinct behaviors were recorded depending on the initial medium. Explants from media 1 and 2 showed organogenesis capacity, with percentages ranging between 6.9% (**Ștefănești 24**) and 27.5% (**Argeș 20**) on medium 5 (0.5 mg·L^{-1} IAA + 0.25 mg·L^{-1} Z) and between 0% (**Argeș 20**) and 18.5% (**Costate 21**) on medium 6 (2.0 mg·L^{-1} BAP + 1.0 mg·L^{-1} IAA). Explants from media 3 and 4 (with 2,4-D) formed only calluses, which went into necrosis regardless of the differentiation medium used subsequently.

After five months, the formed shoots were transferred to hormone-free media, where they achieved efficient rooting (Fig. 2). Experiments repeated for three years revealed a stable regenerative behavior, specific to the genotypes analyzed. The genotypes **Argeș 11** and **Ștefănești 24** generated calluses with embryogenic potential, ´Ștefănești 22´ showed root differentiation, and the genotypes **Argeș 20** and **Costate 21** regenerated complete, morphologically normal plants.

These plants were successfully acclimatized under *ex vitro* conditions, maintaining viability and morphological stability during two consecutive growing seasons. The results obtained demonstrate the essential influence of the type of growth regulator on the direction of morphogenetic development, as well as the need to adapt culture protocols depending on the specific response of each genotype. This aspect is crucial for streamlining *in vitro* regeneration processes within tomato breeding programs.

Morphological characterization of plants regenerated by anther culture

Following regeneration by anther culture, plants obtained from the cultivars **Costate 21** and **Argeș 20** showed significant morphological variability, with obvious differences compared to the donor cultivars from which the flowering buds were taken.

Morphological characterization was performed after acclimatization of the seedlings under ex vitro conditions and their transfer to the greenhouse, using phenotypic descriptors specific to tomatoes, following the international *Lycopersicon* (*Solanum lycopersicum*) descriptor standards (IPGRI, 1996). The traits monitored included plant height, leaf density, leaf shape, floral characters, and fruit morphology. For the cultivar **Costate 21**, out of the 36 plants regenerated *in vitro*, 20 were successfully acclimatized and transferred to the soil in the greenhouse. Plant height ranged between 140 and 250 cm, with only 9 plants registering a large size (code 7), similar to the donor cultivar. The others were medium (code 5) or small (code 3), often correlated with necrotic inflorescences or seedless fruits. The leaf type varied between the standard leaf (code 3) and the peruvianum type (code 4), but a plant with a dwarf leaf type (code 1) was also identified. The position of the stigma, essential for the self-fertilization capacity, was different in the regenerants: from the inserted style (code 1) to the highly exerted style (code 4), which may influence fertility. The shape of the fruits varied from flattened to round, the color at maturity being predominantly red, except for two plants in which the fruits remained green until late autumn. Only 9 of the 17 plants that reached maturity produced fruits with viable seeds.

In the case of the variety **Argeș 20**, 10 seedlings were transferred to the greenhouse in three stages. Only 5 of these plants showed normal development and produced fruits with seeds. Plants acclimatized later (December and January) showed weaker vegetative development, lower height, and small and uneven fruits. Even though the initial appearance was similar to that of the control, morphological differences were accentuated in the greenhouse, suggesting the influence of the duration of the *in vitro* phase on phenotypic stability.

To summarize these findings, Table 2 presents a comparative overview of the key morphological traits evaluated in regenerants from both varieties.

Table 2. Main morphological features of anther-derived regenerants / *Tabelul 2. Principalele caracteristici morfologice ale regeneranților derivati din antere*

Morphological Trait / Donor genotype	Costate 21 (36 regenerated, 20 acclimatized)	Argeș 20 (10 regenerated, 5 acclimatized)
Number of Acclimatized Plants	20 (55.6%)	5 (50%)
Plant Height (7.1.2.2)	140–250 cm; shorter plants showed reproductive anomalies	Not specified; growth rate varied among regenerants (between 21- 144cm)
Leaf Type (7.1.2.9)	Varied: Dwarf (1), Standard (3), Peruvianum-type (4)	Mainly Standard (3); some variations noted
Foliage Density (7.1.2.6)	Ranged from Sparse (3) to Very Dense (7)	Generally Medium (5); low variability
Style Position (7.2.1.7)	Inserted (1), Same Level (2), Slightly Exserted (3), Highly Exserted (4)	Inserted (1), Same Level (2
Fruit Shape (7.2.2.5)	From Flattened (1) to Highly Rounded (4)	Rounded to Slightly Flattened (2–4)
Fruit Cross-Section Shape (7.2.2.29)	Round (1), Angular (2)	Round (1)
Fruit Color at Full Maturity (7.2.2.11)	Mainly Red (5); plants 1 and 20 retained green, immature fruits	Mostly Red (5); some fruits with incomplete development
Plants with Viable, Seeded Fruits	9 out of 17	3 out of 5
Notable Observations	Unique plants with dense foliage/semi-erect leaves, high vigor, or unique flavor	Somaclonal variation confirmed at the molecular level; varied fertility and vigor

Molecular Analysis of Anther-Derived Lines Using SSRs

To assess the genetic variability induced during *in vitro* regeneration by anther culture and to complement the morphological characterization that may be influenced by environmental factors (Garcia *et al*., 2004; Irikova *et al*., 2011), molecular analyses were performed using Simple Sequence Repeat (SSR) markers (Shehata *et al*., 2009).

These molecular markers provide high-resolution information on genomic polymorphisms and are effective in identifying genetic variation in the analyzed tomato genotypes (*Solanum lycopersicum*) (Khan *et al*., 2020; Bădulescu *et al*., 2022).

SSR markers previously validated for their ability to reveal polymorphism among tomato cultivars regenerated from anther culture. DNA was extracted from androgenetic plants derived from both the cultivars **Costate 21** and **Argeș 20**, as well as from the donor cultivars.

Amplification of these markers generated polymorphic banding profiles, with clear allelic differences observed both between regenerated individuals and between regenerants and their respective donor cultivars (Fig. 3).

These findings demonstrate that regenerated plants are not genetically identical to the donor plants, supporting the hypothesis that the regeneration process originates from microspores rather than somatic tissues in the anther (Bull & Michelmore, 2022; Popescu *et al*., 2022; Sumedrea *et al*., 2024).

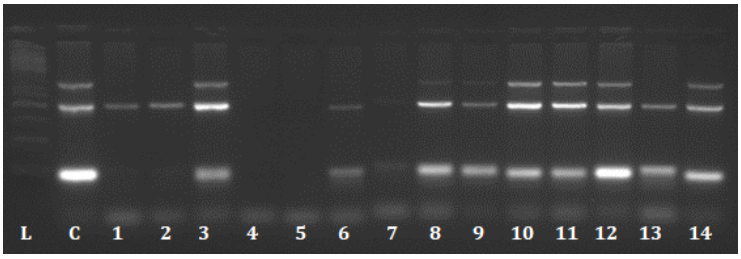

Fig. 3. Electrophoretic profiles of DNA fragments. L = molecular weight ladder; C = donor plant of the cultivar Costate 21; lanes 1–14 represent distinct regenerants obtained through in vitro anther culture / *Fig. 3. Profile electroforetice ale fragmentelor de ADN. L = marker de greutate moleculară; C = plantă donatoare din soiul Costate 21; benzile 1–14 reprezintă regeneranți diferiți obținuți prin cultură in vitro a anterelor.*

For the cultivar **Costate 21**, a wide range of genetic profiles was observed among the 20 androgenetic plants acclimatized to greenhouse conditions. Similarly, in the case of **Argeș 20**, molecular analysis of the five plants that reached maturity confirmed distinct SSR profiles, indicating somaclonal variation.

These results align with the phenotypic variability recorded during the morphological evaluation phase and confirm that genetic changes occurred during *in vitro* culture, probably due to either microspore-derived embryogenesis or spontaneous mutations induced during tissue culture. Furthermore, progeny obtained by seed germination from androgenetic plants of **Costate 21** were also analyzed. SSR profiling revealed uniform banding patterns within the seed-derived lines, but distinct from the original donor variety (Fig. 4). This suggests that the androgenetic lines have achieved genetic stability and that the traits introduced or fixed by *in vitro* regeneration were heritable. This progeny can be regarded as new genotypes, valuable for genetic improvement and selection in tomato breeding programs.

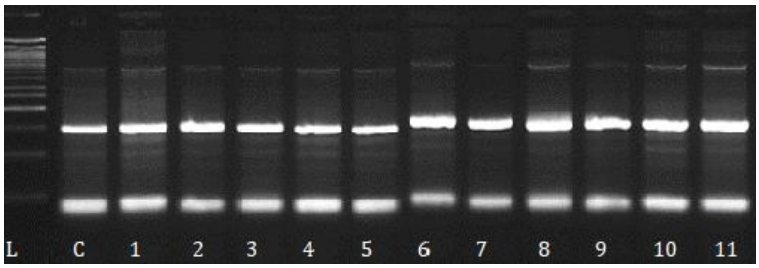

Fig. 4. Electrophoretic analysis of SSR amplified products from second-year plants derived from seeds obtained through in vitro regeneration; L = molecular weight marker; 1-9 = plants; C21 = control, Costate 21 / *Fig. 4 Analiza electroforetică a produselor amplificate SSR din plantele de al doilea an provenite din semințe obținute prin regenerare in vitro; L = marker de greutate moleculară; 1-9 = plante; C21 = control,* ***Costate 21***

The application of simple sequence repeat (SSR) molecular markers in the evaluation of regenerants derived from anther culture of **Costate 21** and **Argeș 20** tomato cultivars revealed clear patterns of induced genetic polymorphism, reflecting significant genomic differences from the

donor plants. SSR profiling enabled the detection of novel allelic configurations and the identification of stable, genetically distinct lines, thereby confirming the efficacy of these markers in characterizing somaclonal variation at a high resolution.

The observed molecular diversity underscores the genomic impact of *in vitro* androgenesis and highlights SSR markers as a powerful tool for monitoring genetic integrity, assessing clonal fidelity, and guiding selection in tomato breeding.

These results emphasize the critical role of marker-assisted analysis in ensuring the traceability and reproducibility of regeneration protocols aimed at developing new, genetically unique tomato lines.

CONCLUSION

The study demonstrated that plant regeneration from anther culture is influenced by genotype, microspore stage at harvest, and the composition of the initiation medium. Cold treatment and optimized media (1, 2, and 5) favored callus formation and regeneration, especially in the **Argeș 20** and **Costate 21** varieties, unlike media with 2,4-D, which generated only non-viable callus (without shoot differentiation or plant regeneration).

In vitro regeneration was successful for two of the tomato cultivars, **Costate 21** and **Argeș 20**, which produced regenerated plants distinct from the donor plants.

The regenerated plants showed morphological differences compared to the donor plants, confirmed by SSR analysis, which revealed genetic variations between the regenerants and the donor cultivars.

Plants regenerated from seeds obtained from the second generation demonstrated genetic stability and uniformity, indicating their potential for use in breeding programs.

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THE GENETIC HERITAGE OF RSFG BĂNEASA: PAST AND FUTURE PERSPECTIVES

PATRIMONIUL GENETIC AL SCDP BĂNEASA, TRECUT ȘI PERSPECTIVE PENTRU VIITOR

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Abstract

Fruit breeding in Romania began with the selection of local genotypes, laying the foundation for systematic breeding programs. A major milestone was achieved in 1949 when Nicolae Constantinescu and Profirie Popa initiated the first peach breeding program at the Research and Development Station for Fruit Growing Băneasa (RSFG Băneasa). Since then, it has remained a leading institution, with notable contributions from Viorica Bălan in apricot breeding and Antonia Ivașcu in peach breeding, enhancing fruit quality, adaptability, and resilience. To expand genetic diversity, RSFG Băneasa integrated germplasm from China, Italy, Spain, and the U.S., focusing on disease resistance, fruit quality, and climatic adaptability. The achievements include disease-resistant selections, flat peaches and dwarf varieties, and cultivars for industrial processing. Looking ahead, fruit breeding must remain a priority, particularly in addressing climate change. Key objectives include conserving genetic diversity and using marker-assisted selection (MAS) to develop varieties resilient to late frosts, drought, and extreme temperatures, phenomena that are increasingly affecting Romanian orchards. Expanding the genetic base through global germplasm integration will be essential for strengthening resistance to diseases, pests, and environmental stressors. Advancements in molecular genetics, digitalization, and mechanization are transforming breeding programs. Identifying molecular markers improves resistance breeding, while systematic data collection and automation optimize orchard management. Despite land and financial constraints, RSFG Băneasa remains a key player in conserving native genetic resources and fruit breeding research. Continued investment in innovation is vital for developing new varieties and ensuring the long-term viability of Romania's fruit sector.

Keywords: *breeding, apricot, peach, climate resilience, genetic diversity.*

Rezumat

Ameliorarea pomilor fructiferi în România a început prin selecția genotipurilor locale, punând bazele programelor sistematice de ameliorare. Un moment important a fost în 1949, când Nicolae Constantinescu și Profirie Popa au inițiat primul program de ameliorare al piersicului la Stațiunea de Cercetare – Dezvoltare pentru Pomicultură Băneasa (SCDP Băneasa). De atunci, stațiunea a rămas o instituție reprezentativă, cu contribuții notabile ale Vioricăi Bălan în ameliorarea caisului și Antoniei Ivașcu în cea a piersicului, îmbunătățind calitatea fructelor, adaptabilitatea și reziliența acestora. Pentru extinderea diversității genetice, SCDP Băneasa a integrat germoplasmă din China, Italia, Spania și SUA, concentrându-se pe rezistența la boli, calitatea fructelor și adaptabilitate climatică. Astfel, au fost obținute selecții rezistente la boli, soiuri de piersic plate și pitice, precum și soiuri destinate industrializării. Ameliorarea trebuie să rămână o prioritate, mai ales în fața schimbărilor climatice. Obiectivele esențiale includ conservarea diversității genetice și selecția asistată de markeri (MAS) pentru dezvoltarea soiurilor rezistente la înghețuri târzii, secetă și temperaturi extreme, fenomene care afectează tot mai mult livezile din România. Integrarea germoplasmei globale va fi esențială pentru creșterea rezistenței la boli, dăunători și factori de stres. Progresele în genetica moleculară, digitalizare și mecanizare transformă programele de ameliorare. Identificarea markerilor moleculari îmbunătățește selecția, iar automatizarea optimizează managementul plantațiilor pomicole. În ciuda constrângerilor legate de pierderea terenurilor și finanțare, SCDP Băneasa rămâne o unitate de referință în conservarea patrimoniului genetic autohton și al cercetării în domeniu. Investițiile continue în inovare sunt esențiale pentru dezvoltarea de noi soiuri și pentru viabilitatea pe termen lung a sectorului pomicol românesc.

Cuvinte cheie: *ameliorare, cais, piersic, reziliență climatică, diversitate genetică.*

INTRODUCTION

Romania has a long tradition of cultivating *Prunus* species, with historical records of apricots and peaches being grown since the 18th century (Bălan et al., 2007). However, systematic breeding efforts began in the mid-20th century. After World War II, organized fruit breeding programs were initiated under the coordination of visionary horticulturists. For peaches (*Prunus persica* (L.) Batsch), foreign cultivars were first introduced to Romania in the late 19th century (*e.g.* the Istrița nursery in 1893) and tested by nurseries and pomologists. A formal peach breeding program was established around 1949 at the Institute of Agronomic Research of Romania (ICAR) under Prof. Nicolae Constantinescu, marking the start of developing local peach varieties. The creation of a dedicated horticultural research institute in 1957 led to a new era: breeders such as Porfirie Popa at Băneasa Research Station began extensive crossing and selection work on peaches (Stănică *et al*., 2021). Similarly, apricot (*Prunus armeniaca* L.) breeding gained momentum later in the 20th century. By 1980, RSFG Băneasa had launched a program focused on apricot disease resistance (Bălan *et al*., 2000), and in 1986, a comprehensive apricot genetic improvement program was underway (Bălan *et al*., 2007).

RSFG Băneasa, located near Bucharest, emerged as a central node for these breeding activities. Together with other stations like Pitesti-Mărăcineni and Constanța, Băneasa spearheaded the development of new peach and apricot cultivars suited to Romania's temperate-continental climate (Bălan & Ivașcu, 1994). The station assembled a wide array of genetic resources, including local genotypes and exotic germplasm from the USA, Western Europe, and China, to extend the breeding pool (Ivașcu & Stinga, 2006; Sansavini *et al*., 2006). Over the decades, breeding objectives expanded from simply introducing foreign varieties to creating Romanian-bred cultivars that combine superior fruit quality with adaptability to local growing conditions (*e.g.* tolerance to cold winters and late frosts) (Bălan & Ivașcu, 1994; Bălan *et al*., 2007). Researchers at Băneasa have particularly focused on traits such as extended ripening season (from very early to late maturing cultivars), improved flavour and nutritional content, resistance to major diseases, and tree traits like reduced vigour and later bloom time to escape spring frosts.

Notably, Dr. Viorica Bălan's team concentrated on apricot genetic improvement from the 1980s onward, releasing numerous cultivars and studying the inheritance of key traits (Bălan *et al*., 2007; Bălan *et al*., 2010). In parallel, Dr. Antonia Ivașcu and colleagues have managed the peach breeding and germplasm program, emphasizing disease resistance and quality in new peach and nectarine varieties (Ivașcu & Stinga, 2006). The collaborative efforts of these scientists and others at RSFG Băneasa have built a rich genetic heritage, a legacy of breeding lines, cultivars, and preserved gene bank accessions. This article synthesizes the past achievements of the Băneasa breeding programs in peaches and apricots and discusses future perspectives. Emphasis is placed on how traditional breeding has been augmented by molecular tools and how the resulting genetic resources can help address upcoming challenges such as climate change, emerging pests, and the need for sustainable orchard management. This paper also aims to document past achievements and outline updated breeding directions focused on resilience to climate change and diversified consumer demands.

MATERIALS AND METHODS

Genetic Resources

The breeding work at RSFG Băneasa has drawn on extensive genetic resources maintained on-site and via collaborations. In apricot, a broad gene pool of **over 650 apricot genotypes** was preserved, representing landraces and diverse cultivars from at least seven geographic regions (Bălan *et al*., 2007). This living gene bank included both Romanian traditional varieties and valuable foreign accessions, ensuring a wide reservoir of traits (fruit characteristics, phenology,

stress tolerances) for breeding. Until the 1990s, the RSFG Băneasa collection of peach and nectarine varieties included approximately 670 elite selections, along with thousands of hybrid seedlings (Ivașcu & Stinga, 2006). Germplasm sourced from China and other centers of diversity was especially important for introducing novel genes for disease resistance and adaptation (Sansavini *et al*., 2006). Wild relatives and exotic *Prunus* species (such as *P. davidiana* and *P. ferganensis*) were also evaluated as potential donors of pest and disease resistance alleles (Ivașcu & Stinga, 2006). Throughout the breeding programs, planned crosses were conducted using selected superior cultivars, combining cold-hardy or flavourful local varieties with disease-resistant or late-blooming exotic genotypes (Bălan & Ivașcu, 1994; Sansavini *et al*., 2006).

Breeding Methods

The Băneasa Research Station employed **classical breeding techniques** for both apricot and peach improvement. Controlled hybridization was the primary method: hundreds of cross combinations were made to generate first-generation (F_1) hybrid populations. For apricots, a diallel crossing scheme was used among selected parents with desirable quality attributes, yielding 12-50 F_1 seedlings per family (Bălan et al., 2006). Subsequent generations were produced to amplify genetic variation: self-pollination of F_1 hybrids created F_2 populations (when self-compatibility allowed), and back-crosses were done by crossing F_1 hybrids with one of their parents to introgress specific traits (Bălan et al., 2006). In addition, **physical mutagenesis** was explored to induce novel traits: for instance, buds of the apricot cultivar **Comandor** were irradiated with 60Co (3000 R) and grafted, producing mutant lines (V_1 and V_2) that were then incorporated into the breeding program (Bălan *et al*., 2006). Open pollination in germplasm orchards provided another source of variable seedlings, especially in peach; thousands of such open-pollinated seedlings were screened for promising traits (Ivașcu & Stinga, 2006).

Selection and evaluation practices

Selection and evaluation practices were rigorous and long-term. Seedling screening for disease resistance was initiated at early stages, for example, young peach hybrids were exposed to pathogens (in greenhouse or nursery conditions) to quickly eliminate susceptible individuals (Ivașcu & Stinga, 2006). Apricot seedlings were often inoculated or tested under high disease pressure for fungal diseases (like *Stigmina carpophila* leaf spot and *Monilinia* fruit rot) and observed for any *Plum Pox Virus* (sharka) symptoms (Bălan *et al*., 2000). Only a small fraction of hybrids with superior resistance and horticultural traits advanced to field trials. At Băneasa and its regional partner stations, field trials (competitive varietal testing plots) were established to evaluate the performance of elite selections under real orchard conditions. For instance, in one apricot breeding cycle, 240 elite hybrids were planted in comparative trials at Băneasa and Constanța; about 40% of these met the stringent standards for fruit quality, yield, and resilience (Bălan *et al*., 2010). Over multiple years, data on flowering time, ripening date, yield, fruit size, flavour, and pest/disease incidence were collected. Standard pomological measurements were taken (fruit weight from samples of 20-30 fruits, soluble solids content, acidity, etc.) to compare selections with commercial cultivar benchmarks (Dumitru *et al*., 2009). Tree habit and productivity (*e.g.* presence of spur-bearing branches, tendency toward alternate bearing) were also assessed in these trials. Promising selections that consistently outperformed existing varieties across seasons and locations were proposed for release as new cultivars.

In addition to conventional hybridization and selection methods conducted within the station and its breeding department, RSFG Băneasa also implemented physical mutagenesis as a complementary breeding strategy. This approach, using cobalt-60 (^{60}Co) at a dose of 0.3 KR, proved effective in expanding phenotypic variability, particularly in the standard apricot cultivar **Comandor**. Notable outcomes included the induction of reduced tree vigour, an important trait for orchard management and high-density planting, as well as alterations in fruit quality attributes such as dry matter, vitamin C, and carotene content. Although the primary goal of mutation breeding was to generate compact tree architecture and improve fruit quality, variations also emerged in

flowering and ripening periods, and in certain cases, pigment intensity in the fruit flesh. These findings demonstrated that induced mutations could be a valuable tool in apricot improvement, offering novel genotypes that would have been difficult to obtain through crossing alone. However, some mutant lines showed reduced resistance to key pathogens such as *Stigmina carpophila* and *Cytospora cincta* compared to the parental genotype, highlighting the importance of integrated screening during selection (Bălan, 1995).

RESULTS AND DISCUSSION

Breeding Achievements and Key Cultivars

Through decades of systematic breeding, RSFG Băneasa has developed an impressive array of apricot and peach cultivars tailored to Romania's needs. In apricot, the program led by V. Bălan between 1983 and 2006 yielded over a dozen new cultivars, many of which have been officially homologated (registered) and released for commercial cultivation (Bălan *et al*., 2007). These include **Rareș**, **Valeria**, **Carmela**, **Viorica**, **Nicușor**, **Adina**, **Alexandru**, **Bucovina**, **Siret**, **Atractiv**, **Dacia**, **Excelsior**, **Favorit**, **Comandor**, and **Olimp** from the Băneasa station, among others (Bălan *et al*., 2007). Each of these varieties embodies specific improvements targeted by the breeding program. For example, **Rareș** (derived from a Băneasa hybrid cross B12/6 × NJA13) is an early-ripening apricot that matures in the first half of June, with large (60-65 g) elongated-spherical fruits of excellent taste and aroma (Bălan *et al*., 2010). 'Rareș' also has a compact growth habit (shorter internodes and fruiting on spurs), allowing higher-density plantings and earlier bearing (fruiting begins in the 3rd year) (Bălan *et al*., 2010). On the other end of the season, cultivars like **Excelsior** and **Comandor** extend apricot harvest into late summer (August), helping to extend the consumption period of fresh apricots, a major breeding goal (Bălan *et al*., 2006; Bălan *et al*., 2007). Several of the Băneasa apricots also have improved post-harvest characteristics (firm flesh, better storability) and attractive appearance (bright ground and blush colours), aligning with market demands for quality (Bălan *et al*., 2007).

In peach breeding, significant advancements were achieved at RSFG Băneasa, particularly during the 1990s and early 2000s, under the leadership of Antonia Ivașcu and Viorica Bălan. The program capitalized on an extensive gene pool of over 670 cultivars and breeding lines from Romania and abroad, and focused on developing new peach and nectarine varieties tailored to Romanian environmental challenges and market demands (Ivașcu & Stinga, 2006). By recombining diverse germplasm, including materials from China, the U.S., and Southern Europe, breeders released a wide array of cultivars, each exemplifying specific typological and commercial improvements. These included **Congres** (white flesh), **Victoria** (late ripening), **Triumf** (firm flesh), and **Amalia** (intensely coloured flesh), as well as nectarines such as **Tina** and **Dida** for improved storability, and **Antonia** for superior texture (Ivașcu & Buciumanu, 2006; Ivașcu & Stinga, 2006).

The breeding strategy targeted not only fruit quality and ripening calendar extension (from early June to late September), but also resistance to critical biotic stressors like *Taphrina deformans*, *Cytospora cincta*, *Monilinia spp.*, *Sphaerotheca pannosa*, and *Plum Pox Virus* (Ivașcu & Stinga, 2006). Disease-free cultivars such as **Triumf**, **Superba de Toamnă**, and **Victoria** were confirmed through systematic pathogen screening, including the Dienes staining technique for mycoplasma detection (Ivașcu & Stinga, 2006). Moreover, the program addressed abiotic stress tolerance, especially frost and spring fluctuation resilience, by using genitors with proven hardiness like **Amsden**, **Hardyred**, and **Canada 55111** (Ivașcu & Buciumanu, 2006; Ivașcu & Stinga, 2006).

A notable innovation was the inclusion of dwarfing traits for high-density orchard suitability, achieved via hybridization with compact genotypes (*e.g.*, **Albertina**, **Compact Redhaven**), and the development of selections like **BII 85.9.23** and **89 BII 03.21**, characterized by reduced vigour and stable productivity (Ivașcu & Stinga, 2006). To extend utility, the breeding efforts also targeted industrial traits, leading to clingstone cultivars suited for processing applications such as jams, juices, and canned fruit, derived from crosses with **Loadel**, **Andross**, and **Babygold 9** (Ivașcu & Stinga, 2006).

Altogether, the Băneasa breeding program stands out for its multidimensional approach, integrating classical hybridization, selection from open pollination, mutagenesis, and pathogen screening, resulting in more than 17 modern cultivars officially registered and adopted in Romania. These contributions reinforce RSFG Băneasa's pivotal role in shaping resilient and commercially competitive peach germplasm for southeastern Europe (Ivașcu & Stinga, 2006).

