AGRICULTURAL AND FORESTRY SCIENCES ACADEMY

"GHEORGHE IONESCU - SISESTI"

ACTA AGRICOLA ROMANICA

Volume 7, Year 7, No. 7

August 2025

BUCHAREST

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The papers were presented at the Anniversary Conference of the Academy of Agricultural and Forestry Sciences "Gheorghe Ionescu-Șișești", May 29, 2025

ACTA AGRICOLA ROMANICA

Volume 7, Year 7, No. 7
August 2025

Field Crops, Natural Resources and Mechanization

BUCHAREST

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The magazine appears annually, in the second semester of the year

ISSN – L 2784 – 0948

RESEARCH ON THE EFFICIENCY OF PHYTOSANITARY TREATMENTS AT DIFFERENT PHENOLOGICAL STAGES FOR CONTROLING PATHOGENS IN WHEAT CROPS

CERCETĂRI PRIVIND EFICIENȚA TRATAMENTELOR FITOSANITARE ÎN DIFERITE FENOFAZE PENTRU CONTROLUL PATOGENILOR ÎN CULTURA DE GRÂU

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Abstract

In recent years, mild winters have contributed to an increased pressure of pathogens on wheat crops. Determining the optimal timing for applying plant protection treatments is essential to achieve a healthy crop and ensure high production, both in terms of quantity and quality. Since pathogens are active throughout the entire growing season, applying phytosanitary treatments becomes a necessity. However, it is crucial to consider economic efficiency, as applying numerous treatments, if they are costly and ineffective, does not bring real benefits.

The objective of this research is to identify the optimal phenological stages for applying treatments against the pathogen complex in wheat crops, using two complex fungicides. Treatments were applied in three main phenological stages (tillering, booting, and heading) as well as in combinations of these stages.

The results obtained indicate that applying three treatments in the tillering+booting+heading stages represents the most effective strategy for significantly reducing the degree of pathogen attack.

From a production perspective, the most effective treatment timing was found to be the tillering + booting combination, resulting in a 19% yield increase compared to the untreated control.

Rezumat

În ultimii ani, iernile blânde au favorizat intensificarea presiunii patogenilor asupra culturii de grâu. Determinarea momentului optim pentru aplicarea tratamentelor fitosanitare este esențială pentru obținerea unei culturi sănătoase și a unei producții satisfăcătoare, atât din punct de vedere cantitativ, cât și calitativ.

Deoarece patogenii se manifestă pe tot parcursul perioadei de vegetație, aplicarea tratamentelor fitosanitare devine o necesitate. Totuși, este important să luăm în considerare eficiența economică, aplicarea unui număr mare de tratamente, dacă acestea sunt costisitoare și ineficiente, nu aduce beneficii .

Obiectivul acestei cercetări este de a identifica fenofazele optime pentru aplicarea tratamentelor împotriva complexului de patogeni la cultura de grâu, utilizând două fungicide complexe. Tratamentele au fost aplicate în trei fenofaze principale (înfrățit, burduf și înspicar) și în combinațiile acestora.

Rezultatele obținute au evidențiat că aplicarea a trei tratamente în fenofazele de înfrățit+burduf+spic este cea mai eficientă strategie pentru reducerea semnificativă a gradului de atac al agenților patogeni.

Din perspectiva producției, cele mai eficiente momente de aplicare s-au dovedit a fi înfrățit+burduf, înregistrându-se un spor de producție de 19% comparativ cu martorul netratat.

Keywords: winter wheat, foliar pathogens, fungicide treatment, phenophase
Cuvinte cheie: grâu de toamnă, patogeni foliari, tratament fungicid, fenofază

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a cornerstone of global agriculture, serving as a primary food source for a significant portion of the world's population. However, its cultivation is continually challenged by a myriad of fungal pathogens that can severely compromise both yield and grain quality. Effective disease management is thus pivotal to ensure food security and the economic viability of wheat production.