Fruit Quality and Nutritional Improvements

A hallmark of RSFG Băneasa's genetic program has been the steady enhancement of fruit quality parameters in both apricots and peaches. Quality encompasses sensory attributes (flavour, sweetness, texture) as well as nutritional content (vitamins, sugars) and technological traits (firmness, transportability). Breeding for these traits can be challenging due to complex inheritance and trade-offs, yet the Băneasa programs achieved notable progress. In apricot, by inducing greater genetic variability across generations (F_1, F_2, backcrosses, and even mutant lines), researchers observed significant gains in certain quality traits. Notably, the later-generation apricot populations (F_2, backcross, V_2 mutants) showed increased levels of soluble solids and vitamin C compared to both the original parent varieties and the F_1 generation (Bălan *et al*., 2006). This indicates successful transgressive segregation for sugar accumulation and nutritional quality, presumably by combining complementary alleles from different genitors. For example, using high-sugar cultivars like '**Excelsior**' and '**Dacia**' as standards, the breeding program was able to produce descendants surpassing those standards in sweetness and dry matter content (Bălan *et al*., 2006). Such improvements are crucial for modern apricot cultivars to meet consumer expectations for richer taste and for processing uses (dried apricots, juices) requiring high soluble solids (Dumitru *et al*., 2009). Peach breeding similarly puts emphasis on flavour and texture. Through careful selection, new peaches like '**Eugen**' (a melting-flesh type with aromatic flavour) and firm-flesh types like '**Triumf**' were obtained, balancing the need for excellent taste and aroma with firmness adequate for handling (Ivașcu & Stinga, 2006). The incorporation of diverse parental lines (including some heirloom flavourful peaches) helped ensure that improved yield or disease resistance did not come at the expense of flavour, a common risk in breeding (Dumitru *et al*., 2009). By measuring °Brix (sugar content) and titratable acidity in hundreds of hybrid fruits each year, the breeders could select those with a high sugar/acid ratio conferring a sweet, well-balanced taste. Additional recent studies confirmed that both genotype and applied agrotechnologies significantly influence fruit firmness and productivity in apricot. For instance, Oltenacu *et al*. (2025) evaluated three apricot cultivars at RSFG Băneasa and demonstrated that varietal differences and management practices jointly affect key traits such as yield per tree and pulp firmness, reinforcing the role of integrated breeding and orchard management approaches in fruit quality improvement. The end result is that many of the Baneasa-derived cultivars are recognized for superior eating quality, a testament to the breeders' success in marrying quality with other agronomic traits.

Disease and Pest Resistance

A major component of the Băneasa genetic heritage is enhanced resistance to diseases that commonly afflict *Prunus* species. In apricots, the program explicitly targeted a range of fungal and viral diseases, aiming to create multi-resistant cultivars, a visionary goal at a time (1980s) when most apricot breeding worldwide focused on single disease resistance. By the late 1990s, Băneasa breeders had achieved selections that combined resistance to four or more diseases in one genotype

(Bălan *et al*., 2000). For example, two promising apricot selections from that era, coded '83.34.6 BI' and '83.34.7 BI', exhibited strong resistance to brown rot (*Monilinia laxa*), shot-hole disease (*Stigmina carpophila*), cankers (*Cytospora cincta*), and *Alternaria* fruit rot, and importantly showed no symptoms of Plum Pox Virus (PPV, sharka) on leaves or fruit even under both artificial inoculation and natural exposure (Bălan *et al*., 2000). This breadth of resistance is remarkable, effectively addressing the most economically serious apricot diseases in Romania. Although achieving the ideal combination of high resistance and top fruit quality in a single cultivar is difficult (some of those selections had only moderate fruit size or flavour) (Bălan *et al.*, 2000), the work demonstrated that the resistant gene sources could be successfully combined. Indeed, crosses of resistant x resistant or even resistant x susceptible parents were pursued to accumulate resistance genes, and biochemical markers were explored to track resistance alleles in progeny. The program was pioneering in also recognizing emerging threats: for instance, in 1997 a new apricot breeding subprogram was initiated to improve resistance to *Schizophyllum commune*, a wood decay fungus causing trunk rot in orchards (Bălan *et al*., 2000). By proactively breeding for such traits, RSFG Băneasa has been able to release apricot cultivars with substantially better disease profiles than older varieties. Cultivar **Comandor** is one example often noted for its field tolerance to major apricot diseases while delivering large, high-quality fruit; it has become a preferred cultivar in Romanian orchards partly for this reason (Bălan et al., 2007).

Peach and nectarine breeding at Baneasa similarly integrated disease and pest resistance objectives. Peach leaf curl caused by *Taphrina deformans*, powdery mildew (*Sphaerotheca pannosa var. persicae*), brown rot (*Monilinia*), bacterial canker (*Cytospora/Leucostoma*), green aphid (*Myzus persicae*), and Oriental fruit moth (*Cydia molesta*) are among the challenges for peach growers (Ivașcu & Stinga, 2006). By collecting and preserving gene sources from around the world, the Baneasa collection included resistance donors for many of these issues (Ivașcu & Stinga, 2006). For example, Chinese wild peach (*P. davidiana*) accessions, which are known to carry genes for leaf curl and powdery mildew resistance, were utilized to transfer those traits into new (Sansavini *et al*., 2006). One outcome of this approach is the creation of peach lines with significantly improved spring health: some Băneasa-bred peaches show high field resistance to leaf curl, a disease that can otherwise defoliate trees in spring and reduce yield (Ivașcu & Stinga, 2006). The use of *P. davidiana* and other wild species has also been crucial in efforts to breed for Plum Pox Virus resistance in peach, a particularly difficult goal since most commercial peaches are highly susceptible to this virus. In fact, conventional breeding for PPV resistance in peach is so challenging that transgenic approaches have been researched internationally as a potential solution (Sansavini *et al*., 2006). While Romania has not deployed transgenic fruit trees, the Băneasa program has identified a few parent lines (such as certain *Prunus* hybrids from INRA France) that carry partial PPV tolerance (Sansavini *et al*., 2006), and these have been included in crossing. Additionally, green aphid resistance, important for reducing virus transmission and minimizing tree damage, was actively pursued. For instance, the French selection S2678, noted for its resistance to *Myzus persicae*, was identified as a valuable parent in breeding programs (Sansavini *et al*., 2006). By 2006, the peach improvement program at Băneasa had produced a considerable pipeline of breeding material: over 2500 F_1 hybrids, 1250 self-pollinated lines, 412 F_2, and 625 mutant lines were under evaluation (Ivașcu & Stinga, 2006), many of them being screened specifically for pest and disease resistance traits. The breeding objectives between 1990-2000 were firmly centered on obtaining cultivars resistant to the main diseases causing crop losses in Romania (leaf curl, canker, mildew, PPV, brown rot) (Ivașcu & Stinga, 2006). Subsequently, in 2000-2006 the objectives expanded to also include resistance to abiotic stresses and orchard performance (yield consistency, tree form) (Ivașcu & Stinga, 2006), as discussed below. The concerted focus on pathology in breeding has ensured that the modern varieties coming out of Băneasa's programs are far more robust than older cultivars, reducing the need for chemical controls and contributing to more sustainable fruit production.

Adaptation to Climate and Environmental Conditions

Adapting fruit cultivars to local environmental stresses has been a defining theme of the RSFG Băneasa breeding strategy. Romania's climate, with cold winters, late spring frosts, and hot summers, can be challenging for peaches and apricots, which originate from warmer or more stable climates. Thus, breeding for cold hardiness and frost avoidance has been critical. One approach has been to select for late-flowering genotypes in apricot, so that bloom occurs after the most dangerous spring frost period. The breeding program successfully obtained new apricot hybrids whose bloom time is significantly later than that of their parent varieties, without losing early ripening of fruit (Bălan *et al*., 2007). This was achieved by crossing standard cultivars with late-blooming wild or Central Asian apricot sources, and by cumulative selection over generations. The result is cultivars that often bloom several days (or even a week) later than traditional Romanian apricots, greatly reducing the risk of flower bud damage from late frosts, an adaptation of great value to growers in colder regions. Additionally, the winter hardiness of flower buds and wood was improved. Many Băneasa apricot releases can tolerate winter temperature drops that would have killed or injured older varieties; for instance, they can withstand -20°C to -25°C without serious bud loss, a reflection of genes from hardy genitors and selection under Romania's continental winters (Bălan *et al*., 2007). In peaches, a similar attention was given to frost resistance: breeding objectives explicitly included improving resistance to frost and spring temperature fluctuations, acknowledging the reality of climate volatility (Ivașcu & Stinga, 2006). Modern Băneasa-bred peaches like **Triumf** and **Congres** are noted to have survived severe winter conditions and late frosts better than standard varieties, ensuring more reliable yields year to year (Ivașcu & Stinga, 2006). To address high summer temperatures and periodic droughts (which are increasing with climate change), selection for robust tree health and water-use efficiency is indirectly achieved by choosing only those seedlings that fruit consistently under the relatively low-input conditions of the research station (minimal irrigation, typical Romanian soil conditions). The objective of creating cultivars that do not exhibit alternate bearing, *i.e.* that crop reliably each year despite weather stresses, has been part of the breeding goals since 2000 (Ivașcu & Stinga, 2006). Achieving regular bearing is partly genetic and partly management, but by selecting genotypes that set flower buds even after a heavy crop or a weather shock, Băneasa breeders have developed varieties with more stable productivity. Indeed, field trials noted that for certain new apricot selections, the genotype had a greater influence on yield than the seasonal effect, meaning some breeds consistently yielded well across different years (Dumitru *et al*., 2009). This yield stability is a key indicator of good environmental adaptation.

Another aspect of adaptation is tree architecture and vigour. In high-density modern orchards, more compact trees with spur-type fruiting (short fruiting branches) and reduced vigour are desirable, as they better tolerate mechanical pruning and facilitate harvest. The apricot program introduced spur-bearing traits (short fruiting twigs) into new selections, in part via cytoplasmic inheritance and mutagenesis, resulting in some dwarf or semi-dwarf phenotypes (Bălan *et al*., 2007). Meanwhile, the peach program explicitly pursued dwarfing traits by selecting or hybridizing dwarf genotypes (Ivașcu & Stinga, 2006). By incorporating such traits, new cultivars like **Alexia** and others can be grown on their own roots or on semi-dwarf rootstocks with a smaller canopy, fitting modern orchard systems. Rootstock development itself, while outside the scope of this article, complements scion breeding; ongoing work at Băneasa includes evaluating new rootstock genotypes that confer improved adaptability (*e.g.* tolerance to calcareous soil or drought) to grafted peach trees (Bălan & Ivașcu, 1994). Overall, the genetic improvements in phenology (bloom and ripening time), stress tolerance, and growth habit have produced cultivars finely tuned to Romania's environmental conditions, reflecting the value of long-term local breeding versus direct import of foreign varieties.

Future Perspectives – Challenges and Opportunities

The genetic heritage developed at RSFG Băneasa forms a strong foundation to confront future challenges in fruit growing. One looming challenge is climate change, which is expected to cause shifts in temperature patterns, more frequent extremes, and new pest/disease pressures. Fortunately, many traits already emphasized in the breeding programs directly contribute to climate resilience. For instance, late blooming and frost-hardiness will be even more crucial if winters continue to fluctuate and spring frosts become less predictable (Ivașcu & Stinga, 2006). The work done to accumulate frost tolerance genes in the breeding stock (Ivașcu & Stinga, 2006) will help in developing new cultivars that can cope with erratic winter weather and sudden cold snaps. Similarly, tolerance to heat and drought conditions, while not explicitly a trait targeted in older programs, can be found in some of the diverse germplasm (such as hardy apricots from Central Asia, or peaches from the Mediterranean) that Băneasa has preserved. These can be utilized in future crosses to introduce traits like deeper root systems or higher water-use efficiency.

Marker-assisted selection (MAS) will become an essential tool in accelerating the breeding process at Baneasa. It will allow the tracking of key alleles linked to disease resistance, fruit quality, and stress tolerance. By identifying QTLs and associated molecular markers, MAS will enable early selection in large populations, thus improving efficiency and reducing the time and cost of traditional phenotypic screening.

Another concern with climate change is the potential range expansion of pests and diseases. Insect vectors and pathogens not previously seen in Romania may become problematic. The broad-spectrum disease resistance breeding approach at Băneasa will need to continue expanding to address issues such as *Xanthomonas* bacterial spot (already a peach issue in warmer regions) or new viruses. Here, modern molecular breeding will be a crucial ally: advanced genomic tools could enable quicker identification of resistance genes from wild species and marker-assisted introgression of these genes into elite lines.

International collaboration will also play a role, as Romanian researchers can exchange germplasm and data with other breeding centers tackling similar issues. The ever-evolving consumer preferences pose another set of challenges. Tastes may shift towards even more convenient and novel fruit types, and there is growing interest in very large apricots, or peaches with unique flesh colours and nutraceutical benefits. As consumer preferences evolve toward new fruit types with attractive flavour, texture, and appearance, diversification of the breeding targets is becoming increasingly relevant. The Romanian experience with nectarine cultivars, such as those developed at the Constanța Research Station, demonstrates the importance of selecting cultivars suited for both fresh markets and processing, with appealing attributes such as white/yellow flesh, semi-dwarf growth, and good storability (Gavăț *et al*., 2022). The genetic variability maintained in Băneasa's collections (including, for example, apricot varieties with red flesh or high antioxidant content) offers opportunities to breed such novel fruits. Breeders will need to be creative "breeder's ingenuity and imagination" as Sansavini noted (Sansavini *et al*., 2006) in combining these traits to keep the fruit industry dynamic and appealing.

Finally, sustaining this genetic heritage requires ongoing support for research and conservation. The accomplishments to date, dozens of high-quality, locally adapted cultivars, underscore the value of long-term breeding programs. In light of ongoing land disputes and urban expansion in the Băneasa area of Bucharest, RSFG Băneasa has initiated the relocation of its genetic collection to the Moara Domnească Experimental Base. This site, located northeast of the capital in Ilfov County, approximately 17 km from Bucharest, lies within the Vlăsia Plain, a subunit of the Romanian Plain. The farm belongs to the Research and Development Station for Fruit Growing Băneasa and offers improved agroclimatic and logistical conditions for the long-term conservation, restoration, and development of valuable *Prunus* germplasm.

Continuing these programs will require training new generations of plant breeders, investing in genomic and phenotyping infrastructure, and maintaining rich field collections. In summary,

RSFG Băneasa's past and present work has positioned Romania as a key source of valuable apricot and peach germplasm. By leveraging both traditional breeding knowledge and cutting-edge science, the station is well-prepared to enhance this genetic heritage and address the future needs of fruit growing in a changing world. The cultivars and knowledge developed at Băneasa will not only benefit Romanian agriculture but may also contribute globally, as breeders everywhere seek germplasm resilient to climate stress while delivering top-quality fruit (Ivașcu & Stinga, 2006; Sansavini *et al*., 2006). This genetic heritage is thus a living legacy, one that continues to grow and evolve with each new challenge and innovation.

CONCLUSIONS

Rich Germplasm Foundation

RSFG Băneasa has amassed a broad genetic base of apricot and peach germplasm (including hundreds of local and foreign accessions), which underpinned the successful creation of new cultivars adapted to Romania's climate. This extensive gene pool remains a critical resource for future breeding efforts.

Successful Breeding Programs

Classical breeding at Băneasa (hybridization, selection, and even mutagenesis) has yielded numerous high-quality apricot and peach cultivars with desirable traits, from improved fruit quality and extended ripening seasons to enhanced disease resistance and cold hardiness. These cultivars (*e.g.* **Rareș** apricot, **Congres** peach, among many others) exemplify the genetic progress achieved in the last decades.

Improved Disease Resistance

The breeding programs have effectively addressed major biotic stresses by combining multiple disease resistance genes in new varieties. Apricot selections with combined resistance to *Monilinia*, *Stigmina*, *Cytospora* and Plum Pox Virus were developed, and peach breeding integrated resistance sources for leaf curl, powdery mildew, canker, and pests. This results in more robust cultivars requiring fewer chemical inputs.

Adaptation to Local Conditions

Băneasa's cultivars are notable for their adaptation to Romania's environment, featuring late bloom times to avoid frost, tolerance to winter cold, and regular bearing even under variable conditions. Traits like reduced tree vigour (spur-type growth) and a wide harvest window (early and late maturing varieties) have been incorporated to support modern, intensive orchard systems and market demands.

Integration of Modern Tools

Advancements in genetic and digital technologies are being embraced to further enhance breeding outcomes. Molecular markers and QTL mapping are increasingly used to inform crosses and assist in selecting seedlings with desired traits, thereby accelerating breeding cycles. Additionally, tools like the CROM expert system support optimal orchard management by assessing site factors and guiding improvements, indirectly benefiting the selection and performance of new cultivars.

Future-Ready Strategies

The genetic heritage at RSFG Băneasa provides a strong platform to face future challenges such as climate change and evolving pests. Ongoing and future breeding strategies will focus on incorporating stress resilience (drought, heat, frost) and novel quality attributes, leveraging both the rich germplasm and molecular breeding techniques. By continuing the synergy of traditional breeding expertise with modern science, RSFG Băneasa is well positioned to sustain and expand its contributions to fruit breeding in Romania and beyond.

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EXPERIMENTAL RESEARCH OF A MULTI-FUNCTIONAL QUICK-FREEZING EQUIPMENT

CERCETAREA EXPERIMENTALĂ A UNUI ECHIPAMENT MULTIFUNCȚIONAL DE CONGELARE RAPIDĂ

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Abstract
The fastest method for cooling food products is the use of liquid nitrogen, which comes into direct contact with the food to be frozen. The paper addresses the experimental research of the multi-functional quick-freezing equipment – ICR, developed by INMA within a national research program. The aim was to determine the qualitative working indices and energy indices of the equipment during the quick-freezing process of three species of berries, namely blueberries, strawberries and raspberries. Following the analysis of the experimental data, there were found the following: the minimum total freezing time was recorded in the case of blueberries and the maximum total freezing time was recorded for strawberries; for blueberries and strawberries, the average linear freezing rates recorded values corresponding to a very fast freezing process and for raspberries, the average linear freezing rate recorded a value corresponding to a fast freezing process; the liquid nitrogen consumption recorded a minimum value for blueberries and a maximum value for raspberries. It was also found that the solution of utilizing the "exhausted" thermal agent (nitrogen gas) obtained from the quick-freezing process, in order to reduce the thermal load of the refrigeration unit within the classic freezing chamber, leads to a reduction in hourly electricity consumption by 30.96%.

Keywords: *highly perishable, quick-freezing, cryogenic freezing, liquid nitrogen, berry species*

Rezumat
Cea mai rapidă metodă pentru răcirea produselor alimentare constă în utilizarea azotului lichid, care intră în contact direct cu alimentul de congelat. Lucrarea abordează cercetarea experimentală a echipamentului multifuncțional de congelare rapidă – ICR, dezvoltat de INMA în cadrul unui program național de cercetare. S-a urmărit determinarea indicilor calitativi de lucru și a indicilor energetici ai echipamentului, pe parcursul desfășurării procesului de congelare rapidă a trei specii de fructe de pădure, respectiv afine, căpșune și zmeură. În urma analizei datelor experimentale s-au constatat următoarele: timpul total minim de congelare s-a înregistrat în cazul afinelor iar timpul total maxim de congelare s-a înregistrat pentru căpșune; pentru afine și căpșune vitezele medii liniare de congelare au înregistrat valori corespunzătoare unui proces de congelare foarte rapidă iar pentru zmeură viteza medie liniară de congelare a înregistrat o valoare corespunzătoare unui proces de congelare rapidă; consumul de azot lichid a înregistrat o valoare minimă pentru afine și maximă pentru zmeură. Deasemenea, s-a constatat faptul că soluția de valorificare a agentului termic ”epuizat” (azot gaz) obținut în urma procesului de congelare rapidă, pentru a reduce sarcina termică a agregatului frigorific din incinta de congelare clasică, conduce la o reducere a consumului orar de energie electrică cu 30,96 %.

Cuvinte cheie: *foarte perisabil, congelare rapidă, congelare criogenică, azot lichid, specii de fructe de pădure*

INTRODUCTION

Horticultural products play a very important role in human nutrition through their intake of vitamins, mineral substances and antioxidants (Gherghi, 1994; Hoffmann *et al*., 2014; Cirillo *et al*., 2023), contributing to a better functioning of metabolic processes in the human body. These products contain water in proportion of 80–90% (Khan *et al*., 2017), which favours microbial activity and enzymatic reactions within the cells, resulting in chemical degradation and loss of quality. Taking into account the previously presented conditions, horticultural products are highly

perishable, often requiring preservation processes. Preservation technologies aim to lower the intensity of metabolic processes like respiration and transpiration and also the activity of pathogenic microorganisms which represent the main cause of decomposition processes.

To extend the valability period of perishable products, various preservation methods can be applied (Ingeau *et al*., 2015; Biglia *et al*., 2016; Sousa-Gallagher *et al*., 2016; Alhamdan *et al*., 2018a; Alhamdan *et al*., 2018b; Bilbao-Sainz *et al*., 2019): decreasing the water content of the product (concentration, dehydration); using high temperatures (sterilization, pasteurization); using inhibitors of deterioration phenomena (chemical preservatives, bacteriocins, natural antimicrobials, acidification, sugar addition etc.); using low temperatures (modified atmosphere, freezing, refrigeration); using non-conventional techniques (high pressure, high voltage electrical pulses, ionizing radiation, non-ionizing radiation, ultrasound, photodynamics, ozone etc.). The best results in terms of keeping the attributes and quality of the horticultural products at a level as close as possible to that of the fresh product, are obtained when using artificial cold (refrigeration, freezing), despite all other methods (Aghdam and Bodbodak, 2014; Cao *et al*., 2018; Jha *et al*., 2018; Gales *et al*., 2022).

Freezing, as a method of preservation, increases the permissible storage time of food products by more than 5...50 times compared to preservation by refrigeration (Niculita, 1998). The increase in long-lasting quality obtained by freezing is based on the effects of low temperatures of strongly slowing down or completely inhibiting the development of microorganisms, of reducing or stopping metabolic processes in the case of living products, and of reducing chemical and biochemical reactions.

Depending on the value of the average linear freezing rate, Wm, the International Institute of Refrigeration (Institut International du Froid - IIF) recommends the following classification of freezing methods:

- slow freezing Wm = 0.5 cm/h;
- quick freezing Wm = 0.5...3 cm/h;
- very quick-freezing Wm = 3...10 cm/h;
- ultra-quick-freezing Wm = 10...100 cm/h.

The usual freezing of products, during which their average temperature drops below -10°C in a period of time that does not allow the triggering of unwanted enzymatic and microbiological reactions, is characterized by the adoption of average linear freezing rates of 0.1...0.5 cm/h.

If the decrease in temperature is achieved slowly, a progressive formation of small and large ice crystals is obtained, which can destroy the cellular structure of the product and irreparably destroy the tissues upon defrosting (Delgado and Rubiolo, 2005; Buggenhout *et al*., 2006; Chassagne-Berces *et al*., 2009; Kotwaliwale *et al*., 2012; Chaudhary, 2021; Parandi *et al*., 2022; Loayza-Salazar *et al*., 2024). If, on the contrary, these temperatures are reached quickly, crystallization can be avoided by creating an amorphous phase, characterized by the formation of small ice crystals (Li and Sun, 2002; Van der Sman *et al*., 2013; James *et al*., 2015; Zhu *et al*., 2020; Li *et al*.,2025), that favours the stability of the products during the following storage period (Kennedy, 2003; Fellows, 2017), while limiting weight loss due to dehydration (Mulot *et al*., 2019).

The main methods of freezing food products are:

- cooled air freezing;
- freezing by contact with cold metal surfaces;
- freezing by contact with intermediate agents;
- freezing with cryogenic agents.

The cooled air freezing method is the most widespread due to the fact that most food products lend themselves to this type of preservation. In general, the application of the cooled air freezing method requires the existence of a closed, thermal insulated space, an air-cooling aggregate and a system of distribution of cooled air over the products.

In the case of freezing by contact with metal surfaces, the heat is taken from the products, by direct transfer, by the cooled surface. Surface cooling is achieved either with a vaporizing refrigerant or with an intermediate agent. Heat transfer is carried out in most cases exclusively by thermal conductivity.

Freezing by contact with intermediate agents consists in cooling the product by contact with a cooled intermediate agent, such as for example an aqueous solution of propylene glycol, and it is generally suitable for hermetically packaged products.

The method of freezing by contact with cryogenic agents consists in using the latent heat of vaporization at atmospheric pressure of some cryogenic liquids as well as the sensible heat that the formed vapours absorb by increasing their temperature from the very low level of vaporization to a level close to the temperature at which the product is frozen. The cryogenic agents used in this case are: liquid nitrogen, nitrogen oxide, carbon dioxide.

Liquid nitrogen freezing equipment can be of discontinuous (cryogenic cabinet) or continuous (linear tunnel, spiral freezer, immersion tunnel) operation:

- Cryogenic cabinet: is equipment designed for discontinuous operations and intended for users whose production needs (100...500 kg of products per hour) do not justify the installation of continuous operation equipment. The product must be placed in trays positioned on a stainless-steel rack. An automated system regulates the introduction of nitrogen, depending on the temperature and duration of the desired cycles.

- Linear tunnel: consists of an insulated room in which the product is introduced with the help of a stainless-steel conveyor belt. In the pre-cooling zone, the product is covered in countercurrent, by a cold flow of nitrogen gas; in the middle zone, a first superficial freezing takes place where a partial contact occurs between the product and the liquid nitrogen which, through vaporization, takes the heat of the product causing its quick freezing. In the last zone, the temperature of the product is homogenized, being brought to the desired value in the centre of the product. The tunnel is equipped with an automated system that regulates the introduction of cryogenic fluid, so as to maintain a regulated temperature inside. In addition, a conveyor belt speed control system and a series of homogenizing fans and exhaust gas extractors are provided.

- Spiral freezer: is a compact equipment that allows obtaining very high production capacities occupying an extremely small space. It consists of an insulated room, in which the product is introduced by means of a loop conveyor belt, made of stainless steel, which moves in a spiral. Liquid nitrogen is introduced at the top where, by vaporization, it creates a quick-freezing zone. With the help of a ventilation system, cold gases are directed to the bottom, where they pre-cool the incoming product. The spiral freezer is equipped with an automated system for regulating the temperature and speed of the conveyor belt.

- Immersion tunnel: is the ideal system if the available space is limited, when a very quick freezing is required or a superficial crust is desired. The product is introduced into the tunnel using a stainless-steel conveyor belt and passes through the liquid nitrogen bath. In this way, the surface crust is formed instantly, while freezing inside requires very short times.

The use of liquid nitrogen for the quick freezing of food is one of the most common applications of gases in the food industry, being carried out with the aim of maintaining the qualities of the product for as long as possible, both from an organoleptic and nutritional point of view, through the quick lowering of the temperature to values lower than -18°C inside the product, so as to inhibit the activity of microorganisms.

The fastest method for cooling food products is to use liquid nitrogen, which comes into direct contact with the food to be frozen. Nitrogen, the main component of the atmosphere, is odourless, colourless, tasteless and inert, and has no harmful effect on food. At atmospheric pressure, liquid nitrogen is found at a temperature of -196°C, its main characteristic being the ability to absorb a large amount of heat even at lower temperature, allowing high refrigeration efficiencies and heat transfer coefficients far superior to mechanical systems.

In the case of nitrogen, 48% of the total refrigerating capacity is represented by latent heat of phase change, and the remaining 52% is represented by the sensible heat of the vapours, which, for this reason, are recirculated in the freezing room in order to make maximum use of cooling capacity.

Unlike usual freezing methods, the cryogenic technique has the following benefits: rapid freezing, reduction of bacterial growth, minimal dehydration, significant decreasing of quantitative losses due to dehydration, optimum conservation of nutritional value, maintenance of the appearance and taste of food products, significant reduction of investment costs in production facilities.

The experimental research presented within this paper aims to determine the working qualitative indices and the energy indices in the cryogenic freezing of some berrie species, using a multifunctional quick-freezing technical equipment with liquid nitrogen, having a discontinuous operation and an automatic working regime.

The present paper addresses the experimental research of the multi-functional quick-freezing equipment – ICR, cabinet type, developed by INMA within a national research program. The aim was to determine the qualitative working indices and energy indices of the equipment during the quick-freezing process of some berrie species.

MATERIALS AND METHODS

The experimental research was carried out using three species of berries purchased from a hypermarket, respectively blueberries, strawberries and raspberries (Figure 1).

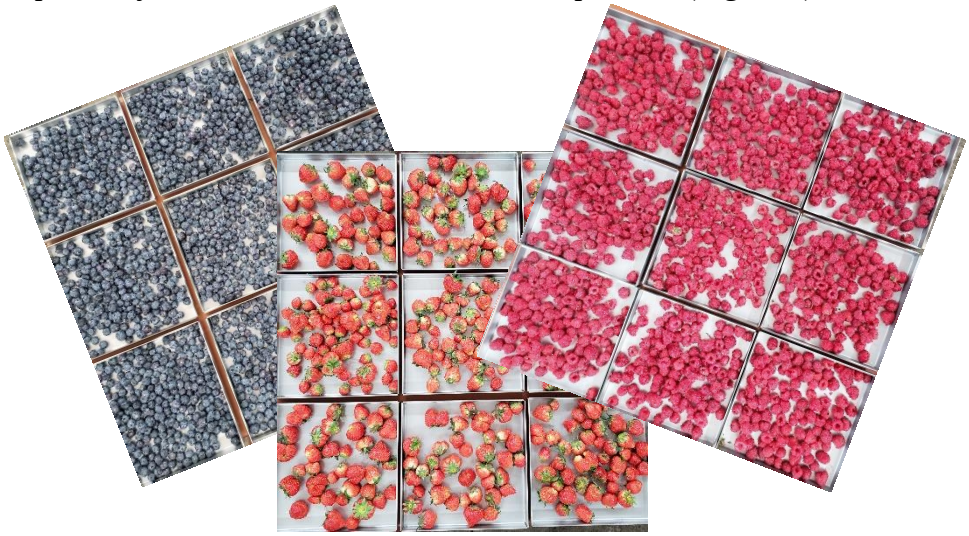

Fig. 1. Species of berries used in the experimentation */ Specii de fructe de pădure utilizate la experimentare*

The berries were subjected to a quick-freezing process using an experimental model of multifunctional quick-freezing equipment, with discontinuous operation, that uses liquid nitrogen as a thermal cooling agent, developed by INMA within a national research programme (Figure 2). At present, the equipment is subject to a national patent application (CBI A-00054/07.02.2023) and a European patent application (EPC EP23020352.3/25.07.2023).

The ICR equipment uses the latent heat of vaporisation at atmospheric pressure of liquid nitrogen, in order to reduce the temperature of the products to the frozen storage temperature. The technical equipment includes new solutions for the distribution of liquid nitrogen by using high precision nozzles and homogenising the exposure to the liquid nitrogen jets by driving in a continuous rotation movement of the rack with trays on which the products subjected to quick freezing will be positioned. The equipment also ensures the superior recovery of the "exhausted"

coolant, by reusing the cold nitrogen vapor (-30 °C) discharged from the quick-freezing room, when cooling an adjacent room for pre-cooling/temporary storage in frozen state. To prevent the formation of ice and facilitate the access to the freezing rooms at the end of the quick-freezing/pre-cooling or temporary storage in frozen state process, the access doors are equipped with sealing gaskets accompanied by defrost resistors.

Fig. 2. Experimental model of multifunctional quick-freezing equipment – ICR */ Model experimental de echipament multifuncțional de congelare rapidă – ICR*

The experimental model is endowed with temperature probes that permit continuous monitoring and control of process parameters:

- Thermocouple (chromel–alumel), type K, with rod diameter φ = 1.5 mm, measurement domain -100 °C...+30 °C, performs temperature measuring in the center of the product;
- Thermocouple, type K, with temperature transmitting segment, measurement domain -100 °C...+30 °C, performs temperature measuring on the outer surface of the product;
- Type TTR Pt 100 thermal resistance, measurement domain -200 °C...+30 °C, performs temperature measuring within the freezing room.

In order to determine the qualitative working indices and energy indices of the multifunctional quick-freezing equipment, the following measuring and control devices were used, the characteristics of which are presented in Table 1:

Table 1. The characteristics of the measuring and control devices */ Caracteristicile echipamentelor de măsură și control*

No. crt.	Name of the instrument or device	Measurement range	Measurement uncertainty/ Tolerable error
1.	Digital caliper	0÷150 mm	0,007 mm
2.	Kern electronic scale	0÷10000 g	precizie: 0,1 g
3.	Adjustable arm scale	max. 350 kg	50 g
4.	Thermohygrometer P330	-40 °C...+70 °C 0 % RH...99 % RH	incert.: 0,5 °C incert.: 1,8 % RH
5.	Digital multimeter type CA 8334	0÷10 kA; 0÷1000 V	1 %

The main measuring and control devices are presented in Figure 3.

Fig. 3. Measuring and control devices / *Echipamente de măsură şi control*

Each sample had a mass of 5000 g, 500 g on each of the 10 trays (nine trays on the rack and one tray on the floor of the quick-freezing room where there were positioned the product temperature probes: for measuring temperature in the center of the product and for measuring temperature on the outer surface of the product) (fig. 4). The average mass of a berry was determined, for each sample, as the mean of five aleatory weighings from the mass of the product. The average height and maximum equatorial diameter of the berries corresponding to each sample of the three species were also measured. This was achieved as a mean of five random measurements from the product mass.

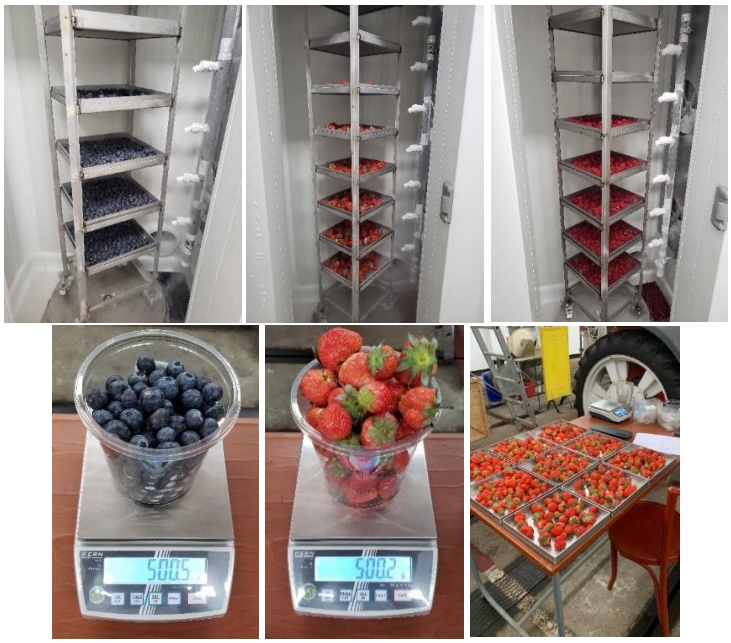

Fig. 4. Preparation of samples for experimentation / *Pregătirea probelor pentru experimentare*

Taking into consideration the geometric shapes of the three fruit species, the minimum distance between the thermal center and the external surface of the product was calculated. Assuming that, for every specie taken into account, the tissue of the fruit is homogeneous, having constant thermal properties throughout its mass, it is considered that the thermal center coincides with the mass center of the fruit.

For the fruits having regular geometric shapes, the thermal center (CT) coincides with their geometric center. The minimum distance between the thermal center CT and the external surface of the fruit is denoted by δ_0, this being a significant parameter to determine the average linear freezing

rate (fig. 5). For blueberries, δ_0 was approximated by h/2, for strawberries it was approximated by h/3 and for raspberries it was approximated by h'/2.

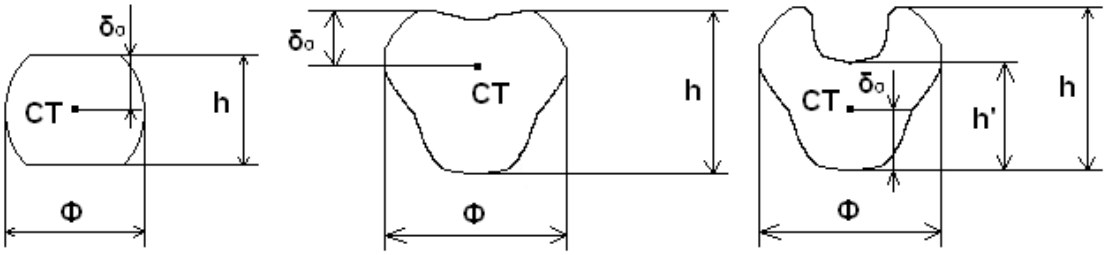

Fig. 5. Dimensional characteristics according to the geometric shape of the analyzed fruits */ Caracteristicile dimensionale în funcție de forma geometrică a fructelor analizate*

The freezing process is considered complete only when the temperature in the product thermal centre reaches -15 °C. During the quick-freezing process there were followed the main parameters, such as:

- Freezing time from 0 °C to -15 °C;
- Temperature within the quick-freezing room, at the end of the freezing process;
- Temperature on the surface of the product at the end of the freezing process;
- Temperature in the thermal center of the product, at the end of the freezing process;
- Total freezing time;
- Mass of the liquid nitrogen container used for freezing;

After processing the experimental data, the following indexes were determined:

- Average linear freezing rate;
- Liquid nitrogen consumption for a freezing cycle.

To classify a freezing process depending of cooling intensity, the average linear freezing rate is considered to be an appropriate indicator and is defined by the formula:

$$w_m = \frac{\delta_0}{\tau_0}, \, [cm/h] \tag{1}$$

where: δ_0 is the smallest distance between the thermal centre and the outer surface of the product, [cm];

τ_0 - the freezing time from a uniform initial temperature of 0 °C to the temperature to be achieved in the thermal centre, [h].

The difference between the mass of the liquid nitrogen container before and after the freezing process was completed, represents the liquid nitrogen consumption for a freezing cycle.

To evaluate the influence of the supply of coolant – "exhausted" nitrogen gas on the energy consumption of the refrigeration unit serving the pre-cooling / temporary storage room in a frozen state, the following procedure was followed:

- the refrigeration unit used for classic freezing was started and the temperature inside the enclosure was allowed to reach -18 °C;
- the experimentation within the quick-freezing room, with liquid nitrogen, was started;
- at the end of the quick-freezing cycle, the products frozen in this way were stored in the classic freezing room;
- the operating times and the rest times of the classic refrigeration unit were monitored, while the experimentation with liquid nitrogen continued in the adjacent quick-freezing room;
- after completing the quick-freezing tests, the air exhaust valves were closed, both the one in the common wall of the freezing enclosures and the one on the roof of the classic freezing room (so that there is no cold nitrogen gas supply from the quick-freezing room and no warm air from outside the classic freezing room);
- the monitoring of the operating times and the rest times of the classic refrigeration unit continued for the working of the refrigeration unit without "exhausted" nitrogen gas supply;

- both, for the working of the refrigeration unit with "exhausted" nitrogen gas supply and for the working of the refrigeration unit without "exhausted" nitrogen gas supply, the power absorbed from the electrical network was measured, in operation and at rest.

The electrical network voltage and current intensity were measured and the power absorbed (P_a) from the electrical network was determined by direct reading on the screen of the measuring instrument. The energy consumption was calculated with the formula:

$$W = \frac{P_a \cdot t}{3600} \ [kWh], \tag{2}$$

where: P_a is the power absorbed from the electrical network, [kW];

t - the time for which energy consumption is calculated, [s].

RESULTS AND DISCUSSION

Regarding the characterization of the three species of berries used, after the processing and interpretation of the experimental data, the following results were obtained:

Table 2. The characteristics of the three species of berries used for experimentation / *Caracteristicile celor trei specii de fructe de pădure utilizate pentru experimentare*

No. crt.	Characteristic	UM	Value of parameters determined in the tests		
			Blueberries	**Strawberries**	**Raspberries**
1.	Sample mass	*g*	5000	5000	5000
2.	Average mass of a berry fruit	*g*	2.220	15.367	4.313
3.	Berries maximum equatorial diameter, Φ (average of five random measurements)	*mm*	17.2	35.2	23.1
4.	Berries average height, h	*mm*	13.3	40.5	*h*=18.8 / *h'*=4.1
5.	The smallest distance between the thermal centre and the outer surface of the product, δ_0	*mm*	6.65	13.50	2.05

Inside the quick-freezing room, the limit temperature was set to -30 °*C* for all of the three species of berries used. During the quick-freezing cycle, the process parameters can be determined by direct reading on the touch screen of the command and control panel or by post-processing the data recorded on the SD memory card. Freezing times were determined as follows:

- the freezing time from 0 °*C* to -15 °*C* was determined by the difference between the total timer recorded at the end of the freezing process (-15 °*C*) and the timer recorded when the temperature reached 0 °*C* in the product's thermal centre;

- the temperature within the quick-freezing room, the temperature on the surface of the product and the temperature in the thermal center of the product, all measured at the end of the freezing process, were determined by directly reading the values indicated by the respective temperature sensors, at the end of the freezing process;

- the total freezing time was determined by direct reading of the total timer recorded at the end of the freezing process.

Aspects during the experimental research are presented in Figure 6 and 7:

Fig. 6. Determination of the working qualitative indices */ Determinarea indicilor calitativi de lucru*

Fig. 7. Determination of the environmental parameters and energy indices */ Determinarea parametrilor de mediu și a indicilor energetici*

The parameters of the freezing process and the determined indexes are shown in Table 3.

Table 3. The parameters of the freezing process and the determined indexes */ Parametrii procesului de congelare și indicii determinați*

No. crt.	Characteristic	U.M.	Value of parameters determined in the tests		
			Blueberries	Strawberries	Raspberries
1.	Freezing time from 0 °C to -15 °C	*s*	379	773	457
2.	Temperature within the quick-freezing room, at the end of the freezing process	°C	-30	-32	-33
3.	Temperature on the surface of the product at the end of the freezing process	°C	-28	-30	-31
4.	Temperature in the thermal center of the product, at the end of the freezing process	°C	-15	-15	-15
5.	Total freezing time	*s*	498	965	603
6.	Average linear freezing rate for an operating cycle	*cm/h*	6.32	6.29	1.62
7.	Liquid nitrogen consumption for an operating cycle	*kg*	6.90	7.05	7.20

Following the analysis of the experimental data, there were found the following: the minimum total freezing time, 498 s, was recorded for blueberries and the maximum total freezing time, 773 s, was recorded for strawberries; for blueberries and strawberries the average linear freezing rates recorded values of 6.32 cm/h and 6.29 cm/h corresponding to a very fast freezing process and for raspberries the average linear freezing rate recorded a value of 1.62 cm/h corresponding to a fast freezing process; liquid nitrogen consumption recorded a minimum value of 6.90 kg for blueberries and a maximum value of 7.20 kg for raspberries.

Regarding the influence of the supply of coolant – "exhausted" nitrogen gas on the energy consumption of the refrigeration unit serving the pre-cooling / temporary storage room in a frozen state, after the processing and interpretation of the experimental data, the following results were obtained:

Table 4. The comparative energy consumption of the classic refrigeration unit, with or without "exhausted" nitrogen gas supply */ Consumul energetic comparativ al instalației frigorifice clasice, cu sau fără aport de azot gaz „epuizat".*

No. crt.	Characteristic	U.M.	Value of parameters determined in the tests
The working of the refrigeration unit with "exhausted" nitrogen gas supply			
1.	Power absorbed in operation	*kW*	1.235
2.	Operating time	*s*	1668
3.	Electricity consumption in operation (based on a 1-hour working period)	*kWh*	0.572
4.	Power absorbed in rest	*kW*	0.160
5.	Rest time	*s*	1932
6.	Electricity consumption in rest (based on a 1-hour working period)	*kWh*	0.086
7.	**Electricity consumption based on a 1-hour working period**	***kWh***	**0.658**
The working of the refrigeration unit without "exhausted" nitrogen gas supply			
1.	Power absorbed in operation	*kW*	1.235
2.	Operating time	*s*	2656
3.	Electricity consumption in operation (based on a 1-hour working period)	*kWh*	0.911
4.	Power absorbed in rest	*kW*	0.160
5.	Rest time	*s*	944
6.	Electricity consumption in rest (based on a 1-hour working period)	*kWh*	0.042
7.	**Electricity consumption based on a 1-hour working period**	***kWh***	**0.953**

On average, extrapolated to a working period of 1 hour, for the working variant of the refrigeration unit with "exhausted" nitrogen gas supply, an operating time of 1668 s and a rest time of 1932 s was obtained (6 complete on-off cycles plus an extra operating time of 162 s). For the working variant of the refrigeration unit without "exhausted" nitrogen gas supply, an operating time of 2656 s and a rest time of 944 s was obtained (8 complete on-off cycles plus an extra operating time of 168 s). The environmental conditions (temperature and humidity) at the time of the tests were as follows: Tair=26 °C and Uair=49.2 %.

Analyzing the results obtained, it is found that the solution of using the "exhausted" nitrogen gas supply obtained from the quick-freezing process, in order to reduce the thermal load of the refrigeration unit in the classic freezing room, led to a reduction in hourly electricity consumption by 30.96 %.

Aspect of the samples before and after freezing are presented in Figure 8:

Fig. 8. Aspect of the samples before and after the quick-freezing */ Aspectul probelor înainte și după congelare rapidă*

Following the visual analysis of the state of frozen products' outer surface it was found that, as a consequence of thermal shock, in the case of blueberries, fissures and cracks appeared in fruit epidermis and pulp during freezing. Strawberry and Raspberry fruits reacted better, with no deterioration of the outer surface condition. Although quick-freezing was associated with an improved internal microstructure of individual berries, it may also cause a fracture on the berry skin, as a consequence of thermal shock, if the limit temperature set inside the quick-freezing room has a higher negative value. For a thermo-sensible berry skin, the limit temperature set inside the quick-freezing room should have a little lower negative value to avoid the damage produced by thermal shock (for example -25 °C instead -30 °C).

CONCLUSIONS

1. The best results in terms of keeping the attributes and quality of the horticultural products at a level as close as possible to that of the fresh product, are obtained when using artificial cold (refrigeration, freezing), despite all other methods.
2. Among the existing freezing methods, freezing with cryogenic agents allows obtaining average linear freezing rates superior to the other methods.
3. The fastest method for cooling food products is to use liquid nitrogen, which comes into direct contact with the food to be frozen.
4. Although quick-freezing is regarded as the best technique to maintain the texture of frozen products, very high average linear freezing rates may lead to epidermis and pulp damage, as a consequence of thermal shock.
5. Using the "exhausted" nitrogen gas supply obtained from the quick-freezing room, led to a reduction in hourly electricity consumption by 30.96 % of the classic refrigeration unit within the pre-cooling / temporary storage room in a frozen state.

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BIOTECHNOLOGIES USED IN THE PROPAGATION OF FRUIT ROOTSTOCKS AT RIFG PITEȘTI

BIOTEHNOLOGII UTILIZATE ÎN PROPAGAREA PORTALTOILOR POMICOLI LA ICDP PITEȘTI-MĂRĂCINENI

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Abstract

*Biotechnology applied in in vitro propagation is a valuable tool for the fruit nursery industry. At RIFG Pitești, were established protocols with success rates for '**Mirobolan dwarf**' (plum rootstock), '**Adaptabil**' (peach and plum rootstock), '**IPC 3'** (sweet cherry rootstock). Protocols for technologies or technological sequences were also developed for other rootstocks created by RIFG Pitești: for apricot rootstocks: '**Baroc**' and '**Apricor**', pear rootstocks: '**Sălișteː**', '**Tileș**', '**P10/94'**, '**P1/91**' and, peach rootstock: '**Miroper**'. Regarding the international assortment were established the protocols for '**Gisela 5**' and '**PHLC**' (cherry rootstocks). The culture media were represented by Murashige & Skoog (M&S-1962), Lee & Fossard (L&F-1977), Quoirin & Lepoivre (Q&L-1977), vitamins Walkey, 1972 and Driver, J., A. Kuniyuki (DKW-1984). Our results show the best values for '**Mirobolan dwarf'** (95-100%), '**Adaptabil**' (98%), '**Gisela 5**' (94%) and, '**IPC 3**' (73.33%).*

Keywords: *in vitro, meristem, propagation, rooting*

Rezumat

*Biotehnologia aplicată în propagarea in vitro este un instrument valoros pentru industria pepinieristică pomicolă. La ICDP Pitești-Mărăcineni au fost stabilite protocoale de înmulțire cu rată ridicată de succes pentru o serie de portaltoi pomicoli: '**Mirobolan dwarf**' (prun), '**Adaptabil**' (piersic și prun), '**IPC 3**' (cireș). De asemenea, s-au dezvoltat protocoalele specifice și pentru alți portaltoi creați la ICDP Pitești-Mărăcineni: pentru cais '**Baroc**' și '**Apricor'**, pentru păr: '**Sălișteː**', '**Tileș**', '**P10/94**', '**P1/91**' și pentru piersic: '**Miroper**'. În ceea ce privește sortimentul internațional au fost stabilite protocoalele pentru portaltoii de cireș: **Gisela 5** și **PHLC**. Mediile de cultură au fost reprezentate de Murashige & Skoog (M&S-1962), Lee & Fossard (L&F-1977), Quoirin & Lepoivre (Q&L-1977), vitaminele Walkey, 1972 și Driver, J., A. Kuniyuki (DKW-1984). Cercetările arată că cele mai bune rezultate s-au obținut prin propagarea in vitro la următoarele genotipuri: '**Mirobolan dwarf**' (80-100%), '**Adaptabil**' (98%), '**Gisela 5**' (94%) și 'IPC 3' (73,33%).*

Cuvinte cheie: *in vitro, mertistem, propagare, înrădăcinare*

INTRODUCTION

For many fruit species/genotypes that are targeted for large-scale propagation have been established protocols for *in vitro* propagation due to the advantages offered by this technique. However, conditions must be optimized for most species or genotypes (Damiano *et. al*., 2000). The use of *in vitro* propagation technique allows a considerable increase in the yield of propagation but although a current application for some species the efficiency of micropropagation is dependent on a number of factors. The differentiated cells of the explant retain their meristematic state potential, which is however inhibited or repressed. In order for such a cell to return to the state of active division, it is necessary to create conditions that allow the removal of inhibition. The most commonly used explants for *in vitro* pear establishment and multiplication are shoot tips, axillary

buds and single nodes, especially those obtained from grafted plants grown in greenhouses (Thakur, *et al.*, 2008, Ružić *et al.*, 2008).

The type of basal culture medium, the type and concentration of plant growth regulators, and the parameters of the explants are the most important factors for the success of each *in vitro* propagation approach. Murashige and Skoog (MS), 1962, Lepoivre (LP), Driver-Kuniyuki (DKW) Quoirin, M.; Lepoivre, P., 1977, and Woody Plant Medium (WPM) Lloyd, G.; McCown, 1981, have been the most commonly applied media for tissue culture in various species.

Regarding to phytohormones, α-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 2,4-dichlorophenoxyacetic acid (2,4-D), N6 benzyladenine (BA), 6-benzylaminopurine (BAP), thydiazuron (TDZ), 2-isopenteniladenine (2-iP), zeatin (Zt) and gibberellic acid (GA_3) have been mainly used varying in concentration or the combination (Thakur *et al*., 2008; Bommineni *et al*, 2001; Bell *et al*., 2002; Anirudh *et al*.,2008). In other species as well as in *Prunus*, the type of cytokinin, its concentration are important factors for the rate of multiplication and elongation. The genotype response is different in addition to the intended effect, and undesirable phenomena may occur as a result of hyperacidity. Many authors report that although it is well known that cytokinins promote cell division and implicitly shoot multiplication, the association with the appropriate auxins is of great importance (Ruzic and Vujovic, 2016).

The rooting stage for *in vitro* culture is also related to certain culture conditions. The response of cuttings to exogenous application of auxins is dependent on several internal and external factors. Both the concentration and type of the applied phytohormone and above all, the genotype response can be limiting factors. Moreover, the concentration and mineral composition of the culture medium affect the evolution of the behavior of the biological material *in vitro*. Some researchers have proposed reducing the normal concentration by half to improve the rooting capacity (Plopa *et al*., 2012).

Considering that tissue culture propagation usually aims for high multiplication rate and good rhizogenesis, the objective of this study was to evaluate the appropriate *in vitro* culture conditions.

MATERIALS AND METHODS

The biological material was represented by the pear selections: **Sălișteˮ**, **Tileș**, **P10/94**, **1/91**; the plum rootstock **Mirobolan dwarf**; cherry rootstocks: **IPC 3**, **Gisela 5**; **PHL**'; apricot rootstocks: **Apricor** and **Baroc** and peach rootstocks: **Adaptabil** and **Mirope'**.

Explant source was represented by annual branches that were harvested in February. The explants inoculated on the culture media were obtained from meristems excised from the axillary buds on the annual branches.

Disinfection of biological material consisted of:

-washing with water and liquid detergent;

-immersion in 6% (w/v) $Ca(OCl)_2$ for 20 minutes;

-immersion in 96 vol% alcohol for 10 minutes;

-washing with distilled and sterile water 3 x 10 minutes.

Culture media was represented by the media from the specialized literature, based on macroelements, microelements and vitamins Murashige&S Skoog (MS -1962), Lee & Fossard (LF - 1977), Quoirin & Lepoivre (QL - 1977) + Walkey vitamins (1972), (QL - 1977) + Lloyd and McCown vitamins (WPM vitamins - 1981), Driver&Kuniyuki (DKW-1984) to which optimizations regarding the concentration of macro and microelements in different phases of culture of some genotypes and to which the hormonal balance was added (Table 1).

Table 1. Culture media variants tested for micropropagation of pear, plum, apricot, peach and cherry genotypes/
Variante de mediu testate pentru micropropagarea genotipurilor de păr, prun, cais, piersic și cireș

Genotypes	*Regeneration phase*					
	Variants	**Basal medium**	**Vitamins**	**Growth regulators (mg/l)**		
				GA_3	**IBA**	
'Mirobolan dwarf', 'Adaptabil' 'Miroper' 'IPC 3', 'Gisela 5' 'PHLC'. 'Săliște', 'Tileș', 'P10/94', 'P1/91', 'Apricor', 'Baroc'	**V1**	QL	Walkey	0.1	0.01	
	V2	QL	Walkey	0.1	0.1	
	V3	MS	MS	0.1	0.01	
	V4	MS	MS	0.1	0.1	
	V5	DKW	DKW	0.1	0.01	
	V6	DKW	DKW	0.1	0.1	
	V7	LF	LF	0.1	0.01	
	V8	LF	LF	0.1	0.1	
	Multiplication phase					
	Variants	**Basal medium**	**Vitamins**	**Growth regulators (mg/l)**		
				BAP	**ANA**	**GA_3**
	V1	MS	MS	0.5	0.2	0.1
	V2	MS	MS	0.5	0.5	0.2
	V3	MS	MS	1.0	0.2	0.1
	V4	MS	MS	1.0	0.5	0.2
	V5	MS	MS	1.5	0.2	0.1
	V6	MS	MS	1.5	0.5	0.2
	V7	QL	Walkey	0.5	0.2	0.1
	V8	QL	Walkey	0.5	0.5	0.2
	V9	QL	Walkey	1.0	0.2	0.1
	V10	QL	Walkey	1.0	0.5	0.2
	V11	QL	Walkey	1.5	0.2	0.1
	V 12	QL	Walkey	1.5	0.5	0.2
	V 13	LF	LF	0.5	0.2	0.1
	V 14	LF	LF	0.5	0.5	0.2
	V 15	LF	LF	1.0	0.2	0.1
	V 16	LF	LF	1.0	0.5	0.2
	V17	LF	LF	1.5	0.2	0.1
	V 18	LF	LF	1.5	0.5	0.2
	V 19	QL	WPM	0.5	0.2	0.1
	V 20	QL	WPM	0.5	0.5	0.2
	V 21	QL	WPM	1.0	0.2	0.1
	V 22	QL	WPM	1.0	0.5	0.2
	V 23	QL	WPM	1.5	0.2	0.1
	V 24	QL	WPM	1.5	0.5	0.2
	Rooting phase					
	Variants	**Basal medium**	**Vitamins**	**Growth regulators (mg/l)**		
				GA_3	**IBA**	**ANA**
	V1	MS	MS	-	1.5	-
	V2	½ MS	MS	-	1.5	-
	V3	MS	MS	0.01	1.5	-
	V4	½ MS	MS	0.01	1.5	-
	V5	MS	MS	1.5	1.5	-
	V6	½ MS	MS	1.5	1.5	-
	V7	LF	LF	0.01	1.5	-
	V8	LF	LF	-	1.5	1.5
	V9	LF	LF	0.2	1.0	-
	V10	QL	Walkey	0.01	1.5	-
	V11	QL	Walkey	-	1.5	1.5
	V 12	QL	Walkey	0,01	1.0	-
	V 13	QL +1g/l activated charcoal	Walkey	0,01	1.0	-
	V 14	QL	Walkey	0,01	2.0	-
	V15	QL +1g/l activated charcoal	Walkey	0,01	2.0	-

All culture media contained dextrose 40 g/l, agar 7-8 g/l and Na Fe EDTA 32mg/l.

RESULTS

The response of the genotypes to propagation by biotechnological methods respectively *in vitro* cultures was monitored by the reaction to the tested culture media. In the differentiation stage for the presented rootstocks were established, efficient protocols with the best results on MS, LF and QL culture media (Table 2).

Table 2. Culture medium variants with the best results obtained in the differentiation phase / Variantele de mediu de cultură cu cele mai bune rezultate obținuteîn faza de diferențiere

Genotypes	MS	LF	QL
'Mirobolan dwarf' *(Plopa et. al, 2012)*	-	-	0.01 mg/l IBA + 0.1 mg/l GA_3
'Adaptabil' *(Plopa, et. al., 2012)*	-	-	0.01 mg/l IBA + 0.1 mg/l GA_3
'IPC 3' *(Plopa, et. al, 2009)*	-	0.01 mg/l IBA + 0.1 mg/l GA_3	-
'Gisela 5' *(Plopa, et. al., 2010)*	0.01 mg/l IBA +0.1 mg/l GA_3	-	-
'Săliște'*	-	-	0.01 mg/l IBA + 0.1 mg/l GA_3
'Tileș'*	-	-	0.01 mg/l IBA + 0.1 mg/l GA_3
'P10/94'*	-	-	0.01 mg/l IBA + 0.1 mg/l GA_3
'P1/91'*	-	-	0.01 mg/l IBA + 0.1 mg/l GA_3
'PHLC'*	0.01 mg/l IBA +0.1 mg/l GA_3	-	-
'Apricor' *	-	-	0.01 mg/l IBA + 0.1 mg/l GA_3
'Baroc'*	-	-	0.01 mg/l IBA + 0.1 mg/l GA_3
'Miroper'*	-	-	0.01 mg/l IBA + 0.1 mg/l GA_3

*= *unpublished results*

The same MS, LF and QL media but with hormonal balances adapted for stimulate of the adventitious buds for multiplication provided optimal conditions in the multiplication phase of the biological material studied (Table 3).

Table 3. Culture media variants with the best results obtained in the multiplication phase /Variantele de mediu de cultură cu cele mai bune rezultate obținuteîn faza de multiplicare

Genotypes	MS	LF	QL
Mirobolan dwarf *(Plopa et. al, 2012)*	-	-	1 mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l NAA
Adaptabil *(Plopa, et. al., 2012)*	-	-	1.5 mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l NAA
IPC 3 *(Plopa, et. al, 2009)*	-	1mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l ANA	-
Gisela 5 *(Plopa, et. al., 2010)*	1 mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l NAA	-	-
Săliște*	-	-	1.5 mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l NAA
Tileș*	-	-	1.5 mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l NAA
P10/94*	-	-	1.5 mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l NAA
P1/91*	-	-	1.5 mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l NAA
PHLC*	1 mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l NAA	-	-
Apricor *	-	-	1.0 mg/l BAP + 0.1 mg/l GA_3
Baroc*	-	-	1.0 mg/l BAP + 0.1 mg/l GA_3
Miroper*	-	-	1.5 mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l NAA

*= *unpublished results*

The good results for *in vitro* rooting are represented in Table 4 based on GA_3, IBA, NAA in different concentrations and MS, ½ MS, QL and QL culture media with added 1g/l activated charcoal.

Table 4. Culture media variants with the best results obtained in the rooting phase / Variantele de mediu de cultură cu cele mai bune rezultate obținute în faza de înrădăcinare

Genotypes	MS	½ MS	QL	QL +1g/l activated charcoal
Mirobolan dwarf *(Plopa et. al, 2012)*	-	1.5 mg/l IBA	-	-
Adaptabil *(Plopa, et. al., 2012)*	-	-	-	1mg/l IBA + 0.01 mg/l GA_3
IPC 3 *(Plopa, et. al, 2009)*	1.5 mg/l NNA	-	-	-
Gisela 5 *(Plopa, et. al., 2010)*	1.5 mg/l IBA + 0.01 mg/l GA_3	-	-	-
PHLC*				0.2 mg/l GA_3+1.0 mg/l IBA
Săliște*	-	-	1.0 mg/l IBA + 0.02 mg/l GA_3	-
Tileș*	-	-	-	1.0 mg/l IBA + 0.01 mg/l GA_3
P10/94*	-	-	-	-
P1/91*	-	-	-	-
Apricor*	-	-	-	-
Baroc*	-	-	-	-
Miroper*	-	-	-	-

*= *unpublished results*

The values established in the 3 phases carried out *in vitro* by rootstocks in our research varied from a response indicating efficiency through this method of multiplication to a poor multiplication capacity and lack of rooting on the tested media (Table 5).

Table 5. Behavior of rootstocks to propagation using *in vitro* biotechnological method /*Comportarea portaltoilor testați la înmulțirea prin metoda biotehnologică-culturi in vitro*

No	Genotypes	Regeneration	Multiplication	Rooting
1	**Mirobolan dwarf** *(Plopa et. al, 2012)*	90%	6.2 (5 subcultures)	80.00-100 %
2	**Adaptabil** *(Plopa, et. al., 2012)*	95%	9.0 (5 subcultures)	98.00 %
3	**IPC 3** *(Plopa, et. al, 2009)*	100%	7.8 (5 subcultures)	73.33 %
4	**Gisela 5** *(Plopa, et. al., 2010)*	95%	7.0 (5 subcultures)	94.00 %
5	**PHLC**	90%	5.0 (5 subcultures)	90.00 %
6	**Sălișteanu***	80%	5.0 (4 subcultures)	80.00 %
7	**Tileș***	70%	4.0 (4 subcultures)	80.00 %
8	**P10/94***	80%	5.0 (3 subcultures)	-
9	**P1/91***	75%	3.0 (3 subcultures)	-
10	**Apricor ***	80%	6.0 (3 subcultures)	-
11	**Baroc***	60%	3.0 (3 subcultures)	-
12	**Miroper***	75%	3.0 (3 subcultures)	-

For **Mirobolan dwarf** plum rootstock (figures 1 and 2), maximum efficiency in the differentiation of meristems was achieved with the formula QL + 0.01 mg/l IBA +0.1 mg/l GA_3 (Plopa *et al*, 2012) with a success rate of 90% shoots.

The multiplication was 6.2 on average of 5 subcultures, QL + 1 mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l NAA in the most favorable variant of culture medium. In the research carried out, it was observed that **Mirobolan dwarf** emits roots from the multiplication phase, but to obtain plants it is necessary to separate the shoots from the multiplication bush and transfer them to a rooting medium represented by macro and microelements MS reduced by half and the addition of 1.5 mg/l IBA.
Another *in vitro* rooting performance of the rootstock is that obtained a good rooting percentage 80-85%, even in the case of a culture medium without phytohormones.

Figure 1. Rooting Mirobolan dwarf / Mirobolan dwarf înrădăcinare

Figure 2 . Mirobolan dwarf-plant fortification obtained from *in vitro* culture/ Mirobolan dwarf fortificare plante obținute *in vitro*

Adaptabil rootstock (figure 3, figure 4) culture for initiation phase showed that explants have a maximum differentiation in terms of 95%. **Adaptabil** performed better in the medium with a lower content of ammonium and nitrate ions (QL – 1977). The variants tested for initiation also

included the medium based on MS components (MS -1962), but unlike QL it had a higher concentration of ammonium and nitrate ions (Plopa, et. al., 2012).

In the multiplication phase, the 'Adaptabil' rootstock obtained the best results, an average of the 5 subcultures of 9 shoots, when 1.5 mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l NAA were added to the basic components QL + Walkey vitamins.

Rooting phase recorded the best results 98% at a hormonal balance of 1mg/l IBA + 0.01 mg/l GA_3. On this working protocol, a significant elongation of the rooted shoots also occurs.

Figure **3. 'Adaptabil'-multiplication phase/**
'**Adaptabil**'-faza de multiplicare

Figure **4. 'Adaptabil' rooting phase/**
'**Adaptabil**'-faza de înrădăcinare

For **IPC 3** (Plopa *et al*., 2009), the best performance of shoot differentiation was given by LF medium supplemented with 0.01 mg/l IBA+ 0.1 mg/l GA_3 when regeneration was 100%. Multiplication (Figure 5) on LF medium + 1mg/l BAP + 0.1 mg/l GA_3 + 0.2 mg/l ANA with an average of the 5 subcultures was of 7.8 shoots issued. Shoots obtained by micropropagation from **IPC 3** had the best rooting rate (73.33 % rooted plants) when using 1.5 mg/l NNA and MS medium (Figure 6).

Figure 5. IPC 3 - Multiplication phase/
'IPC 3' - faza de multiplicare

Figure 6. IPC 3 - Rooting phase /
'IPC 3' -faza de înrădăcinare

The behavior of the **Gisela 5** rootstock (Plopa, et. al., 2010), under *in vitro* conditions, indicates the possibility of propagation by this method on a large scale, the established protocol having good efficiency (Figures 7, Figure 8). The culture medium recommended is the MS medium

(Table 1) which recorded superior results in all 3 stages followed: initiation 95%, multiplication an average of 7 shoots/bush in the 5 subcultures, rooting 94%.

Figure 7. Gisela 5 - multiplication /
Gisela 5 – faza de multiplicare

Figure 8. Gisela 5 - rooting /
Gisela 5 – faza de înrădăcinare

PHLC rootstock had the best behavior in the differentiation phase on the V1 variant (Table 1) with 90% differentiated explants, in the multiplication phase (Figure 9) 5 shoots / bush average out of the 5 subcultures on the V3 variant (Table 1) and in the rooting phase the LF medium components with a hormonal balance represented by 0.2 mg/l GA3 and 1 mg/l IBA offered the best results 90% rooting (Figure 10).

Figure 9. PHLC multiplication /
PHLC - faza de multiplicare

Figure 10. PHLC rooting /
PHLC - faza de înrădăcinare

In vitro propagation performances of the 4 genotypes of pear were different. **Sălișteа** had differentiation in 80% of explants and **Tileș** 70%, **P10/94** recorded 80% and **P1/91** 75% on the same culture medium (Table 2). In the multiplication phase on the same culture medium (Table 3) differences were recorded as follows: **Sălișteа** 5 shoots/bush average of 4 subcultures (Figures 10), **Tileș** 4 shoots/bush, average of 4 subcultures (Figure 11), **P10/94** 5 shoots/bush average of 3 subcultures (Figures 12) and **P1/91** 3 shoots per bush average of 3 subcultures (Figures 13). The rooting media provided optimal conditions (Figures 14, 15, 16, 17, 18) only for **Sălișteа** and **Tileș** with 80% rooted shoots (Table 4, Table 5).

Figure 10. Săliște - multiplication phase/
Săliște – faza de multiplicare

Figure 11. Tileș – multiplication phase/
Tileș - faza de multiplicare

Figure 12. P 10/94 - multiplication phase/
P 10/94 - faza de multiplicare

Figure 13. P 1/91 – multiplication phase/
P 1/91 - faza de multiplicare

Figure 14 – Săliște – rooting culture media/
Săliște pe mediul de înrădăcinare

Figure 15 – Tileș / rooting culture media/
Tileș pe mediul de înrădăcinare

Figure 16. Tileș – rooted plants/
Tileș - plante înrădăcinate

Figure 17. Săliște - rooted plants /
Săliște - plante înrădăcinate

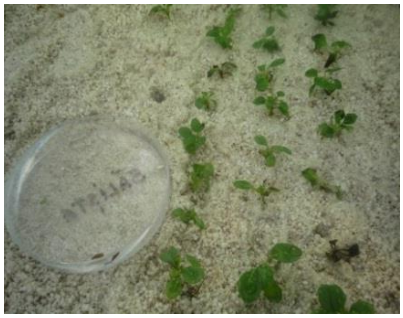

Figure 18. Săliște - aclimatization/
Săliște – aclimatizare

For **P 10/94** and **P1/91**, the rhizogenesis process did not find favorable conditions on the tested media.

Apricor and **Baroc** apricot rootstocks (Figure 19, Figure 20, Figure 21) recorded the best differentiation 80% and 60% respectively and multiplication 6 shoots/bush (3 subcultures) and 3 shoots/bush (3 subcultures) on the same culture media (Table 3 and Table 4). Both rootstocks did not express their rooting capacity on the tested culture media (Table 1).

Figure 19. Apricor differentiation/ Apricor diferențiere

Figure 20. Apricor multiplication/ Apricor multiplicare

Figure 21. Baroc differentiation/ Baroc diferențiere

Miroper peach rootstock (Figure 22, Figure 23) had a differentiation of 75% under the same basic medium and hormonal balance as the **Adaptabil** peach rootstock. The culture medium for multiplication was the same as for the **Adaptabil** rootstock, the average for 3 subcultures being 3 shoots/bush. Regarding the rooting capacity, although the **Adaptabi**l rootstock recorded 98% rooted plants, the **Miroper** rootstock did not root on the tested medium variants.

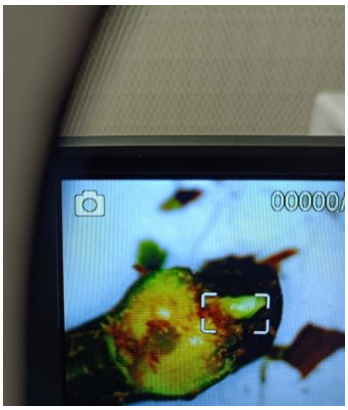

Figure 22. Meristem Miroper/ meristem **Miroper**

Figure 23. Miroper regenerated shoots/ Miroper – lăstari regenerați

CONCLUSIONS

1. Each genotype requires the use of a different culture medium in order to obtain a more efficient *in vitro* culture. **Adaptabil** preferred the medium with a lower content of ammonium and nitrate ions for the initiation phase, **Mirobolan dwarf** recorded the better rooting results at a concentration of macroelements and microelements MS reduced by half.

2. The peach rootstocks **Adaptabil** and **Miroper** had a positive behavior for the differentiation and multiplication phase to the same components of the culture medium, but only the **Adaptabil** rootstock responded to *in vitro* rooting.

3. The pear selections had different reactions to the same environmental conditions, **Sălișteand** **Tileș** emitted roots when 1g/l of activated charcoal was added, while for the **P10/94** and **P1/94** selections, root emission was not activated by the components of the media used.

4. Cherry rootstocks I**PC 3**, **Gisela 5** și **PHLC** have a good result, with foreign genotypes standing out as superior, which are currently the most used as rootstocks.

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THE INFLUENCE OF PHYTOSANITARY TREATMENTS (CONVENTIONAL AND BIOLOGICAL) ON THE QUALITY OF TOMATO FRUITS DURING STORAGE TIME

INFLUENȚA TRATAMENTELOR FITOSANITARE (CONVENȚIONAL ȘI BIOLOGIC) ASUPRA CALITĂȚII FRUCTELOR DE TOMATE, ÎN PERIOADA DE DEPOZITARE

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Abstract

Tomatoes (Solanum lycopersicum) are climacteric fruits that originated in South America and were brought to Europe in the 16th century. Known for their delicious taste, they can be consumed fresh or used in a variety of food preparations. The objective of this experiment was to evaluate the efficacy of some chemical and biological products used in the control of foliar pathogens in tomato crop in greenhouse and their influence on the quality and shelf life of tomato fruits. In controlling pathogens, conventional products have ensured much greater effectiveness compared to biological ones. This research systematically examines variations in water content, total dry matter, total soluble solids, organic acids, and firmness in tomatoes stored at temperatures of 6°C and 22°C, starting from the time of harvest and extending for 3, 5, 7, and 10 days.

Keyword: *Solanum lycopersicum*, chemical and biological control, storage temperatures, shelf life.

Rezumat

Tomatele (Solanum lycopersicum) sunt fructe climacterice, originare din America de Sud, care au fost aduse în Europa în secolul al XVI-lea. Cunoscute pentru gustul lor delicios, pot fi consumate în stare proaspătă sau pot fi folosite într-o mare varietate de preparate culinare. Obiectivul acestei experiențe este evaluarea eficacității unor produse chimice și biologice utilizate în controlul agenților patogeni foliari la cultura de tomate din spații protejate și influența acestora asupra calității și duratei de păstrare a fructelor de tomate. În controlul agenților patogeni, produsele convenționale au asigurat o eficacitate mult mai mare în comparație cu cele biologice. Au fost făcute determinări în ceea ce privește influența tratamentelor fitosanitare asupra următorilor parametri: substanța uscată totală, substanța solidă solubilă, pH și fermitatea fructelor de tomate, depozitate la temperaturi de 6°C și 22°C, la intervale de 3, 5, 7 și 10 zile.

Cuvinte cheie: *Solanum lycopersicum*, combatere chimică și biologică, temperatura de păstrare, durata de păstrare

INTRODUCTION

Tomatoes (*Solanum lycopersicum* L.) are some of the most important vegetables grown worldwide from an economic and nutritional point of view, in open fields and in greenhouses.

Organic agriculture is a dynamic sector in Romania that has seen an upward evolution in recent years. Romanian agricultural research gives special importance to the development of technologies for the cultivation of vegetables grown in conventional and/or ecological agriculture. Good results were obtained in biological control of diseases and pests on tomatoes (Bratu *et al*., 2015, Hogea S.S., 2020), pepper (Calin *et al*., 2017, 2020), eggplants (Iosob and Cristea, 2022), cucumbers (Cenusa *et al*., 2016), melons (Sovarel *et al*., 2024), cabbage (Iosob *et al*., 2023), zucchini (Sovarel *et al*., 2024), onion (Calin *et al*., 2016).

The main diseases that cause damage to tomato crops are: early blight (*Alternaria porri* f.sp. *solani*), gray rot (*Botrytis cinerea*), brown leaf spot (*Fulvia fulva*), late blight (*Phytophthora infestans*), powdery mildew (*Erysiphe* sp.), bacterial leaf spot (*Xanthomonas vesicatoria*), pustular fruit spot (*Pseudomonas tomato*), bacterial wilt of tomatoes (*Clavibacter michiganensis* subsp. *michiganensis*), root, stem and fruit rot (*Phytophthora parasitica*), wilt or fusarium wilt (*Fusarium*

oxysporum f.sp. *lycopersici*), root and crown rot (*Fusarium oxysporum* f. sp. *radicis lycopersici*), verticillium wilt (*Verticillium dahliae*).

MATERIALS AND METHODS

The objective of this experience is to evaluate the efficacy of some chemical and biological products used in the control of foliar pathogens in tomato crops in greenhouses and their influence on the quality and shelf life of tomato fruits.

The experience was conducted at the Research Development Institute for Vegetable and Flower Growing Vidra, in 2024. Planting was made in two greenhouses, on 10 July, using tomato (*Solanum lycopersicum*) variety 'Pink Rock F1', arranged according to the method of randomized blocks, 4 replications. The treatments were applied preventively for *Alternaria solani* and *Fulvia fulva* and *Botrytis cinerea* control.

In the conventional system were made 2 applications to control the pathogens *A. solani* and *F. fulva* (July 19, 26) and 1 treatment for *B. cinerea* (September 6).

The experiment for conventional pathogen control consist on 4 variants treated and untreated control.

Conv. 1: Ortiva Top (azoxistrobin 200 g/L + difenoconazol 125 g/l) 1 l/ha;
Conv. 2: Cidely Top (difenoconazol 125 g/l + ciflufenamid 15 g/l) 1 l/ha;
Botrefin (cyprodinil 375 g/l + fludioxonil 250 g/l) 0.8 kg/ha;
Conv. 3: Amistar (azoxistrobin 250 g/L) 0.75 l/ha;
Sygnum (boscalid 26,7% + piraclostrobin 6,7%) 1.5 kg/ha;
Conv. 4: Dagonis (difenoconazol 50 g/l + fluxapiroxad 75 g/l) 1 l/ha;
Switch (fludioxonil 25% + ciprodinil 37,5%) 0.8 kg/ha;
Conv. 5: Untreated control.

Also, in the biological pathogen control, the experience also includes 5 experimental variants, to which 6 treatments were applied: July 19 (T1), July 26 (T2), August 2 (T3), September 7 (T4), September 14 (T5) and September 21 (T6), as follows:

Biol. 1: Cavaler 600SL (microorganisms *Bacillus pumillus* and *Bacillus subtilis*) 0.3%;
Biol. 2: Amulet (microorganisms *Bacillus thuringiensis, Bacillus subtilis, Bacillus megatherium*) 40 l/ha;
Biol. 3: Zytron (citrus seed extract 20%) 0.15%;
Biol. 4: Mimoten (*Mimosa tenuifolia* 80% extract) 0.3%;
Biol. 5: Untreated control.

Cavaler 600SL is a product based on microorganisms: *Bacillus pumillus* and *Bacillus subtilis*, which populate the entire surface of the plant, preventing pathogens from establishing themselves. When the disease has already established itself upon application, Cavaler 600 SL isolates the pathogens and destroys them. It control most pathogens in tomato crops: late blight (*Phytophthora infestans*), early blight (*Alternaria solani*), gray mold (*Botrytis cinerea*), leaf mold (*Fulvia fulva*), vascular wilt (*Verticillium dahliae*), powdery mildew (*Erysiphe* sp.), root and basal rot (*Phytophthora parasitica*), septoria leaf spot (*Septoria lycopersici*), bacterial canker (*Clavibacter michiganensis*), fusarium wilt (*Fusarium oxysporum*).

Amulet is a biodynamic product formulated on the basis of liquid extract from marigold leaves. The content of the product is based on microorganisms resulting from the natural fermentation process of marigold leaves, namely by populating the resulting liquid with beneficial bacilli (*Bacillus thuringiensis, Bacillus subtilis, Bacillus megatherium*). In tomatoes, it control the following diseases: downy mildew (*Phytophthora infestans*), powdery mildew (*Erysiphe* sp.), gray rot (*Botrytis cinerea*), bacteriosis (Xanthomonas sp.), fusarium wilt (*Fusarium oxysporum*), white rot (*Sclerotinia sclerotiorum*), septoria wilt (*Septoria lycopersici*), early blight (*Alternaria solani*).

Zytron is a product based on 20% citrus seed extract. It is used on tomatoes to control and prevent the following diseases: powdery mildew, grey mold, early blight, fusarium wilt, late blight, bacteriosis and white rot.

Mimoten is a product obtained from 80% *Mimosa tenuifolia* extract whose formulation ensures safe absorption through leaves and roots, stimulating metabolic processes in the plant that generate self-defense reactions. It is used in tomatoes to control and prevent the occurrence of the following diseases: powdery mildew (*Erysiphe* sp.), grey mold (*Botrytis cinerea*), early blight (*Alternaria solani*), fusarium wilt (*Fusarium oxysporum*), late blight (*Phytophyhora infestans*), bacteriosis (*Pseudomonas* sp., *Xanthomonas* sp.), white rot (*Sclerotinia sclerotiorum*).

Observations and determinations were made on the leaves (5 plants/variant) regarding the frequency and severity of the *Alternaria solani*, *Fulvia fulva* and *Botrytis cinerea* pathogens, based on which the efficacy of the products was calculated.

Climate data monitoring in greenhouse was done with the help of thermohygrometers, which record air temperature and humidity at hourly intervals.

The atmospheric humidity in the greenhouse was greatly influenced by the amount of precipitation that fell during this period. Thus, the precipitation that fell on July 17, 20 and 21, in the form of torrential rains, in amounts of 11, 18 and 22 l/sqm, caused an increase in atmospheric humidity to values of over 88%, between July 17 and 29, creating favorable conditions for the evolution of the pathogens *Alternaria solani* and *Fulvia fulva*.

In September, atmospheric humidity began to increase, with values between 85 and 92.4% recorded in 19 days, with a maximum average of 84.1% for the month (fig. 1). The high humidity and lower temperatures in September created favorable conditions for the emergence and development of the pathogen *Botrytis cinerea*.

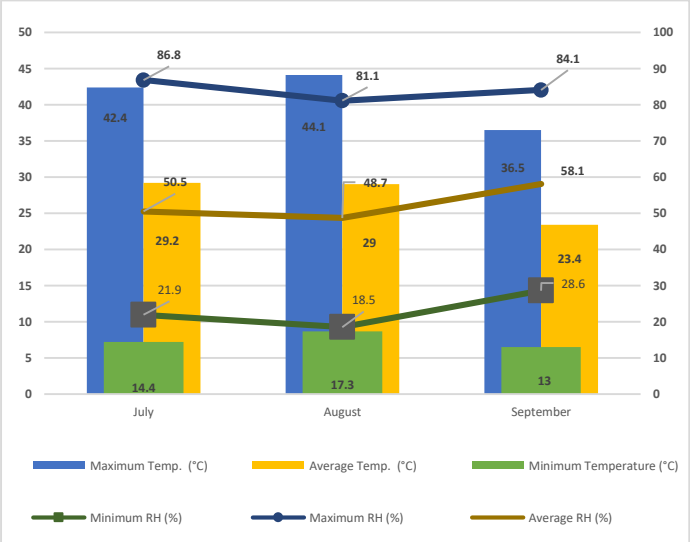

Fig. 1 Greenhouse climate data
for the period July – September 2024 / Datele climatice din seră, în perioada iulie – septembrie 2025

Tomato harvesting took place in Phase VI, according to the USDA tomato ripening stages (Bertin, 2018). The fruits were stored at temperatures of 6°C and 22°C, with a relative humidity of 70%. Evaluations were conducted at harvest and after 3, 5, 7, and 10 days of storage. The analyses included weight loss, dry weight, total soluble solids, firmness, pH and ash content.

The percentage of weight loss was calculated based on the duration of storage, following the methodology presented by Tefera et al. (2007). The formula used was:

Weight loss (%)=$M_0(M_0-M_1)\times100$, where M_0 represents the initial mass of the fruits (g) and M_1represents the mass of the fruits after storage (g). Water and dry weight (%) were determined

using the gravimetric method, total soluble solids (% Brix) were measured using the refractometric method, fruit firmness (kg/cm²) was evaluated with a GY-4 firmness tester, organic acid content was assessed by measuring the fruit pH and ash content (%) was determined using the gravimetric method.

Statistical analyses were performed using SPSS version 26.0.

RESULTS

In the conventional system, all products applied to control pathogens had a very high efficacy ranging between 90.5 and 94.6% for *Alternaria solani* and 86.9 - 90.4% for *Fulvia fulva* (Fig 2).

In the biological control of the pathogen *Alternaria solani*, good results were obtained to the treated variants, with an efficacy between 52.5% (Amulet) and 58.3% (Cavaler 600SL) in controlling this pathogen on leaves. Also, low efficacy was recorded in the control of the pathogen *Fulvia fulva* with values between 40.5% (Amulet) and 48.6% (Cavaler 600SL).

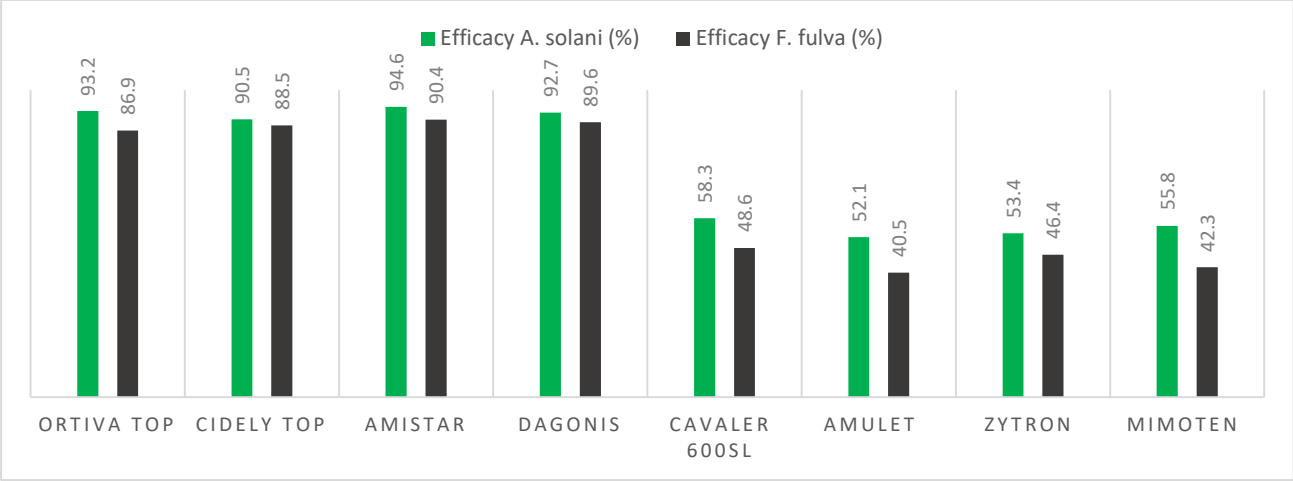

Fig. 2 The efficacy of conventional and biological products on *A. solani* and *F. fulva* / Eficacitatea produselor convenționele și biologice asupra *A. solani* și *F. fulva*

The products Ortiva Top 1 l/ha, Botrefin 0.8 l/ha, Sygnum 1.5 kg/ha and Switch 0.8 l/ha, used to control gray rot, produced by the pathogen *Botrytis cinerea*, had a very good efficacy, between 90.2 and 95.4%. (Fig. 3).

The efficacy of biological products was between 43.7% (Amulet) and 46.1% (Cavaler 600SL) in controlling the pathogen *Botrytis cinerea* on leaves and 63.1% (Amulet) – 65.2% (Cavaler 600SL) on tomato fruits.

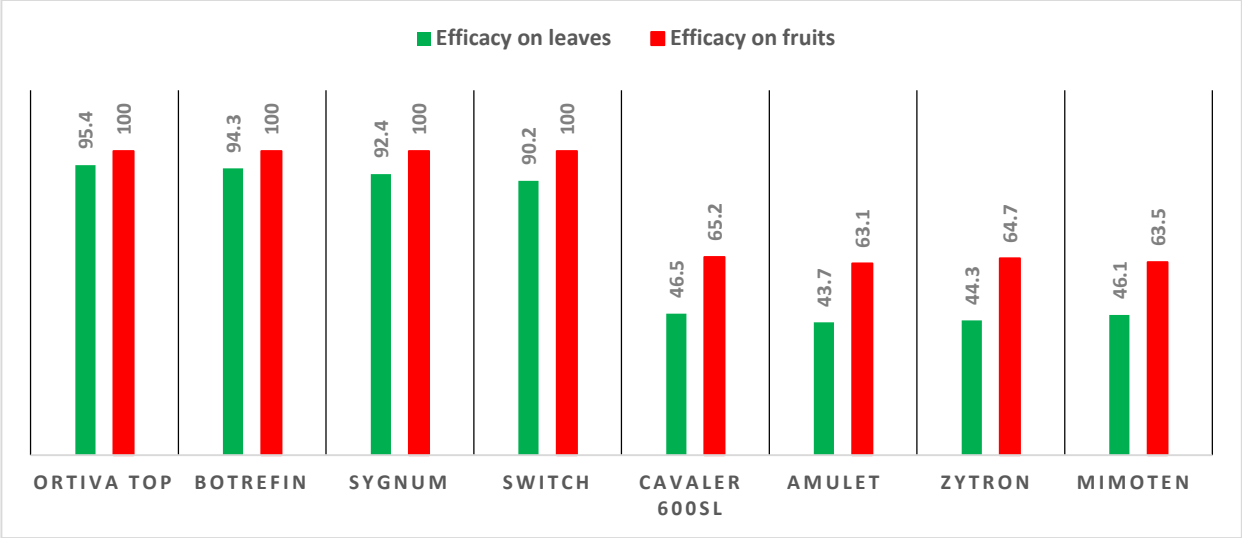

Fig. 3 The efficacy of conventional and biological products on *B. cinerea* / / Eficacitatea produselor convenționele și biologice asupra *B. cinerea*

Results on quality maintenance during storage

Weight loss (%)

Fruit weight loss is mainly determined by respiratory processes, transpiration, and metabolism that occur after harvest (Fatima *et al*., 2022). This loss, primarily caused by dehydration, manifests through changes in the texture, aroma, and appearance of the fruit (Sanford *et al*., 1991; Caleb *et al*., 2012). In the case of tomatoes, the rate of weight loss is significantly influenced by the type of phytosanitary control used during plant development. The study presented revealed average losses of 2.88% for organic products, 3.18% for conventional ones, and 3.48% for the control variant after 10 days of storage at 6°C (Fig. 4). At higher temperatures, weight loss becomes more pronounced; for example, in the Pink Rock F1 hybrid, it reached values of 4.52% for organic products, 4.24% for conventional ones, and 5.10% for the control variant (Fig. 5). This increase is due to the intensification of the respiration rate of the fruits (Gherghi, 1994; Kader, 2002a). The percentage of weight loss during storage also depends on the fruit's maturity stage (Moneruzzaman *et al*., 2009). According to Ben-Yehoshua and Rodov (2002), fruits and vegetables become unmarketable when they lose between 5% and 10% of their initial weight. Excessive water loss not only reduces weight but also accelerates processes of senescence and membrane degradation (Ben-Yehoshua *et al*., 1983). Pre- and post-harvest factors also play a significant role in influencing water loss (Lufu *et al*., 2020). To minimize water loss from fruits during storage, various types of edible coatings have been tested (Peralta-Ruiz *et al*., 2020). Additionally, the use of less ventilated containers may provide more effective protection (Adjouman *et al*., 2018).

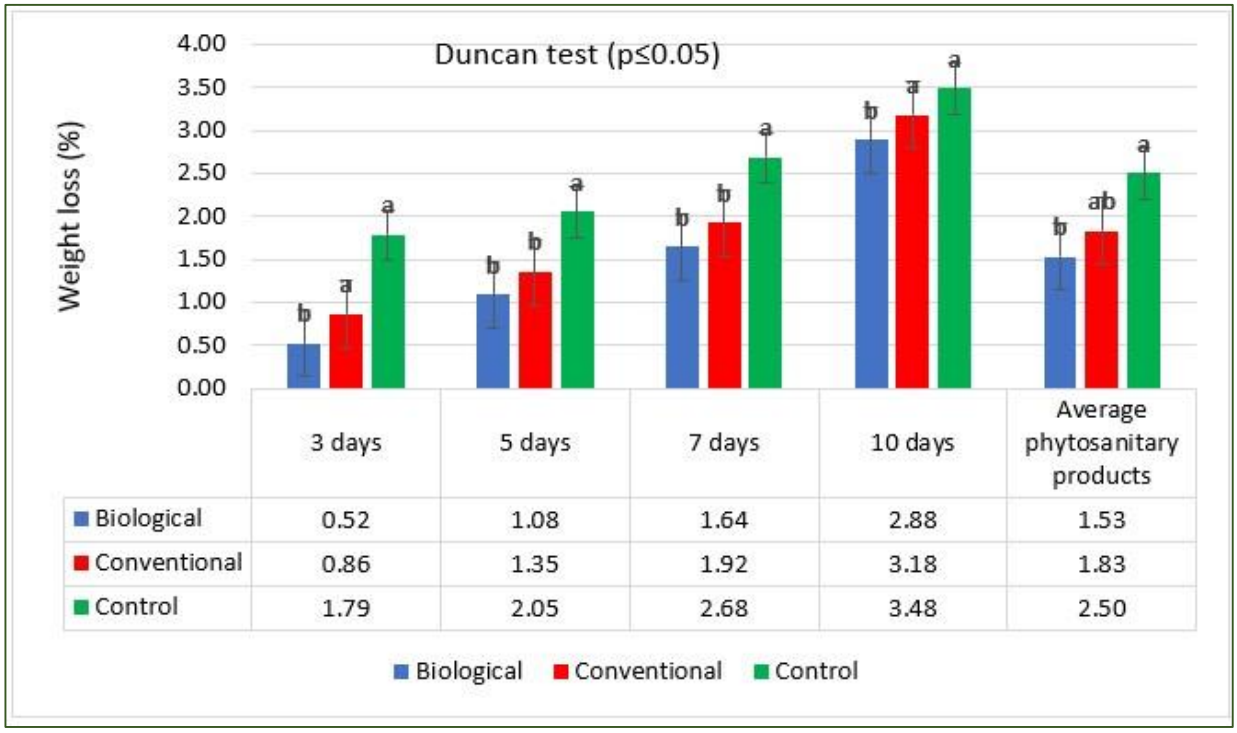

Fig. 4. Influence of phytosanitary control on tomato weight loss (%) depending on the storage period at 6°C / Influența controlului fitosanitar asupra pierderii în greutate a tomatelor în funcție de perioada de depozitare la 6°C

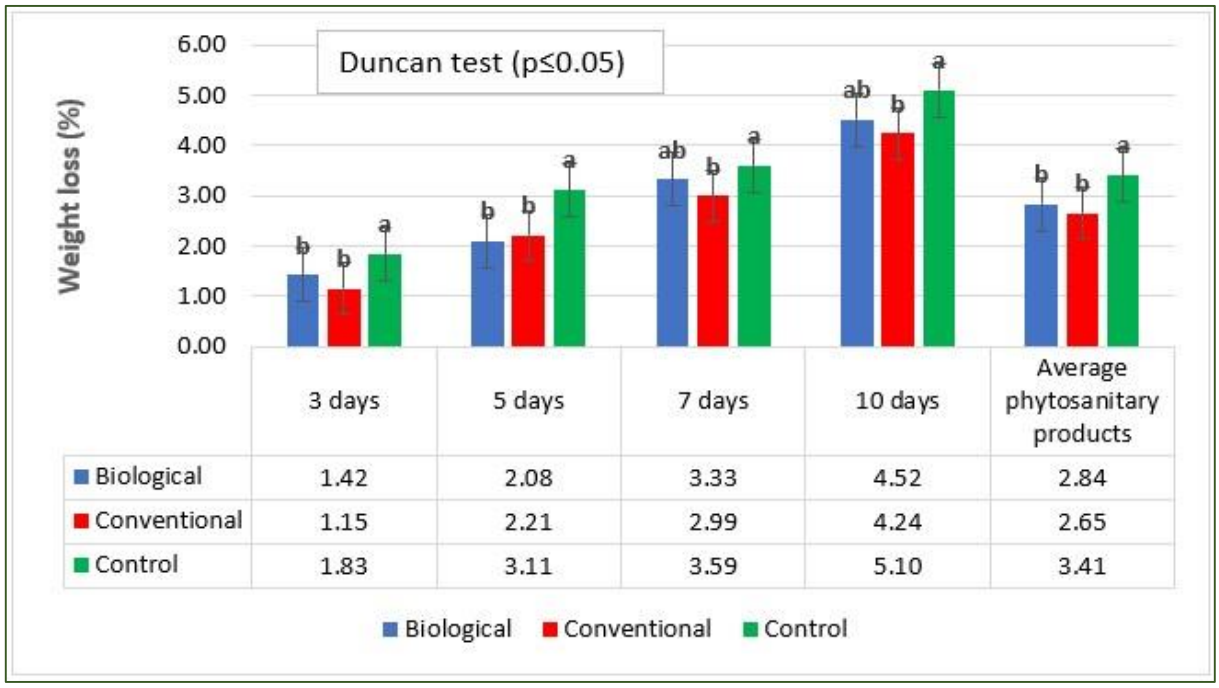

Fig. 5. Influence of phytosanitary control on tomato weight loss (%) depending on the storage period at 22°C / Influența controlului fitosanitar asupra pierderii în greutate a tomatelor în funcție de perioada de depozitare la 22°C

Total Dry Matter Content

The total dry matter (DW) content of fruits is an essential indicator for assessing their quality at harvest. According to research conducted by Davies *et al*. (1981), the water content of tomatoes ranges between 92.5% and 95%. The highest values of dry matter were detected in the control variant after a period of 10 days of storage (Table 1). Factors such as temperature and storage duration significantly influence this dynamic, according to the ANOVA analysis. At a

temperature of 6°C, the total dry matter content during storage was not affected by the phytosanitary treatments administered during the vegetation stage. In contrast, these treatments had a significant effect on this quality parameter only under storage conditions at 22°C. Although this quality indicator is mainly associated with water losses, weight loss provides a more accurate reflection of the effects of treatments applied during the vegetative phase.

Total soluble solids

Total soluble solids (TSS) in fruits mainly reflect the concentration of sugars, organic acids, vitamins, and other soluble substances. A higher value usually indicates a sweeter taste and a more intense aroma. Sugar content is an essential factor in defining the organoleptic quality of fruits (Li *et al*., 2020). During the storage process, an increase in soluble solids is observed. During the first five days of storage at a temperature of 6 °C, this increase is insignificant (from 5.68% to 6.13% Brix in the control variant) (Table 1). Also, at 22 °C, after three days of storage, a similar increase was observed, from 5.65 Brix to 6.13% Brix in the control variant. Subsequently, the total soluble solids content showed a more pronounced increase at both temperatures. This trend of increasing sugar content during storage can be explained by the fruit ripening process and moisture loss. The results obtained support previous observations, according to which fruits can become sweeter as a result of weight loss during storage (Pareek, 2001, cited by Singh *et al*., 2013). Specialized studies suggest that fruit sugars increase in the first days; however, under prolonged storage conditions, they may decrease (Singh *et al*., 2013), because they can be used in respiration processes (Öztürk *et al*., 2019), being transformed into pyruvic acid and citric acid.

Firmness

Tomato firmness is a fundamental aspect of marketing strategy, having a direct impact on consumer perception of quality. This characteristic is determined by cell wall metabolism, moisture level, and cell structure (Al-Dairi *et al*., 2021). Firmness assessment is essential not only for determining fruit quality in the distribution chain but also for breeding programs (Sekse *et al*., 2010). In general, a trend of decreasing tomato firmness is observed as they reach the technical maturity required for consumption. Statistical analysis revealed a decrease in fruit firmness during the ten days of storage. At the beginning of this period, the average firmness of tomatoes ranged between 15.31 N for fruits treated with conventional phytosanitary products and 15.25 N for the control variant. At a temperature of 6 °C, significant differences in fruit firmness were observed depending on the phytosanitary protection methods used during the vegetation period, starting from the seventh day of storage. The lowest firmness values were recorded for fruits in the control variant. At a temperature of 22 °C, significant differences between the phytosanitary control variants were evident starting from the fifth day of storage. Fruit firmness reached the lowest value—6.88 N—at 22 °C after ten days of storage in the control variant. These results suggest that pre-harvest agricultural management conditions can significantly influence tomato firmness during the post-harvest period. According to previous studies, to maintain the commercial characteristics of tomatoes after storage, their firmness should not fall below 4–5 N (Kader, 2002a).

pH

The pH of fruits is closely related to the concentration of organic acids, which is a crucial parameter for preserving their quality. The present study highlights that the pH of tomatoes is affected by various factors, including cultivation technology, storage duration, and temperature. The maximum acid content (corresponding to the minimum pH) was observed in freshly harvested fruits, with values ranging between 4.11 (in fruits treated with biological phytosanitary products) and 4.28 (in fruits from the control variant). In the first three days of storage, a slight increase in pH was recorded, followed by a more pronounced trend in the subsequent days. The most significant reduction in organic acid content was observed between days 7 and 10, when pH values were considerably higher. According to previous studies, the decrease in fruit acidity is due to the involvement of these acids in the metabolic reactions of respiration during storage (Gherghi, 1994; Karadeniz, 2004; Shokrollahfam *et al*., 2012, cited by Zeraatgar *et al*., 2018).

Ash content

The ash content of fruits represents the mineral residues remaining after their complete combustion, thus reflecting the presence of inorganic minerals, including calcium, potassium, magnesium, and phosphorus. This value shows significant variations between different types of fruits and is influenced by factors such as agronomic practices, soil type, environmental conditions, and genetic background (Isack & Lyimo, 2015; Suárez et al., 2011). Fruits with a higher ash content are often considered more nutritious, as they indicate a higher concentration of essential minerals. In the case of the Pink Rock F1 hybrid, no variations in this quality indicator were observed as a result of the use of plant protection products during the growing season.

The intensity of the correlations between the evaluated characteristics, presented in Table 2, suggests that phytosanitary treatments applied during the vegetation period significantly influence the quality of the fruits during storage, especially in terms of weight loss. This result indicates that phytosanitary treatments can contribute to improving the durability of horticultural products in the post-harvest period. Although a slight increase in the dry weight of the fruits and the content of total soluble solids (TSS) is observed, these results are not statistically significant.

Table 1. Influence of temperature and phytosanitary control on the quality of stored tomatoes / Influența temperaturii și a controlului fitosanitar asupra calității tomatelor depozitate

Storage temperature	Storage time (days)	Phytosanitary product	Dry weight. (%)	TSS (% Brix)	Firmness (N)	pH
6°C	At harvest	Biological	5.11±0.37^{a}	5.42±0.29^{b}	15.30±0.75^{a}	4.11±0.04^{b}
		Conventional	5.07±0.27^{a}	5.38±0.20^{b}	15.31±0.72^{a}	4.17±0.08^{ab}
		Control	5.32±0.44^{a}	5.68±0.25^{a}	15.25±0.57^{a}	4.28±0.03^{a}
	3 days	Biological	5.70±0.34^{b}	6.08±0.32^{a}	14.79±0.78^{a}	4.15±0.06^{b}
		Conventional	6.25±0.11^{a}	5.94±0.31^{a}	14.67±0.77^{a}	4.21±0.05^{ab}
		Control	6.18±0.27^{a}	6.08±0.23^{a}	14.77±0.59^{a}	4.32±0.05^{a}
	5 days	Biological	6.62±0.54^{a}	6.37±0.33^{a}	13.45±1.12^{a}	4.19±0.07^{b}
		Conventional	6.69±0.17^{a}	6.46±0.22^{a}	13.39±0.91^{a}	4.25±0.01^{b}
		Control	6.81±0.20^{a}	6.13±0.33^{b}	13.09±0.69^{a}	4.36±0.02^{a}
	7 days	Biological	7.30±0.66^{a}	6.79±0.37^{a}	12.02±0.70^{a}	4.24±0.01^{b}
		Conventional	7.40±0.43^{a}	6.79±0.22^{a}	11.86±0.72^{ab}	4.29±0.02^{b}
		Control	7.92±0.89^{a}	6.62±0.23^{a}	11.57±0.32^{b}	4.39±0.05^{a}
	10 days	Biological	7.59±0.61^{a}	7.60±0.49^{a}	9.96±0.93^{a}	4.31±0.01^{b}
		Conventional	7.95±0.23^{a}	7.23±0.27^{b}	9.84±0.77^{a}	4.38±0.02^{ab}
		Control	8.36±0.78^{a}	6.90±0.13^{b}	9.30±0.58^{b}	4.45±0.05^{a}
22°C	At harvest	Biological	5.14±0.36^{a}	5.40±0.34^{ab}	15.29±0.71^{a}	4.10±0.01^{b}
		Conventional	5.10±0.54^{a}	5.38±0.22^{b}	15.32±0.70^{a}	4.15±0.08^{ab}
		Control	5.32±0.35^{a}	5.65±0.14^{a}	15.18±0.44^{a}	4.25±0.04^{a}
	3 days	Biological	6.01±0.29^{a}	6.24±0.30^{a}	13.52±0.99^{a}	4.18±0.05^{a}
		Conventional	5.93±0.39^{a}	6.08±0.30^{a}	13.21±1.01^{a}	4.25±0.06^{a}
		Control	6.24±0.41^{a}	6.13±0.29^{a}	13.20±1.04^{a}	4.29±0.05^{a}
	5 days	Biological	6.78±0.56^{a}	6.66±0.30^{a}	10.59±0.97^{ab}	4.23±0.07^{c}
		Conventional	6.64±0.25^{a}	6.57±0.21^{ab}	10.85±0.88^{a}	4.30±0.05^{b}
		Control	7.24±0.49^{a}	6.32±0.33^{b}	10.27±0.69^{b}	4.39±0.06^{a}
	7 days	Biological	7.47±0.64^{ab}	7.08±0.40^{a}	9.66±0.57^{b}	4.33±0.06^{b}
		Conventional	7.22±0.29^{b}	6.94±0.24^{ab}	10.14±1.09^{a}	4.30±0.06^{b}
		Control	8.25±0.55^{a}	6.72±0.19^{b}	9.52±0.76^{b}	4.47±0.08^{a}
	10 days	Biological	7.73±0.64^{ab}	7.75±0.51^{a}	7.07±0.75^{ab}	4.40±0.06^{b}
		Conventional	7.51±0.27^{b}	7.41±0.32^{a}	7.24±0.88^{a}	4.36±0.07^{b}
		Control	8.49±0.58^{a}	7.00±0.18^{b}	6.88±0.44^{b}	4.52±0.07^{a}
Total		Biological	6.55±1.08^{b}	6.54±0.85^{a}	12.17±2.0.3^{a}	4.23±0.13^{b}
		Conventional	6.57±0.99^{b}	6.40±0.73^{b}	12.18±1.21^{a}	4.27±0.18^{b}
		Control	7.01±1.26^{a}	6.32±0.50^{b}	11.90±1.07^{a}	4.37±0.18^{a}

*Duncan test: There are no significant differences in the mean values that share the same letter ($p \leq 0.05$).

In addition, a significant positive correlation was identified between the weight loss of tomatoes and dry weight (DW), TSS, and the content of organic acids in the fruits (r=0.582,

r=0.637, respectively). This correlation indicates that as the water volume in tomatoes decreases, the concentration of nutritional and aromatic components increases, influencing the taste, nutritional value, and sensory characteristics of the fruit. There is also a negative and significant relationship between fruit firmness and weight loss (r=-0.751). The interaction between fruit firmness and total soluble solids content, as well as organic acids, shows a significant negative correlation of high intensity (r=-0.876 and r=-0.767, respectively). Essentially, a reduction in fruit firmness corresponds to an increase in total soluble solids and organic acids, in an inversely proportional manner. This phenomenon was also confirmed by Basak *et al.* (2022). Additionally, the positive interdependence between total soluble solids content and fruit pH indicates that as sugars accumulate in the fruit, the acidity level decreases. This finding is supported by several studies (Lavee and Dagan, 1988; Kader, 2002b).

Table 2. Correlation matrix between evaluated component / Matricea de corelație dintre componentele evaluate

		Storage temperature	Phytosanitary product	Weight loss (%)	Dry weight. (%)	Firmness (N)	TSS (% Brix)	pH
Storage temperature	Pearson Correlation	1	0.000	0.359(**)	0.161	-0.182	0.090	0.413(**)
Phytosanitary product			1	0.144(**)	0.135	-0.041	-0.112	0.535(**)
Weight loss (%)				1	0.582(**)	-0.751(**)	0.637(**)	0.826(**)
Dry weight. (%)					1	-0.782(**)	0.778(**)	0.733(**)
Firmness (N)						1	-0.874(**)	-0.767(**)
TSS (% Brix)							1	0.631(**)
pH								1

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

CONCLUSIONS

1. Conventional products: Ortiva Top 0.1%, Cidely Top 0.1%, Amistar 0.1% and Dagonis 0.08% ensure over 90% efficacy in controlling the pathogens *Alternaria solani* and *Fulvia fulva*. Also, Botrefin 0.8 l/ha, Sygnum 1.5 kg/ha and Switch 0.8 l/ha control with very good results *Botrytis cinerea*, in the tomato crop in greenhouses;
2. Biological products (Cavaler 600SL 3 l/ha, Amulet 4 l/ha, Zytron 1.5 l/ha and Mimoten 3 l/ha) have an efficacy of between 40.5 and 65.2% in the control of the pathogens *Alternaria solani, Fulvia fulva* and *Botrytis cinerea*, as a result their use is recommended in case of a low attack or preventive when conditions are favorable for the attack;
3. Phytosanitary treatment applied during vegetation has a significant impact on post-harvest quality, contributing to reducing losses and maintaining the physicochemical characteristics of tomatoes under optimal storage conditions.
4. Additionally, the type of phytosanitary control used influences weight loss and fruit quality differently during storage, with superior results observed for biological treatments at low temperatures and for conventional treatments at higher temperatures.
5. Storage temperature also plays a crucial role, directly affecting weight loss and quality. Maintaining fruits at low temperatures promotes the preservation of firmness and nutrient content.

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VALORIZATION OF RED BEETROOT BY-PRODUCTS, AN INSPIRATIONAL MODEL FOR THE FUTURE OF NATIONAL FOOD SECURITY

VALORIZAREA EFICIENTĂ A SUBPRODUSELOR DE SFECLĂ ROȘIE, UN MODEL INSPIRAȚIONAL DE CONTRIBUȚIE LA ASIGURAREA VIITORULUI SECURITĂȚII ALIMENTARE NAȚIONALE

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Abstract
The innovative valorization of beetroot by-products contributes to reducing waste and supporting a circular economy, ensuring improved resource efficiency, reduced production costs and reduced environmental impact of the food industry. Through their diversity and richness in bioactive phytochemical compounds with beneficial influence on health, beetroot by-products have highlighted a versatility and importance that advocates the need for further research. In this context, it has been demonstrated that beetroot peel powder incorporated into various foods (mayonnaise, meringues, nougat etc) compositional formulas improved their aroma and texture, and increased antioxidant activity due to its high concentration of phenolic compounds, in addition to its function as a natural colorant and the pleasant pink hue it gives to value added food.

Keywords: *red beet by-products, value added food, antioxidant activity, biological active compounds.*

Rezumat
Valorizarea inovativă a subproduselor din sfeclă roșie, contribuie la reducerea deșeurilor și la sprijinirea unei economii circulare, asigurând îmbunătățirea eficienței resurselor, reducerea costurilor de producție și diminuarea impactului industriei alimentare asupra mediului înconjurător. Prin diversitatea și bogăția lor în compuși fitochimici bioactivi cu influență benefică asupra sănătății, subprodusele de la sfecla roșie au evidențiat o versatilitate și o importanță ce pledează în favoarea necesității unor cercetări ulterioare.În acest context, s-a demonstrat că pudra din coajă de sfeclă roșie încorporată în diversele formule compoziționale de produse alimentare (maioneză, bezele, alviță etc) a îmbunătățit aroma și textura acestora, asporit activitatea antioxidantă datorită concentrației sale mari de compuși fenolici, pe lângă funcția sa de colorant natural și nuanța plăcută de roz pe care o conferă produselor alimentare cu valoarea adăugată.

Cuvinte cheie: *subproduse din sfeclă roșie, produse alimentare cu valoare adăugată, activitate antioxidantă, compuși biologic activi.*

INTRODUCTION

Beetroot is a vegetable rich in biologically active compounds (Ninfaliand Angelino, 2013; Clifford, 2015) such as carotenoids, nitrates, flavonoids, vitamins, minerals such as potassium, sodium, phosphorus, calcium, magnesium, copper, iron, zinc, manganese) and water-soluble pigments such as betalains (Guldiken *et al*., 2016), which are divided into two classes, betacyanins (red-purple in color) and betaxanthins (yellow-orange in color) (Panghal *et al*., 2017), being an important food source for human health.

The presence in beetroot of compounds such as polyphenols, carotenoids and some vitamins lead to significantly high values of antioxidant, anti-inflammatory, anticarcinogenic and

hepatoprotective activities, with antidiabetic, antihypertensive, reducing the risks of cardiovascular diseases, and wound healing effects (Slavov *et al*., 2013).

As a result of the technological processing of red beetroot, a significant amount of vegetable by-products results in the form of peels rich in the bioactive compounds already mentioned, which are important sources of flavor compounds, dyes and natural antioxidants, capable of replacing chemically synthesized additives from the compositional formulas of some food products. This is contributing to increase the quality of life through the benefits induced on the state of health and by increasing the attractiveness and the diversity of these products among consumers as a result of the improvement of their sensory properties (taste, aroma, colour) and, equally, to ensure the circular economy worldwide. For example, beetroot by-products, following minimal technological processing, could be incorporated into the compositional formulas of food products such as mayonnaise, meringue and nougat.

Mayonnaise is one of the most popular and appreciated sauce used in a variety of foods with the aim of improving their flavor, texture and taste. The ingredients used in the compositional formulas for the preparation of mayonnaise sauce are whole egg or egg yolk, vinegar, water, mustard and soybean, rapeseed, sunflower or corn vegetable oil (Depree and Savage, 2001). Although it is highly valued for its aroma, texture and taste, mayonnaise is a food product susceptible to the oxidation of lipids in vegetable oil rich in polyunsaturated fatty acids (Raikos *et al*., 2015) so the use of antioxidants is imperative. Although synthetic antioxidants are cheap and effective, they are not widely accepted by consumers.Inthis context, the food industry is currently focusing on replacing synthetic antioxidants with those obtained from natural plant resources (Park *et al*., 2019).

Meringue is a type of dessert associated with Swiss, French, Polish and Italian cuisine, being traditionally prepared from egg whites and sugar, so the main nutrients are proteins and carbohydrates. Since meringue does not have a stable structure, it has a short shelf life of about 2 weeks if stored properly. Being a hygroscopic food, it easily absorbs water from the environment in which it is stored. The increase of the structural stability and implicitly of the shelf life of the marshmallow could be ensured by incorporating into its compositional formula a plant extract with a superior structural protection capacity.

Nougat is a food product with a sweet taste and different textures, obtained from sugar, glucose syrup, honey, starch or egg white, with or without the addition of nuts, almonds, candied fruits, raisins, cocoa, various flavors and certain additives. In order to increase diversity, the classic recipes for obtaining nougat are based on the mentioned ingredients but also on the addition of chemically synthesized additives (thickening and loosening agents, preservatives, taste enhancers, flavors, dyes) that can exert a cumulative negative effect on the health of the human body. In this context, it is imperative to optimize the compositional formulas of the nougat varieties by supplementing them with biologically active compounds from vegetable by-products such as those from beetroot.

Therefore, the objective of the research was to obtain 3 types of value-added food products (mayonnaise sauce, meringue, nougat) by adding beetroot peels powder, which could be considered as an inspirational model in the direction of ensuring the food security of the population in the future.

MATERIALS AND METHODS

Powder preparation. The red beetroot was bought from a local producer in Galati County. It was washed, and with a knife the peels were removed. Subsequently, the beetroot peel together with the pulp soaked in juice was washed with ultrapure water, and frozen in the drying room. The drying operation was carried out by freeze-drying technique (CHRIST Alpha 1-4 LD plus, Germany), at a temperature of – 42 °C, under a pressure of 0.10 mBar, for 48 h. Finally, the dried

pomace and peels with a relative humidity of 10 % were ground using special equipment (MC 12 Producer Stephan, Germany) and then stored in a plastic container with a lid in the dark at the room temperature.

Phytochemical characterization of red beetroot by products powder. The methods used for the content of biologically active compounds were the Folin-Ciocâlteu method for determining the content of total polyphenols (mg EAG/g s.u.) (Giusti and Wrolstad, 2001), the method described by Cai et al. (1998), for the total betalaine content (mg/g s.u.) and the method of reducing the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) in the presence of an antioxidant for the value of antioxidant activity (mMol Trolox/g s.u.). The same methods were used for the phytochemical characterization of all experimental samples with the addition of red beetroot powder.

Physicochemical characterization of value-added foods. The values of the physicochemical parameters of the value-added products (moisture, protein content, fat content, ash content, total sugar content etc) were determined using AOAC methods.

Evaluation of the industrial application potential of red beetroot by products powder. Different technological variants were developed for obtaining 3 types of value-added products (mayonnaise, meringue, nougat), and their functionality was evaluated by determining the content of phytochemical compounds and the value of antioxidant activity. The technological processes were carried out within the Integrated Center for Research, Expertise and Technology Transfer for the Food Industry at the Faculty of Food Science and Engineering, Lower Danube University of Galati (https://erris.gov.ro/FOOD-BIOTECHNOLOGY).

Analysis of the textural profile of value-added products. To examine the textural characteristics of value-added products it was used a CT3-1000 texture analyzer (Brookfield Ametek, Chandler, AZ, USA). Double dispersion in a sample of an acrylic cylinder with a diameter of 38.1 mm was used to obtain a depth of 20 mm. The test speed was set to one millisecond per second, the trigger load to 0.067 N, and the load cell to 9.8 N. The following textural parameters: firmness, cohesiveness, elasticity, adhesiveness, and chewiness were calculated using the TexturePro CT V1.5 software. Each test was subjected to three determinations. The samples were kept at room temperature for 2 h before testing.

Sensory analysis of value-added products. The sensory attributes of the products obtained were evaluated by 20 untrained consumers aged between 25 and 60 years (80% women and 20% men). A training session was offered to the panelists before the sensory analysis. For the mayonnaise samples, the characteristics evaluated were color, aroma, taste, consistency, texture, smell, aftertaste, spreadability and acceptability, using a hedonic 9-point rating scale with scores from 1 (very unpleasant) to 9 (very pleasant), accompanied by an evaluation questionnaire. From a sensory point of view, the samples of marshmallows and nougat were analyzed using a scale with 7 attributes (color, appearance, smell, aroma, taste, texture, sound, aftertaste), based on a unitary numbering. The sensory analysis was performed were the temperature of 20 °C and the relative humidity of the air (45 – 47 %).

Statistical analysis. All determinations were performed in duplicate, and the data were presented as mean and standard deviation. Significant differences between outcomes were identified using analysis of variance (ANOVA). Tukey's test was applied using the Minitab 18 software to determine significant differences between samples. For all tests, p-values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Characterization of red beetroot byproducts powder. Characterization of red beetroot byproducts powder is presented in Table 1 (Lazăr *et al*., 2021).

Tabel 1. Phytochemical characteristics of beetroot by-product powder /
Caracteristicile fitochimice ale pudrei din subproduse de sfeclă roșie

Characteristics	Values
Total betalains (mg/g dw)	2.34 ± 0.12[a]
Total polyphenols (mg EAG/g dw)	282.12 ± 5.09
Antioxidant activity (mM Trolox/g dw.)	46.66 ± 0.21

[a]-standard error

Obtaining mayonnaise sauce with the addition of red beetroot powder. A recipe for obtaining mayonnaise sauce has been developed by combining the following ingredients in the following proportions of weight (g/w): sunflower oil (80%), egg powder (8%), water (7%), vinegar (2%), lemon jus (3%), salt (0.3%) and different proportions (S1-1.5%, S2-3%, S3-5% and S4-7%) of beetroot powder hydrated with water (1:1). A emulsion was made in water by dissolving the powder from egg yolk, lemon jus, salt and vinegar. The mayonnaise was prepared by gradually adding oil to the aqueous mixture and quickly mixing the components with a hand blender for 10-15 min. Beetroot powder was further added to the mayonnaise at four different concentrations of 1.5%, 3%, 5% and 7%, continuing mixing until the samples turned uniform purple and were coded S1, S2, S3 and S4. The control sample of control mayonnaise was produced in the same way as the experimental mayonnaise, but without the addition of red beetroot powder. The mayonnaise samples obtained were kept at 4°C until the measurements were taken.

The phytochemical composition and antioxidant activity of mayonnaise with added beetroot powder together with storage stability over 28 days at a temperature of 4 °C is shown in Table 2. All parameters evaluated, the content of bioactive compounds (betalains and total polyphenols) and antioxidant activity, increased as a larger amount of red beetroot powder was added to the mayonnaise samples (Lazăr *et al*., 2022).

Table 2. Phytochemical characteristics and antioxidant activity of control and value-added mayonnaise samples
Caracteristicile fitochimice și activitatea antioxidantă ale probelor de maioneză analizate

Phytochemical characteristics		Value-added mayonnaise samples				
		S0	S1	S2	S3	S4
Total betalains (mg/100 gdw)	0 days	-	1.31 ± 0.01^{aD}	2.58 ± 0.06^{aC}	4.19 ± 0.09^{aB}	5.61 ± 0.16^{aA}
	14 days	-	1.11 ± 0.04^{abD}	2.22 ± 0.02^{bE}	3.69 ± 0.24^{aB}	4.86 ± 0.09^{bA}
	28 days	-	0.83 ± 0.13^{bD}	1.83 ± 0.02^{cC}	2.84 ± 0.11^{bB}	4.12 ± 0.06^{cA}
Total polyphenols, (mg/100 gdw)	0 days	24.61 ± 0.06^{aE}	197.11 ± 1.91^{aD}	270.4 ± 11.06^{aC}	307.4 ± 4.06^{aB}	325.9 ± 5.61^{aA}
	14 days	20.95 ± 0.64^{bE}	188.11 ± 3.96^{aD}	252.65 ± 1.34^{bC}	278.95 ± 3.75^{bB}	310.50 ± 1.27^{bA}
	28 days	18.15 ± 1.06^{bE}	152.15 ± 2.19^{bD}	227.50 ± 1.98^{cC}	253.85 ± 7.71^{cB}	285.05 ± 0.78^{cA}
Antioxidant activity (mM Trolox/100 gdw)	0 days	1.81 ± 0.01^{aE}	29.5 ± 0.11^{aD}	37.07 ± 0.90^{aC}	45.60 ± 0.61^{aB}	52.09 ± 2.91^{aA}
	14 days	1.77 ± 0.03^{aD}	27.20 ± 0.42^{aC}	34.65 ± 1.77^{abB}	39.25 ± 1.20^{bB}	48.75 ± 1.63^{abA}
	28 days	1.60 ± 0.10^{aE}	21.15 ± 0.6^{bD}	30.35 ± 0.78^{bC}	34.80 ± 1.27^{cB}	46.05 ± 0.21^{bA}

The averages on the same row (uppercase letters) and the same column (lowercase letters) for each sample analysed that do not have a letter in common are significantly different ($p < 0.05$).

The physicochemical composition control mayonnaise (Table 3) revealed results similar to those reported by other authors (Rojas *et al*., 2019) (10), while the physicochemical composition of mayonnaise with powder additions revealed a significant difference ($p < 0.05$) between the samples analysed. The results showed that as the percentage of red beetroot powder in the mayonnaise samples increased, the moisture was lower and the ash content was higher (Lazăr *et al*., 2022). Furthermore, the carbohydrate content was increased by adding red beetroot powder (Table 3).

Tabel 3. Physico chemical characteristics of control and value-added mayonnaise samples / Caracteristicile fizico-chimice ale probelor de maioneză analizate

Physico chemical characteristics	S0	S1	S2	S3	S4
Proteins, g/100 g	5.4 ± 0.01^{a}	5.2 ± 0.01^{b}	5.1 ± 0.03^{c}	5.02 ± 0.01^{d}	4.91 ± 0.01^{e}
Lipids, g/100 g	72.05 ± 0.01^{a}	71.6 ± 0.14^{ab}	71.3 ± 0.14^{b}	71.7 ± 0.14^{ab}	71.5 ± 0.14^{b}
Carbohydrates, g/100 g	2.65 ± 0.01^{e}	3.11 ± 0.03^{d}	3.26 ± 0.01^{c}	3.52 ± 0.01^{b}	3.7 ± 0.01^{a}
Humidity, g/100 g	18.04 ± 0.01^{a}	17.97 ± 0.01^{b}	17.92 ± 0.01^{b}	17.15 ± 0.01^{c}	17.01 ± 0.01^{d}
Ash, g/100 g	1.91 ± 0.01^{e}	2.12 ± 0.01^{d}	2.42 ± 0.01^{c}	2.61 ± 0.01^{b}	2.88 ± 0.01^{a}

Averages on the same row that do not have a letter in common are significantly different ($p < 0.05$).

The sensory characteristics of the mayonnaise samples enriched with different concentrations of red beetroot powder (Figure 1) revealed the following appreciations.

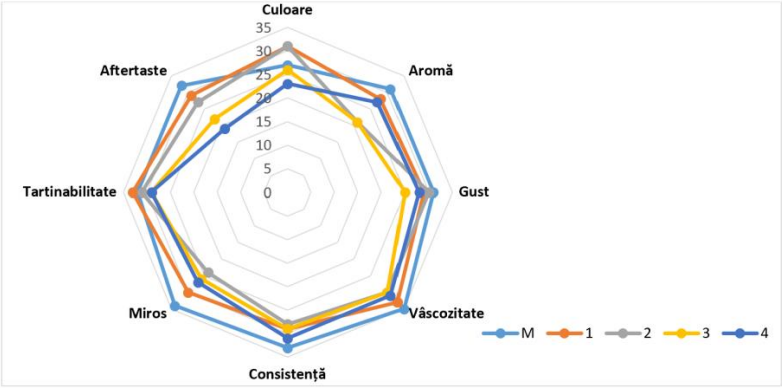

Figure 1. Sensorial evaluation of samples / Evaluarea senzorială a probelor

Color of the mayonnaise samples was considerably influenced by the concentration of red beetroot powder ($p < 0.05$), so that the sample S4 with 7% beetroot husk powder received the highest color score, followed by S3 with 5%, as a result of the increase in the concentration of betalain pigments that generated an attractive red-purple color. Smell, taste and spreadability were not affected by the addition of red beetroot powder (Lazăr *et al*., 2022). The texture of the samples was affected by the addition of a higher percentage of red beetroot powder (7%); the addition of higher concentrations of beetroot powder in mayonnaise led to a slightly increased consistency score. The results obtained were in agreement with those of other authors (Raikos *et al*., 2016) (11), who evaluated the sensory properties of mayonnaise with the addition of microwaved beetroot and found that the addition of beetroot improved the color, textural properties and overall acceptability of mayonnaise.

Obtaining marshmallows with the addition of red beetroot powder. The value-added marshmallows were obtained from the following ingredients: 27 g of egg white powder (equivalent to one egg), hot water (40ºC), 50 g of powdered sugar and red beetroot powder (B1 - 4%, B2 - 7% and B3 - 10%). The process described is simple, involving the mixing of the ingredients presented above, with beetroot husk powder being added as an ingredient. For comparison, a control sample (B) was also performed, which followed the same technology, but in which red beetroot powder was not added. The results presented in Table 4 confirm the added value of marshmallows with the addition of red beetroot powder, by increasing the total content of betalains and polyphenols, which lead to a product with high antioxidant activity. For this reason, beetroot powder can be used as a natural substitute for antioxidants obtained through chemical synthesis. After storage for 21 days at a temperature of 4 ºC, marshmallows with the addition of beetroot powder, a slight decrease in the

content of total betalains and total polyphenols, as well as in the antioxidant activity, was observed in all the variants of marshmallows (Lazăr *et al*., 2022).

Table 4. Phytochemical characteristics and antioxidant activity of control and value-added marshmallow samples / Caracteristicile fitochimice și activitatea antioxidantă ale probelor de bezele analizate

Phytochemical characteristics	Samples				
	Time, days	B	B1 (4%)	B2 (7%)	B3 (10%)
Total betalains (mg/100 gdw)	0 days	-	4.10 ± 0.02^{aA}	6.62 ± 0.05^{aB}	9.93 ± 0.38^{aC}
	7 days	-	3.52 ± 0.04^{bA}	5.19 ± 0.11^{bB}	8.38 ± 0.22^{bC}
	14 days	-	2.85 ± 0.04^{cA}	4.58 ± 0.06^{cB}	7.24 ± 0.11^{cC}
	21 days	-	1.60 ± 0.09^{dA}	3.32 ± 0.18^{dB}	5.67 ± 0.15^{dC}
Total polyphenols, (mg/100 gdw)	0 days	38.36 ± 0.29^{aA}	42.14 ± 1.16^{aA}	52.80 ± 1.23^{aB}	65.90 ± 0.68^{aC}
	7 days	32.59 ± 0.48^{bA}	32.26 ± 1.13^{bA}	40.92 ± 1.44^{bB}	57.09 ± 0.16^{bC}
	14 days	28.87 ± 0.19^{cA}	30.31 ± 0.85^{bcA}	36.87 ± 0.29^{cB}	43.90 ± 0.55^{cC}
	21 days	26.01 ± 0.51^{dA}	28.26 ± 0.41^{cA}	31.69 ± 0.32^{dB}	31.18 ± 1.11^{dB}
Antioxidant activity (mM Trolox/100 gdw)	0 days	4.19 ± 0.02^{aA}	17.15 ± 0.55^{aB}	27.59 ± 0.74^{aC}	39.06 ± 0.52^{aD}
	7 days	3.90 ± 0.12^{aA}	14.29 ± 0.98^{bB}	26.05 ± 1.04^{aC}	35.01 ± 0.68^{bD}
	14 days	2.38 ± 0.18^{bA}	12.49 ± 0.38^{bB}	24.65 ± 0.74^{aC}	29.87 ± 0.87^{cD}
	21 days	2.14 ± 0.07^{bA}	11.94 ± 0.19^{bB}	22.33 ± 2.35^{aC}	27.06 ± 0.38^{dC}

The variation in the concentration of compounds over time is highlighted by small letters on the column.

The differences in concentrations of the compounds between the samples are highlighted by uppercase letters one at a time. Values that divide a lowercase/uppercase letter are not significantly different (p>0.05)

The physicochemical analysis of value-added marshmallows revealed that the addition of beet husk powder resulted in a slight decrease in protein content by up to 4 %, simultaneously with a slight increase in carbohydrate concentration with an increase in the percentage of powder added (up to a 3 % increase in carbohydrate content in sample B3 compared to the control sample) (Table 5).

Table 5. Physico chemical characteristics of control and value-added marshmallows samples / Caracteristicile fizico-chimice ale probelor de bezeleanalizate

Physico chemical characteristics	Bezele			
	B	B1 (4%)	B2 (7%)	B3 (10%)
Proteins, g/100 g	4.81 ± 0.09^{a}	4.63 ± 0.02^{a}	4.61 ± 0.03^{a}	4.60 ± 0.01^{a}
Glucides, g/100 g	84.62 ± 1.52^{a}	85.12 ± 3.14^{a}	86.30 ± 1.09^{a}	87.04 ± 1.88^{a}
Humidity, g/100 g	9.69 ± 0.01^{a}	9.23 ± 0.18^{a}	7.91 ± 0.36^{b}	7.10 ± 0.49^{b}
Ash, g/100 g	0.88 ± 0.02^{a}	1.02 ± 0.01^{b}	1.18 ± 0.01^{c}	1.26 ± 0.02^{d}

Averages on the same row that do not have a letter in common are significantly different (p < 0.05).

Table 6 showing the evolution of the textural parameters of marshmallows shows that the minimum firmness value, 10.44 N, was recorded for the control sample, and for the other experimental samples the firmness values were increasing, simultaneously with the increase in the added beet husk powder content (firmness was expressed as the maximum force recorded during the first penetration cycle) (Bourne, 2002).

Table 6. Texturalparameters of samples / Parametri texturali ai probelor

Parameters	B	B1 (4%)	B2 (7%)	B3 (10%)
Firmness, N	10.44 ± 0.77^{a}	10.50 ± 0.99^{a}	10.57 ± 0.90^{a}	10.92 ± 0.14^{a}
Adhesion, mJ	0.30 ± 0.03^{a}	0.28 ± 0.01^{a}	0.28 ± 0.01^{a}	0.31 ± 0.02^{a}
Cohesiveness, -	0.05 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}	0.06 ± 0.01^{a}
Elasticity, mm	2.12 ± 0.15^{ab}	2.18 ± 0.03^{ab}	2.06 ± 0.11^{a}	2.30 ± 0.07^{b}
Masticability, mJ	1.72 ± 0.10^{a}	1.83 ± 0.01^{ab}	1.83 ± 0.17^{ab}	2.00 ± 0.16^{b}

The differences between the analyzed samples were highlighted by small letters one by one. The values of the averages that divide a letter are not significantly different (p>0.05)

Analysing the results of the sensory assessment of value-added marshmallows (Figure 2), it is noted that the variants of marshmallows with the addition of red beetroot powder were assessed as having a balanced and pleasant colour, corresponding to beetroot, unlike the control variant which was the least appreciated. All the samples proposed for analysis were positively appreciated by the team of tasters, who appreciated the value-added marshmallows as having an easily perceptible beetroot taste and aroma.

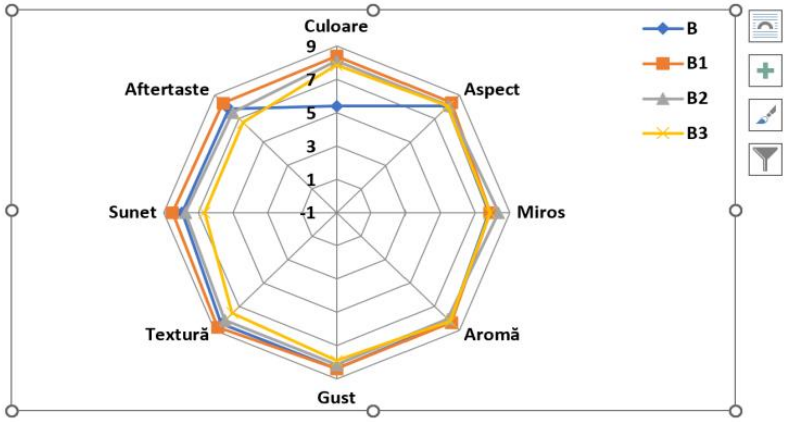

Figure 2. Sensorial evaluation of samples / Evaluarea senzorială a probelor

Obtaining nougat with the addition of red beetroot powder. Value-added nougat is obtained from the following ingredients, expressed in % (w/w): sugar (52 %), honey (26 %), egg white (16 %), lemon juice (2 %), salt (0.2 %) and beetroot powder previously hydrated with water in a ratio of 1:1 (A1 – 2 %, A2 – 4 % and A3 – 6 %), water (the rest up to 100 %).The resulting value-added nougat had a moderate consistency, a remarkable reddish-purple color specific to red beetroot, a sweet and pleasant taste and a fine and homogeneous texture, specific to the conventional product. For comparison, a control sample was also made, which followed the same technology, but in which red beetroot powder was not added.

Table 7 shows the phytochemical profile of value-added nougat samples, obtained by incorporating increasing concentrations (2%, 4% and 6%) of red beetroot powder, which highlights their added value by increasing the total betalains and polyphenols contents, leading to a product with high antioxidant activity.Table 7 shows the results obtained from the evaluation of the stability of the bioactive compounds in the value-added nougat during 21 storage days. The content of bioactive compounds in the value-added nougat shows a slight decrease and implicitly a slightly reduced antioxidant potential, but the nougat samples supplemented with increasing concentrations of beetroot powder show a rich profile of betalains and polyphenols compared to the conventional control product.As for the antioxidant potential, the samples of nougat with the addition of beetroot powder (4% and 6%) showed a higher antioxidant potential than that of the control sample. Therefore, supplementing the nougat with concentrations of over 2% beetroot powder contributes to its enrichment with bioactive compounds that lead to a product with high antioxidant activity.

Table 7. Phytochemical characteristics and antioxidant activity of control and value-added nougat samples / Caracteristicile fitochimice și activitatea antioxidantă ale probelor de alviță analizate

Samples	Phytochemical characteristics	0 days	7 days	14 days	21 days
H	Total betalains (mg/100 g dw)	Nd*	Nd*	Nd*	Nd*
	Total polyphenols (mg EAG/100 g dw)	32.95 ± 4.19^{a}	30.00 ± 1.99^{a}	26.76 ± 1.82^{ab}	21.71 ± 1.20^{b}
	Antioxidant activity(mM Trolox/100g dw)	2.68 ± 0.36^{a}	2.67 ± 0.09^{a}	2.28 ± 0.02^{ab}	2.02 ± 0.05^{b}
H1	Total betalains (mg/100 g dw)	1.78 ± 0.08^{a}	1.49 ± 0.03^{b}	1.17 ± 0.06^{c}	1.02 ± 0.09^{c}
	Total polyphenols (mg EAG/100 g dw)	38.63 ± 1.26^{a}	33.36 ± 1.81^{b}	30.73 ± 0.93^{b}	25.46 ± 0.90^{c}
	Antioxidant activity(mM Trolox/100g dw)	25.20 ± 0.81^{a}	24.51 ± 0.78^{a}	19.40 ± 1.02^{b}	16.21 ± 0.25^{c}
H2	Total betalains (mg/100 g dw)	2.86 ± 0.03^{a}	2.56 ± 0.04^{b}	2.19 ± 0.16^{c}	1.99 ± 0.06^{c}
	Total polyphenols (mg EAG/100 g dw)	53.44 ± 1.33^{a}	50.87 ± 0.62^{a}	46.45 ± 1.67^{b}	40.48 ± 0.76^{c}
	Antioxidant activity(mM Trolox/100g dw)	54.94 ± 2.67^{a}	50.55 ± 0.71^{b}	46.42 ± 0.97^{c}	41.47 ± 1.17^{d}
H3	Total betalains (mg/100 g dw)	3.77 ± 0.09^{a}	3.52 ± 0.09^{b}	3.25 ± 0.11^{c}	2.83 ± 0.08^{d}
	Total polyphenols (mg EAG/100 g dw)	69.48 ± 2.88^{a}	66.42 ± 1.82^{ab}	61.55 ± 1.34^{b}	53.65 ± 0.89^{c}
	Antioxidant activity(mM Trolox/100g dw)	73.89 ± 3.65^{a}	66.86 ± 1.59^{b}	59.91 ± 2.37^{c}	53.33 ± 1.92^{d}

*Nd undetectable

*The different letters (a-b) per row for the same analyzed parameter show significant differences between the means ($p < 0.05$).

The results of the texture analysis of the samples are presented in Table 8. It was found that the addition of red beetroot powder led to an increase in the firmness and adhesion of the samples. If the differences between the control sample and the sample with 2 % added powder are not significant, when the amount of powder added increases, they become more obvious, so that the sample with 6 % added beetroot powder showed a firmness value almost 3 times higher compared to the control sample.

Table 8. Textural parameters of samples / Parametri texturali ai probelor

Parameters	H	H1	H2	H3
Firmness, N	0,54±0,05^{a}	0,56±0,01^{a}	1,06±0,12^{b}	1,51±0,03^{c}
Adhesion, mJ	1,00±0,03^{a}	1,11±0,18^{a}	1,42±0,02^{a}	1,55±0,03^{a}
Cohesiveness	0,57±0,03^{a}	0,45±0,01^{a}	0,43±0,01^{a}	0,38±0,005^{a}
Elasticity, mm	3,65±0,005^{a}	3,23±0,05^{a}	2,32±0,15^{a}	1,28±0,01^{a}
Gumminess, N	0,29±0,005^{a}	0,26±0,04^{a}	0,23±0,02^{a}	0,17±0,05^{a}
Chewability, mJ	1,06±0,06^{a}	0,70±0,02^{ab}	0,52±0,01^{ab}	0,41±0,01^{c}

The differences between the analyzed samples were highlighted by small letters one by one. The values of the averages that divide a letter are not significantly different (p>0.05)

The evolution of firmness is explained by the increase in the density and consistency of the nougat when adding powder. Firmness and adhesion positively influence the preservation of the shape of the product during the storage period. At the same time, the particles in the beetroot powder led to the fragmentation of the protein matrix and the weakening of the internal bonds, which is demonstrated by the decrease in cohesiveness with the increase in the amount of powder added. This finding explains the easier disintegration of samples in the oral cavity during

mastication. In conclusion, it can be said that the addition of beetroot powder has a positive effect on the texture of the beetroot by improving firmness and facilitating mastication.

Analyzing the results of the sensory evaluation of the value-added samples (Figure 3), it is noted that the variants of nougat with the addition of beetroot powder were evaluated as having a balanced, pleasant color, corresponding to the beetroot, unlike the control variant which was the least appreciated.

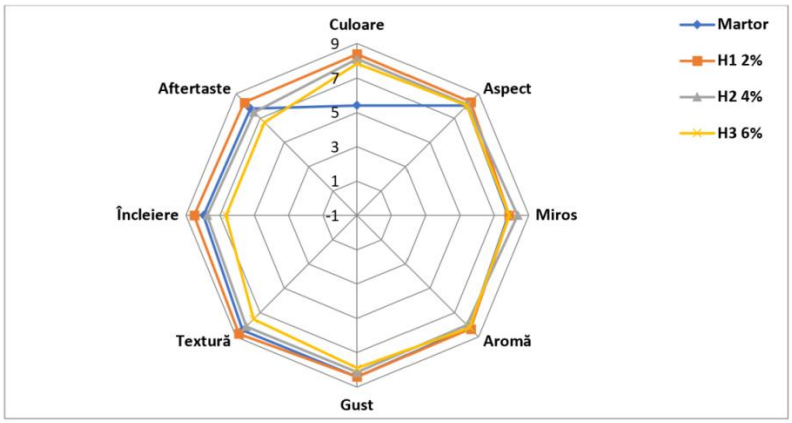

Figure 3. Sensorial evaluation of samples / Evaluarea senzorială a probelor

Positive appreciations were received from the tasters, who appreciated the value-added nougat as having an easily perceptible taste and aroma of roasted beets. The most popular variant was H1, the one with a 2% powder content. It was observed that as the percentage of powder in beetroot powderincreased, tasters appreciated their texture and aftertaste less. However, obtaining a new assortment of nougat with the addition of beetroot powder was distinguished by a reddish-purple color conferred by the addition of powder rich in pigments (betalains) from beetroot, very attractive for consumers and especially for children. The added value of the product is evidenced by the high intake of natural antioxidants present in beetroot by products, as well as by the lack of toxicity.

CONCLUSIONS

1. The use of by-products obtained from the industrial processing of beetroot can become a viable alternative to the variants of dyes, flavorings and antioxidants obtained by chemical synthesis, due to the rich content of biologically active compounds and a higher value of antioxidant activity. In this context, these by-products can have multiple uses in the food industry, can contribute to waste reduction and the implementation of a circular economic model beneficial for environmental protection and, last but not least, to the reduction of food waste, serving as an inspirational model for ensuring future food security.
2. The addition of red beetroot powder in the mayonnaise samples resulted in an improvement in firmness, adhesion and chewiness, giving the product a soft texture as a result of the significant improvement in viscosity. The sensory evaluation of the value-added mayonnaise indicated an improvement in the color attributes of the product and did not affect the overall consumer acceptance score.
3. The results obtained for the meringue type product support the multifunctionality of red beetroot powder, as a source of natural dyes with antioxidant activity, which significantly improve the sensory characteristics of this type of product.
4. The nougat product demonstrates the multifunctionality of the powder obtained from red beetroot powder in its compositional formula, as an important source of natural

compounds with antioxidant, coloring and flavoring activity, so that they improve sensory characteristics such as color, aroma and texture of the product, directly contributing to increasing diversity and attractiveness to consumers.

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ANALYTICAL STUDY ON THE PRODUCTION OF MEDICINAL PLANT SEEDLINGS WITH A VIEW TO OPTIMIZING THEIR VALUE

STUDIU ANALITIC ASUPRA PRODUCERII RĂSADURILOR DE PLANTE MEDICINALE ÎN SCOPUL OPTIMIZĂRII VALORIFICĂRII LOR

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Abstract
The use of medicinal plants has a long tradition in Romania, both for therapeutic purposes and in supporting biodiversity. The potential of our country is considerable and the research and development stations play an important role, contributing to the research, production, and capitalization of medicinal plants with economic and public health impact. The present study, of a comparative type, analyses the evolution of sales of medicinal plant seedlings at VRDS Buzău over a period of 4 years (the analysis of the economic efficiency of the technological sequence of seedling production includes the stages of substrate preparation, sowing, care and hardening, all essential for obtaining quality products). The sale of seedlings to farmers and institutions is analysed in the context of market trends and economic efficiency (comparison of production costs with the income obtained). The results show a constant increase in demand for medicinal plants, which suggests a significant development potential. The authors emphasize the importance of optimizing technological and commercial processes with the aim of sustainably increasing the profitability of this sector, essential for Romania.

***Keywords**: seedlings, medicinal plants, capitalization, optimization*

Rezumat
Utilizarea plantelor medicinale are o tradiție îndelungată în România, atât în scopuri terapeutice cât și în susținerea biodiversității. Potențialul țării noastre este considerabil, iar stațiunile de cercetare-dezvoltare joacă un rol important, contribuind la cercetarea, producția, valorificarea plantelor medicinale cu impact economic și asupra sănătății publice. Prezentul studiu, de tip comparativ, analizează evoluția vânzărilor de răsaduri de plante medicinale la SCDL Buzău pe o perioadă de 4 ani (analiza eficienței economice a secvenței tehnologice de producere a răsadurilor include etapele de pregătire a substratului, semănat, îngrijire și călire, toate esențiale pentru obținerea unor produse de calitate). Vânzarea răsadurilor către fermieri și instituții este analizată în contextul tendințelor pieței și al eficienței economice (compararea costurilor de producție cu veniturile obținute). Rezultatele arată o creștere constantă a cererii pentru plantele medicinale, ceea ce sugerează un potențial semnificativ de dezvoltare. Autorii subliniază importanța optimizării proceselor tehnologice și comerciale cu scopul creșterii sustenabile a profitabității acestui sector, esențial pentru România.

***Cuvinte cheie:** răsaduri, plante medicinale, valorificare, optimizare*

INTRODUCTION

Medicinal plants are a resource increasingly valued in the current context, in which human health, organic food and agricultural sustainability are becoming strategic priorities. Romania has a valuable botanical heritage and research centres capable of generating real impact on local communities. Buzău Vegetable Research and Development Station (VRDS) actively contributes to this endeavour by developing a complete value chain dedicated to medicinal plant seedlings.

MATERIALS AND METHODS

This review is based on a PubMed search using the terms “seedlings”, “medicinal plants”, “capitalization”, “optimization”. The papers were checked for relevance and the most up-to-date information was selected for inclusion. Based on a comparative analysis, the increase in demand and the importance of optimizing technological stages, as well as expanding the impact through dissemination and training actions for the general public, are highlighted. The *“Small Green Pharmacy”* initiative actively supports health, biodiversity and ecological rural entrepreneurship.

This study analyses the value chain of medicinal plant seedling production, with a focus on economic efficiency and sustainable development potential. The analysis was based on economic and technological data recorded in the period 2021–2024 within VRDS Buzău. The following stages of the seedling production process were evaluated. (Fig.1) - a), b), c); (Fig.2); (Fig.3) - a), b), c), d); (Table 1).

(a) (b) (c)

Figure 1. a) Substrate preparation b) Sowing and sprouting c) Transplanting plants in the seedling phase / a) Pregătire substrat b) Semănat și răsărire c) Repicatul plantelor în faza de răsad

Table 1. Averages of biometric characteristics, by species / Mediile caracteristicilor biometrice, pe specii

Specie	*Nr. Frunze*	*Frunză lungime*	*Frunză latime*	*Plantă lungime*	*Plantă lățime*	*Rădăcină lungime*	*Rădăcină lățime*	*Nr. zile până la răsărire*
	(cm)	*(cm)*	*(cm)*	*(cm)*	*(cm)*	*(cm)*	*(cm)*	*(anul-2024)*
Ocimum basilicum Aromat de Buzău	8.0	3.83	1.63	10.07	7.17	5.53	3.07	7
Stevia rebaudiana	10.0	4.47	1.83	5.83	8.83	8.93	3.27	7
Melissa officinalis	8.0	4.7	3.57	7.67	7.2	5.0	4.23	10
Sideritis scardica	6.33	2.67	1.97	5.33	4.87	6.67	4.83	7
Hyssopus officinalis	15.0	3.27	0.63	7.5	6.0	8.0	3.33	7
Ocimum basilicum Busuioc Serafim	8.33	3.53	2.2	12.0	5.5	5.0	3.8	7
Salvia officinalis	9.67	6.83	1.8	11.67	10.43	8.53	5.0	22
Satureja hortensis L	16.33	4.07	1.33	10.37	7.73	4.5	3.5	10
Thymus serpyllum	20.0	1.5	0.6	8.67	2.6	6.0	1.67	13

Figure 2. Plant care from seedling stage, hardening process and preparation for delivery / Îngrijirea plantelor de la faza de răsad, procesul de călire și pregătirea pentru livrare

(a) (b) (c) (d)

Figure 3. Seedlings of: a**) *Salvia officinalis* b) *Melissa officinalis* seedling c) *Ocimum basilicum* d) *Satureja hortensis* L.** / Răsaduri de: a) *Salvia officinalis* b) Răsad de *Melissa officinalis* c) Răsad de *Ocimum basilicum***,** d) Răsad de *Satureja hortensis L.*

At the same time, there were evaluated the followings: the dynamics of sales, the structure of beneficiaries (institutions, farmers, small producers), as well as the market reaction to the diversification of the assortment.

RESULTS AND DISCUSSION

The results indicate a constant increase in demand for medicinal plant seedlings. This reflects both the growing interest in natural prevention and alternative medicine, as well as the need for diversified crops among small producers. Optimized technological stages have significantly contributed to increasing economic efficiency, reducing losses and ensuring consistent quality. Sales doubled during the analysed period, confirming a favourable trend among consumers and farmers (Figure 4, 5, 6, 7).

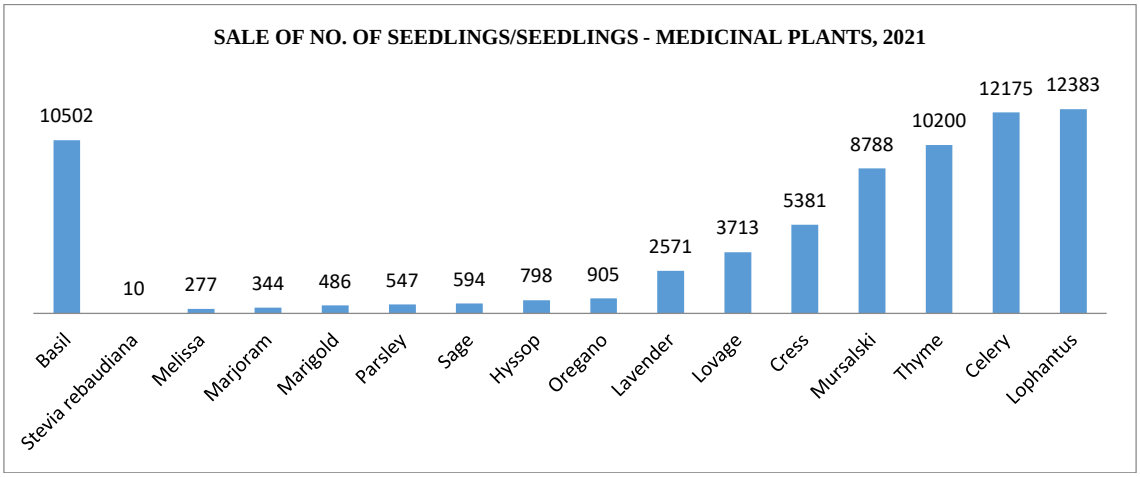

Figure 4. Dominance of classics and low presence of rare plants (2021) / Dominanța clasicelor și prezența scăzută a plantelor rare (2021)

Dominant species:

- *Agastache foeniculum* (12,383),
- *Apium graveolens* (12,175),
- *Satureja hortensis* L (10,200),
- *Sideritis scardica* (8,788),
- *Tagetes patula* (5,381),
- *Ocimum basilicum* (10,502).

Niche species with modest presence: *Stevia rebaudiana* (10), *Melissa officinalis*, *Origanum majorana.*

Observations: sales focused on traditional, easily recognizable plants, many with a food and decorative role; functional plants with a therapeutic role had extremely low sales.

Species on the rise:

- *Satureja hortensis L (8,521),*
- *Hyssopus officinalis (7,734),*
- *Salvia officinalis (7,476),*
- *Tagetes patula (5,533),*
- *Apium graveolens (5,402).*

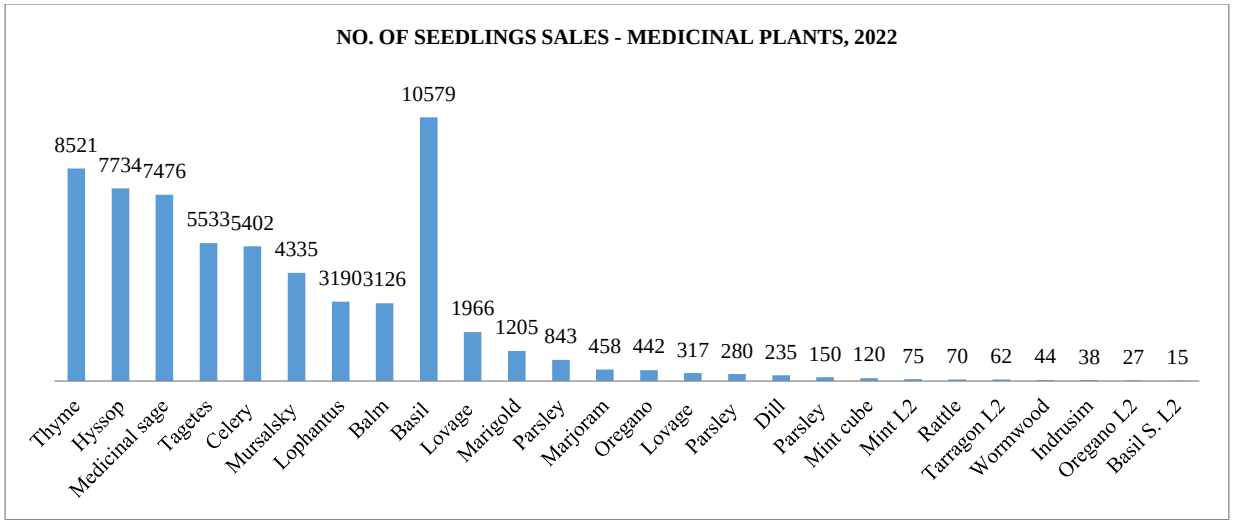

Figure 5. Significant diversification, beginning of the transition to specialized medicinal plants (2022) / Diversificare semnificativă, începerea tranziției la plante medicinale specializate (2022)

New plants: *Stevia rebaudiana*, *Artemisia ludoviciana* and *Artemisia annua.*

Observations: The expansion of the assortment to over 25 species is noticeable, as well as a more curious and educated public.

The promotion of the "Little Green Pharmacies" project/strategy is starting to have its effect.

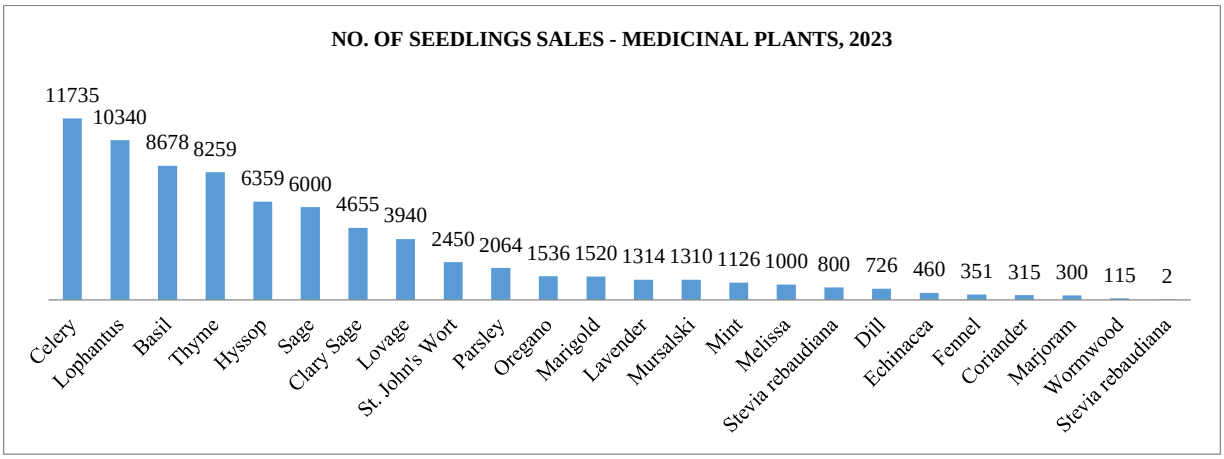

Figure 6. Specialization and balance between culinary, aromatic and medicinal plants (2023) / Specializare și echilibru între plante culinare, aromatice și medicinale (2023)

Dominant species:

- *Apium graveolens (11,735),*
- *Agastache foeniculum (10,340),*
- *Ocimum basilicum (8,678),*
- *Satureja hortensis L (8,259),*
- *Hyssopus officinalis (6,359),*
- *Salvia officinalis (6,000).*

Plants with stable demand: *Tagetes patula*, *Levisticum officinale*, *Hypericum perforatum*, *Calendula officinalis* L., *Lavandula angustifolia* Mill., *Mentha x piperita*.

Species with low sales: *Origanum majorana*, *Artemisia ludoviciana* and *Artemisia annua*, *Echinacea purpurea* (460), *Stevia rebaudiana* (2)

Observations: The emergence of a balance between practical (culinary) and phytotherapeutic uses. Rare plants remain niche, but are beginning to be demanded by a specialized audience.

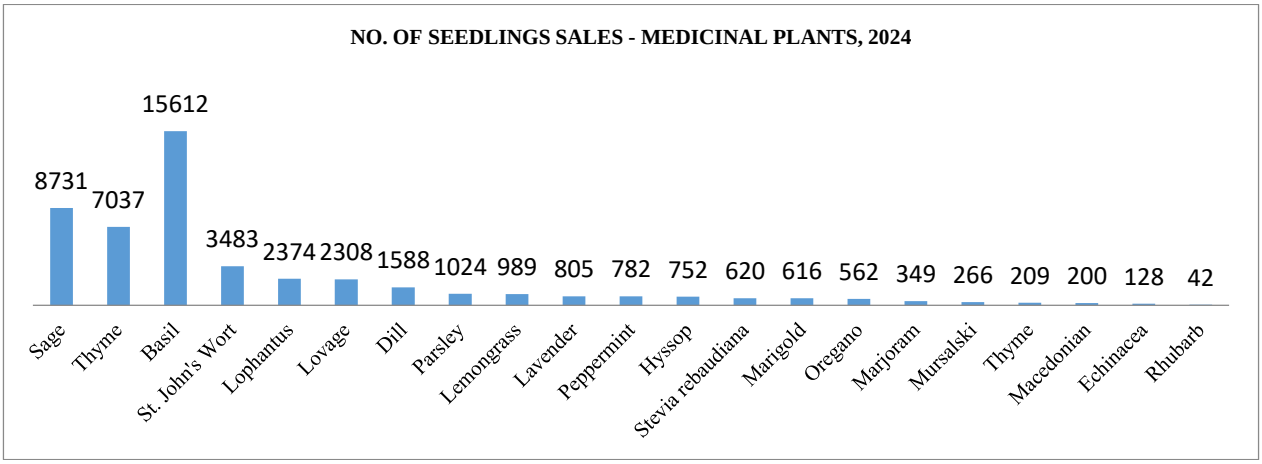

Figure 7. Market maturation and acquisition of rare/valuable species (2024) / Maturizarea pieței și obținerea unor specii rare/valoroase (2024)

Dominant species:

- *Ocimum basilicum* - (15.612 – absolute record),
- *Salvia officinalis* - (8.731)
- *Satureja hortensis L* - (7.037)
- *Hypericum perforatum* - 3.483).

Valuable presences: *Echinacea purpurea* white, *Thymus serpyllum* L., *Origanum vulgare* L., *Calendula officinalis* L.

Observations: diversified demand, multiple species. Plants with therapeutic, anti-inflammatory, adaptogenic or digestive roles are on the radar of gardeners and small producers.

Based on the data recorded in the period 2021-2024, Table 2, Figure 8 and Figure 9 were generated with the evolution of sales for the most representative species of medicinal plants.

Table 2. Comparative evolution of sales in the period 2021–2024 / Evoluția comparativă a vânzărilor în perioada 2021–2024

Specia	**Număr răsaduri vândute**			
	2021	**2022**	**2023**	**2024**
Basil – *Ocimum basilicum*	10.502	10.579	8.678	15.612
Celery – *Apium graveolens*	12.175	5.402	11.735	6.580
Lophantus – *Agastache foeniculum*	12.383	3.190	10.340	2.374
Sage – *Salvia officinalis*	594	7.476	6.000	8.731
Hyssop – *Hyssopus officinalis*	798	7.734	6.340	752
Tyme – *Satureja hortensis L*	10.200	8.521	8.259	7.037
Mursalski chai – *Sideritis scardica*	8.788	4.335	1.310	266
Lavender – *Lavandula angustifolia*	2.571	-	1.314	752
St. John's wort – *Hypericum perforatum*	-	70	2.450	3.483
Lovage – *Levisticum officinale*	3.713	1.966	3.940	2.308

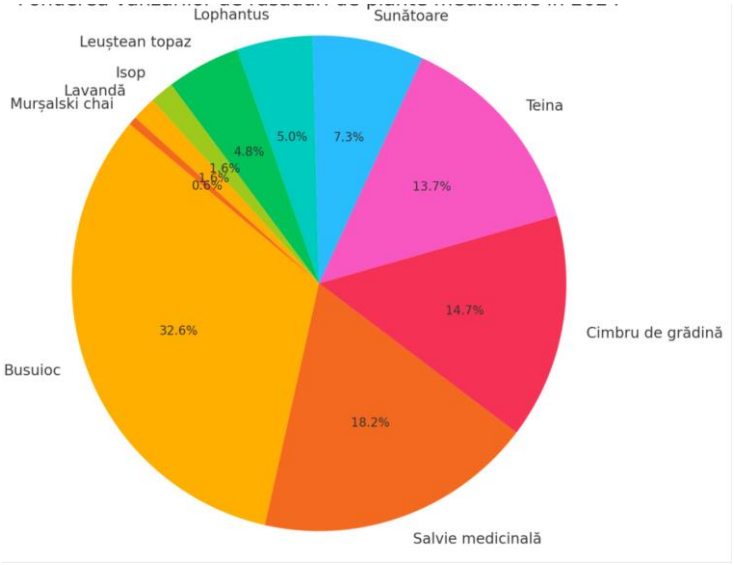

Figure 8. Comparative evolution of sales in the period 2021–2024 / Evoluția comparativă a vânzărilor în perioada 2021–2024

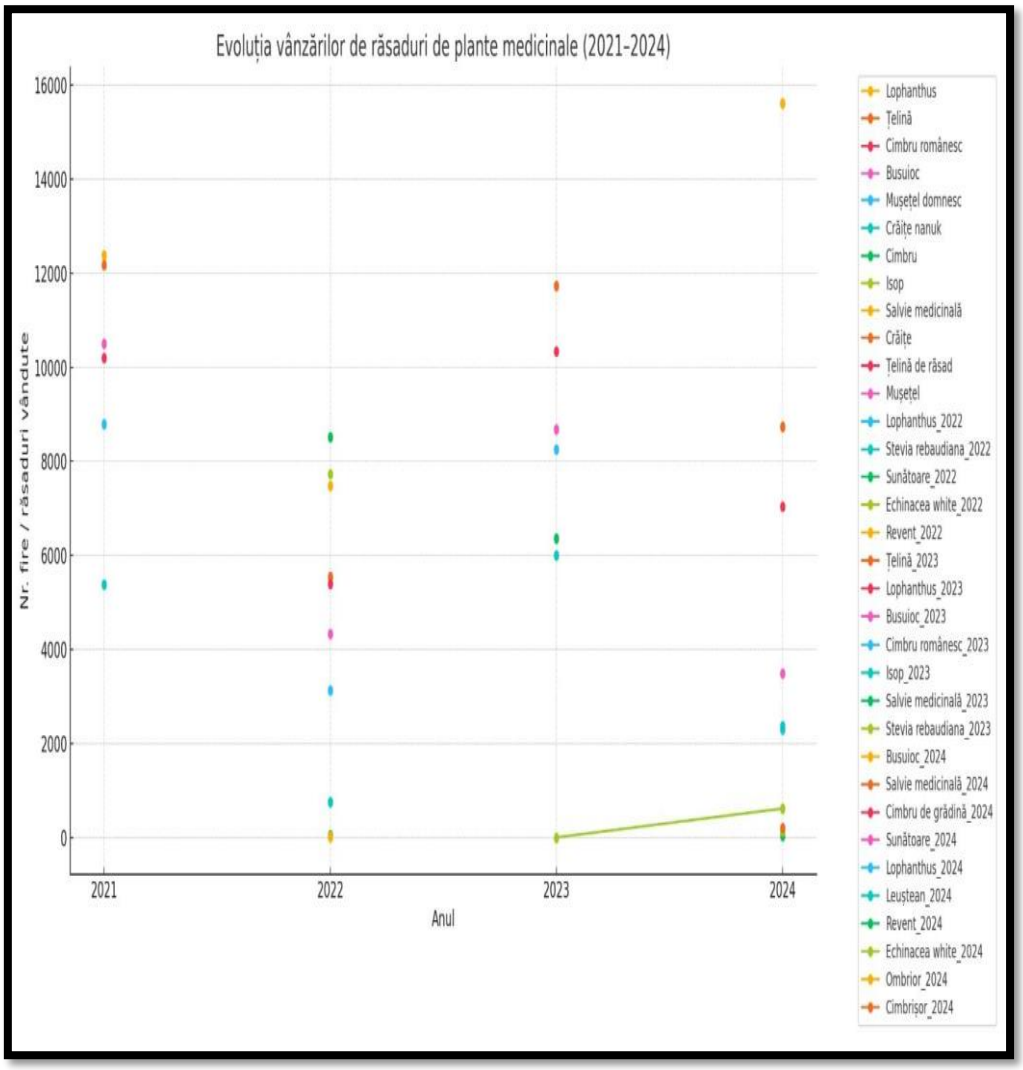

Figure 9. Evolution of seedlings sales of from the medicinal plant collection (2021-2024) / Evoluția vânzărilor de răsaduri din colecția de plante medicinale (2021-2024)

VRDS Buzău not only produces and researches, but also actively disseminates the results to the community, becoming a true training centre. Projects and practical actions supported the expansion of the results among the final beneficiaries – consumers and farmers.

A concrete example of dissemination and community education initiative is the "Small Green Pharmacy" project, through which VRDS Buzau promoted the concept of a personal garden (with 30–50 climatically adapted medicinal plants), useful for health and the protection of other plants. The project was well received in rural areas, in schools and within activities for farmers.

This direction offers clear opportunities for:

- educating the population on the use of medicinal plants;
- diversifying sources of income in households;
- new lines of financing for small businesses;
- integration into short, ecological and sustainable value chains.

Analysis of knowledge regarding the use of medicinal plants

In order to assess the participants' level of knowledge regarding the use of medicinal plants for therapeutic and culinary purposes, a 10-question grid-type test was applied, entitled "The Green Pharmacy in the Garden". The questionnaire was administered to 50 people, and the cumulative score obtained was 355.33 points. This corresponds to an average of 7.11 points out of 10, respectively 71.066%, extrapolated for a sample of 100 people.

The questions aimed at recognizing medicinal plants used to combat common conditions such as insomnia, anxiety, digestive disorders, colds, skin lesions, but also familiarizing themselves with their effects (calming, antibacterial, benefits on memory, uses in aromatherapy). Among the plants analysed were: Thyme (*Thymus serpyllum*), Marigolds (*Calendula officinalis*), Lemon balm (*Melissa officinalis*), Lavender (*Lavandula angustifolia*), Sage (*Salvia officinalis*), Oregano (*Origanum vulgare*), Basil (*Ocimum basilicum*), Thyme (*Satureja hortensis*), Marjoram (*Origanum majorana*).

The results suggest a moderate to satisfactory (good) level of familiarity with medicinal plant species, their traditional and current uses, with most respondents answering correctly to questions that targeted plants frequently found in personal gardens or in natural products.

CONCLUSIONS

1. The study provides that the production of medicinal plant seedlings can be not only a profitable activity, but also a strategic vector for health, education and rural development. Through technological optimization and efficient dissemination of results, research and development stations can play a key role in the transformation of green agriculture in Romania.

2. The general trends observed (2021–2024) were:

- increased diversification: from 15 species/2021 to > 30/2024;
- stable species in the tops: *Ocimum basilicum* L., *Satureja hortensis* L, *Salvia officinalis*, *Hyssopus officinalis*, *Tagetes patula*, *Hypericum perforatum*;
- rare species such as *Stevia rebaudiana*, *Echinacea purpurea*, *Satureja hortensis*, *Thymus serpyllum* L. were present in small but constant volume, indicating a niche potential that can be exploited in the future;
- rare but promising species: *Echinacea purpurea*, *Stevia rebaudiana* (slightly returns in 2024);
- *Ocimum basilicum* L. recorded a constant increase, reaching a peak in 2024 (15,612 threads), which makes it the absolute leader;
- *Agastache foeniculum* and *Apium graveolens* dominated in 2021 and 2023, but recorded a visible decrease in 2024;
- *Satureja hortensis* L, *Hyssopus officinalis* and *Salvia officinalis* had constant sales, being clearly preferred by ordinary or professional beneficiaries.

3. Analysis of the evolution of sales of medicinal plant seedlings (2021–2024) shows that the Buzau Vegetable Research and Development Station recorded a significant evolution in the structure of demand for medicinal plant seedlings, which highlights:

- changes in beneficiaries' preferences;
- portfolio diversification;
- the emergence of rare or niche species;
- the impact of education and dissemination activities.

4. The role of the research station was essential not only in production, but in training, testing and national promotion of phytotherapy (with applicability in households).

5. The population segment surveyed proved to be more educated and health-oriented (thanks to the "Little Green Pharmacy" initiative, VRDS's involvement in educational dissemination and promotion projects and the establishment of medicinal plant crops on larger areas by Romanian producers.

ACKNOWLEDGEMENTS

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RESEARCH ON THE SUITABILITY OF CERTAIN VARIETIES OF BLUEBERRY AND CURRANT FOR PROCESSED PRODUCTS

CERCETĂRI PRIVIND PRETABILITATEA UNOR SOIURI DE AFIN ŞI COACĂZ ÎN PRODUSE PRELUCRATE

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Abstract

Social and economic changes in Romania are transforming the fruit and vegetable sector into an important pillar of economic growth, contributing to the population's food security and safety. Recently, there has been a growing trend among consumers towards national Romanian products, enhancing the importance of the processing segment within the horticultural value chain. The biggest advantage of consuming processed products is their year-round availability, and with the help of modern processing technologies, fruits can be preserved for long periods. To support farmers and processors, this study was conducted to analyze the suitability for industrial processing of several currant and blueberry varieties provided by RDIFG Piteşti – Mărăcineni. The varieties studied were **Jonkheer van Tets** *for the red currants and* **Poli 51** *for the black currants. For blueberries, the varieties were* **Delicia** *and* **Lax***. These were processed into preserves (jam, preserve, compot) and evaluated through sensory analysis. From the point of view of processing suitability, both currant varieties were appropriate, with the mention that for the jam production,* **Poli 51** *was more appreciated. The blueberries' nutritional characteristics led to the production of high-quality products, and both varieties considered for the analysis proved suitable for processing.*

***Keywords*:** *sensory analysis, fruit processing, organoleptic characteristics, preservation*

Rezumat

Schimbările socio-economice din România transformă sectorul legume-fructe într-un punct important al creşterii economice, contribuind la asigurarea securităţii şi siguranţei alimentare a populaţiei. In ultima perioadă s-a remarcat o creştere tot mai accentuată a consumatorului către produsele naturale româneşti, potenţând importanţa segmentului de procesare în lanţul de valorificare horticol. Avantajul major al consumului de produse prelucrate este disponibilitatea lor pe tot parcursul anului iar cu ajutorul tehnologiilor moderne de procesare fructele pot fi păstrate timp îndelungat. In sprijinul fermierilor şi a procesatorilor s-a efectuat prezentul studiu în care s-a analizat pretabilitatea de industrializare a unor soiuri de coacăz şi afin furnizate de către ICDP – Piteşti – Mărăcineni. Soiurile studiate au fost **Jonkheer van Tets** *pentru coacăzul roşu şi* **Poli 51** *pentru soiul de coacăz negru. Pentru afin au fost soiurile* **Delicia** *şi* **Lax***. Acestea au fost prelucrate sub formă de conserve (gem, dulceaţă, compot) şi analizate senzorial. Din punct de vedere al pretabilităţii ambele soiuri de coacăz se pretează pentru prelucrare cu menţiunea că pentru varianta de gem soiul* **Poli 51** *a fost mai apreciat. Caracteristicile nutritive ale afinelor au condus la obţinerea de produse calitative, ambele soiuri luate în analiză s-au pretat pentru prelucrare.*

Cuvinte cheie: *analiză senzorială, procesare fructe, caracteristici organoleptice, conservare*

INTRODUCION

Fruits are important sources of essential vitamins, minerals and antioxidants, having beneficial effects on the human body. They are recommended by the international organizations to be consumed daily in to support a healthy lifestyle, reduce the risk of sickness and increase the quality of life (Dobre-Baron, 2020; Pelegrine Guimarães *et al*., 2019).

Although the fruits can be consumed fresh, due to the perishability of many of them, they are processed, thus extending their shelf life and reducing food waste. The total processing of fruits represents an important aspect in Romanian economy, and source of income and development for communities, by adding value to food productions from already established fruit-growing areas (Alboiu, 2024; Budău, 2024). According to a study conducted by Budău (2024) there is a notable preference among farmers for blueberries (32,5%) and raspberries (28,2%), followed by the interest for blackberries (9,1%) and currants (4,8%). Other shrubs such as hazelnuts, sea buckthorn, and aronia are present in smaller proportions. In what concerns the storage spaces, only 22,7% of farmers own refrigeration units for temperature and humidity control, which is why there is a pressing need for improved fruit preservation. On the other hand, regarding processing, only 14,9% of farmers carry out this activity, suggesting a potential for development in this sector.

Processing is the solution through which the fruits are efficiently utilized, reducing waste and providing consumers with the benefits of fruits throughout the year. The production of jams, preserve and compotes can be done using relatively simple methods, requiring only a small number of equipment, with very good results (Pelegrine Guimarães *et al*.,2019).

In a period of growing competitiveness, with food's quality being an important driver in the consumer's choices, the sensory analysis is a useful tool for every producer. Therefore, the sensory analysis is a method to determine the consumer's preferences regarding a certain product, based on the use of senses, thus contributing to the evaluation of the product's taste quality. It contributes, at the same time, to its differentiation from competition and positioning on the market, leading to enhanced quality and the fulfillment of consumer taste preferences (Mărcuţă *et al*.,2020).

This paper aimed to perform a sensory analysis of the products obtained in the laboratory of Horting Institute from Bucharest, and to determine the processing suitability of two blueberry varieties and two currant varieties, providing useful information for both fruit growers and processors.

MATERIALS AND METHODS

The research was conducted on two blueberry varieties (**Delicia** and **Lax**) and two currant varieties (**Jonkheer Van Tets** and **Poli 51**), provided by RDIFG Piteşti – Mărăcineni. These were analyzed organoleptically and characterized biometrically.

The fruits of the **Delicia** variety (figure 1) are large, with a round-flattened shape, light blue in color, covered with a thick layer of bloom, and rich in bioactive compounds.

The fruits of the **Lax** variety (figure 1) are medium-sized, deep blue in color, firm, sweet, slightly tart, aromatic, and loosely arranged in clusters. In terms of biochemical content, the fruits are richer in compounds with antioxidant activity (anthocyanins, polyphenols, citric acid).

Figure 1. Delicia and Lax varieties / Soiul Delicia şi soiul Lax

Table 1. Biometric characteristics of the blueberry varieties /
Caracteristicile biometrice la soiurile de afin

Variety/ Soiul	Production/bush (kg)/ Producţie/tufă (kg)	Average weight/Fruit (g) / Greutate medie/fruct (g)	Firmness (N)/ Fermitate (N)	pH/ pH
Delicia	2,53	2,05	20,50	3,27
Lax	2,40	1,90	19,23	3,70

The red currant (**Jonkheer van Tets**) is an early and intensive variety with remarkable production potential, yet less demanding in terms of cultivation technology than the black currant (**Poli 51**). The **Jonkheer van Tets** variety (Figure 2) is a distinct red currant type, appreciated for its early fruit ripening and high productivity, and is widely cultivated in Romania. The fruits grow in fairly abundant clusters and are juicy. The plant is highly productive, with vigorous and upright growth.

Figure 2. Poli 51 and Jonkheer van Tets varieties / *Soiul Poli 51 şi soiul Jonkheer van Tets*

Table 2. Biometric characteristics of the black and red currant varieties /
Caracteristicile biometrice la soiurile de coacaz negru si rosu

Variety / Soiul	Production/bush (kg)/ Producţie/tufă (kg)	Average weight/Fruit (g) / Greutate medie/fruct (g)	Firmness (N) / Fermitate(N)	pH / pH
Poli 51	2,38	1,27	12,23	3,10
Jonkheer van Tets	0,98	0,88	11,87	3,07

The fruits were processed in the laboratory of Horting Institute into preserve, jam, and compote varieties, following technological parameters and without the addition of preservatives or other food additives.

After the minimum 21-day stabilization period, the products were analyzed according to STAS 12656-88, Method A, to evaluate their organoleptic properties: appearance, consistency, taste, and aroma. The evaluation of each organoleptic characteristic was carried out by comparing it to scoring scales ranging from 0 to 5 points, and the average score given by the tasting panel was recorded. Weighted average scores were then calculated, summed to obtain the total average score, and the organoleptic qualities of the products were determined based on the total average principle, using a 0–20 point scale. Finally, ratings were assigned for each variety and product.

Based on the grades given by the tasters, each product received a rating and was classified into one of the five corresponding quality classes:

- Very good (18,1-20,0 points)
- Good (15,1-18,0 points)
- Satisfactory (11,1-15,0 points)
- Unsatisfactory (7,1-11,0 points)
- Inadquate (0-7,0 points)

RESULTS AND DISCUSSION

Fruit preserve (Figures 3 and 4) – the method used for preserve was fruit preservation in sugar syrup. Through boiling, the water content was reduced, achieving the desired concentration of dry matter. For preserve, the species, pulp firmness, ripeness, and method of division are taken into account.

According to STAS 3750-90, the preserve must contain 45-55% fruit, 72% soluble dry matter, and 0.7% acidity (expressed as malic acid). The results of the sensory analysis are presented in Table 3.

Figure 3. Currant preserve: Jonkheer van Tets and Poli 51 /
Dulceaţa de coacăze: Jonkheer van Tets şi Poli 51

Figure 4. Blueberry preserve: Lax and Delicia / *Dulceaţa de afine:* ***Lax*** *si* ***Delicia***

Table 3. Sensory analysis of currant preserve / *Analiza senzorială la dulceaţa de coacăze*

Specification /Specificaţie	Variety / Soiul	
	Jonkheer Van Tets	Poli 51
Appearance	4,8	4,8
Color	4,8	5
Consistency	4,4	4,4
Taste	4,8	4,6
Average score	18,8	18,8
Rating	Very good	Very good

It was observed after tasting (Table 3) that both products obtained received a very good sensory score (18.8). The appearance and consistency received the same score, 4.8 and 4.4, respectively. The difference is in taste, as the preserve from the **Jonkheer Van Tets** variety has noticeable seeds (4.8), while the preserve from the **Poli 51** variety has fruits partially soaked in syrup (4.6).

Table 4. Sensory analysis of blueberry preserve / *Analiza senzorială la dulceaţa de afine*

Specification /Specificaţie	Variety /Soiul	
	Delicia	**Lax**
Appearance	4,8	4,8
Color	5	5
Consistancy	4	4,6
Taste	4,4	4,8
Average score	18,2	19,2
Rating	Very good	Very good

Blueberry preserve is appreciated for its appearance and color, both receiving the same score, 4.8 and 5, respectively. Regarding the consistency of the **Delicia** variety, the fruits are slightly disintegrated (4) compared to the **Lax** variety (4.6), but this does not negatively affect the taste. Both were highly appreciated.

Jam (Figures 5 and 6) is a concentrated gelled product made from fresh fruit with the addition of sugar and lemon juice (if needed), packed in hermetically sealed and pasteurized containers. From an organoleptic point of view, the jam must comply with STAS 3183/90: appearance of the product: fruit, pieces of fruit, in a gelled mass, without syrup separation, and without signs of fermentation or mold. The color must be characteristic of the fruit variety, with a pleasant taste and aroma, typical of the fruit variety, and without any foreign taste or smell. The jam made from the fruits studied was obtained according to the corresponding technological instructions.

Figure 5. Currant jam: Jonkheer van Tets and Poli 51/ *Gem de coacăze: Jonkheer van Tets şi Poli 51*

Figure 6. Blueberry jam: Delicia and Lax / *Gem de afine: Delicia şi Lax*

The results of the sensory analysis of the currant jam are presented in Table 5. It is noted that the best results were obtained for the **Poli 51** variety, which received the maximum score for

appearance and color, with an overall average score of 18.4 points. This variety was rated 'very good'.

Table 5. Sensory analysis of currant jam/ *Analiza senzorială a produsului gem de coacăze*

Specification / Specificaţie	**Variety / Soiul**	
	Jonkheer Van Tets	**Poli 51**
Appearance	4,4	5
Color	4,8	5
Consistency	3,6	4
Taste	4,4	4,4
Average score	17,2	18,4
Rating	Good	Very good

For the red currant jam, the **Jonkheer Van Tets** variety, the appearance is in accordance with the standard (fruits in a gelled mass), compared to the black currant variety, **Poli 51**, where the presence of pectic substances gives it a more gelled consistency.

In the **Jonkheer Van Tets** variety, the presence of seeds is unpleasant compared to the black currant variety, **Poli 51**. It is recommended to process the fruit through a pulper to obtain a puree. The appearance will not be the same as the original product, but it will be much more pleasant for consumption.

Following the tastings, the **Poli 51** currant jam was more appreciated in terms of appearance, color, taste, and consistency compared to the **Jonkheer Van Tets** red currant variety (Table 5).

Table 6. Sensory analysis of blueberry jam / Analiza senzorială a produsului gem de afine

Specification/ Specificaţie	**Variety / Soiul**	
	Delicia	**Lax**
Appearance	4,8	4,4
Color	5	5
Consistency	4,8	4,6
Taste	4,8	4,6
Average score	19,4	18,6
Rating	Very good	Very good

The nutritional characteristics of the blueberry varieties lead to the production of high-quality jam, appreciated for its appearance, color, consistency, and taste. As shown in Table 6, both varieties received the rating 'very good'.

Compote (Figures 7 and 8) is a product made from fruits in sugar syrup, with the addition of lemon juice (if needed), packed in hermetically sealed and pasteurized containers. From an organoleptic point of view, it complies with STAS 3164/90: appearance of the fruits: fruits covered with syrup, not broken, in the same container, the fruits must be of the same variety with similar ripeness and size; appearance of the syrup: clear, with fine fruit particles lightly suspended; consistency of the fruit: firm enough, cooked fruit, but not broken; color of the fruit: characteristic of the variety and the degree of ripeness; taste and smell: pleasant, characteristic of cooked fruit, with no foreign taste or odor (mold, fermentation, sourness).

Figure 7. Currant compote from the Jonkheer van Tets and Poli 51 varieties / Compot de coacăze soiul Jonkheer van Tets şi Poli 51

Figure 8. Blueberry compote from the Lax and Delicia varieties / Compot de afine soiul Lax şi Delicia

Table 7. Sensory analysis of currant compote / Analiza senzoriala la compot de coacăze

Specification / Specificaţie	**Variety /Soiul**	
	Jonkheer Van Tets	**Poli 51**
Appearance	4,8	4,8
Color	5	4,8
Consistency	4,4	4,4
Taste	4,6	4,5
Average score	18,8	18,5
Score	Very good	Very good

As shown in Table 7, the sensory analysis of the two varieties received the rating 'very good'. Due to the tart taste of the fruit, there was no need to add lemon juice to the syrup, resulting in a pleasant, aromatic compote. For fruit compote, the fruits must be visually appealing in terms of color, uniform, and have good firmness to withstand thermal processes. For this variety, the processing technology is short, contributing to the preservation of the valuable nutrients in the fruit, as well as its taste properties, color, and aroma.

***Table 8. Sensory analysis of blueberry compote** / Analiza senzoriala a produsului compot de afine*

Specification / Specificaţie	**Variety / Soiul**	
	Delicia	**Lax**
Appearance	4,8	4,8
Color	5	4,8
Consistency	4,6	4,8
Taste	5	4,8
Average score	19,4	19,2
Rating	Very good	Very good

The compote has a taste close to the original fruit. The compotes from the two varieties were very similar, both being rated 'very good'.

CONCLUSIONS

1. Following the organoleptic analysis performed, all products met the quality standards, receiving the rating of 'very good'. An exception was the red currant jam, which received the rating of 'good'. This indicates that all fruit varieties are suitable for processing into fruit preserve, jam, and compote.
2. In red currant preserve, the seeds are present in the syrupy mass, which may pose some inconveniences; therefore, it is recommended to prioritize processing the fruit into varieties from which the seeds can be removed (such as syrup or jelly).
3. The compote made from the **Delicia** variety received the highest score (19.4) among all the products, followed by the compote from the **Lax** variety (19.2). This is because the thermal processing time required to obtain these products was shorter, thus better preserving the existing bio-compounds.
4. The **Poli 51** currant jam, compared to the red currant jam, had a more gelled consistency due to the natural pectic substances present in the fruit.
5. The correct use of sensory analysis leads to quick, easy, and cost-effective quality control of raw materials and finished products.
6. In addition to being a means of ensuring quality, sensory analysis also serves as a market analysis tool that provides a better understanding of consumer behavior.
7. The quality, taste, and aroma of the local fruits are essential factors in the production process, contributing to the increase in commercial value and profit throughout the entire value chain.
8. The suitability of fruits for processing is a characteristic of the variety.
9. Products processed into compote are the most demanding in terms of raw material quality; therefore, varieties with technological properties and chemical composition that meet processing requirements are selected.

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