Among the prevalent fungal diseases affecting wheat are Septoria tritici blotch (*Septoria tritici*), (https://cropprotectionnetwork.org/) leaf rust (*Puccinia sp.*), powdery mildew (*Blumeria graminis* f. sp. *tritici*), and Fusarium head blight (*Fusarium spp*.). (Edward, 2020; Fernandez et al. 2020). These pathogens exhibit varying infection patterns and optimal conditions for proliferation,

necessitating tailored management strategies. (https://cropprotectionnetwork.org/) For instance, Septoria tritici blotch thrives in humid conditions and can lead to significant yield losses if not promptly addressed (Brown et al., 2002). Similarly, Fusarium head blight not only reduces yield but also contaminates grain with mycotoxins, posing health risks to consumers (Bai et al, 2004).

Fungicide application remains a cornerstone in the integrated management of these diseases. However, the efficacy of such treatments is profoundly influenced by the timing of application relative to the wheat's phenological stages. Research indicates that applications synchronized with specific growth stages, such as the flag leaf emergence or heading, can maximize disease control and yield benefits (Madden et al., 2007). Conversely, misaligned applications may result in suboptimal disease suppression and unnecessary economic expenditure. (https://cropwatch.unl.edu/)

In this context, our study aims to evaluate the efficiency of fungicide applications at distinct phenological stages—specifically tillering (BBCH 21–29), booting (BBCH 41–49), and heading (BBCH 51–59)—as well as their combinations, in controlling prevalent wheat pathogens. The research was conducted at the Agricultural Research and Development Station (ARDS) in Livada, Satu Mare County, Romania. The experimental site is characterized by an acidic, low-fertility albic luvisol, representative of the region's soil profile.

The experimental design encompassed eight treatment variants: individual applications at each of the three phenological stages, three combinations of two-stage applications, a comprehensive three-stage application, and an untreated control. The primary objectives were to, determine the most effective phenological stage or combination thereof for fungicide application in terms of disease suppression, assess the impact of these treatments on wheat yield and grain quality and evaluate the economic efficiency of each treatment strategy, considering both input costs and yield returns.

By elucidating the optimal timing for fungicide applications, this study seeks to provide actionable insights for wheat producers aiming to enhance disease management, optimize yields, and improve profitability.

MATERIAL AND METHOD

The objective of the research is to determine the most effective and efficient treatment timings (phenophases) against the pathogen complex in wheat cultivation.

The influence of the timing (phenophase) of fungicide application on the wheat crop, cultivar Glosa, was assessed by setting up an experiment based on the randomized block design, using the fungicide Ortiva Top at a dose of 0.5 L/ha.

The experiment was conducted during the 2023–2024 agricultural year at ARDS Livada on an albic Luvisol and included the variants presented in Table 1.

This experiment involved the application of a complex fungicide to the wheat crop at different phenological stages. The objective was to cover the three critical phenophases of wheat—tillering, booting, and heading—as well as their combinations: tillering + booting; tillering + booting + heading; tillering + heading; booting + heading, in order to determine the most optimal technology for achieving both high-quality and high-quantity yields.

Table 1. Experimental variants//Variantele experienței

Nr. Crt.	Application phenophase	Fungicide	Doze L/ha	Application date
1	tillering (BBCH 29-30)	Ortiva Top (200 g/l azoxistrobin 125 g/l difenoconazol)	0,5	08.04.2024
2	tillering+booting	Ortiva	0,5	
3	tillering+heading	Ortiva	0,5	
4	tillering+booting+heading	Ortiva	0,5	
5	booting (BBCH 39-40)	Ortiva	0,5	23.04.2024
	booting+heading	Ortiva	0,5	
7	heading	Ortiva	0,5	13.05.2025

Nr. Crt.	Application phenophase	Fungicide	Doze L/ha	Application date
	(BBCH 49-50)			
8	control	Netratat	-	-

This experiment involved the application of a complex fungicide to the wheat crop at different phenological stages. The objective was to cover the three critical phenophases of wheat—tillering, booting, and heading—as well as their combinations: tillering + booting; tillering + booting + heading; tillering + heading; booting + heading, in order to determine the most optimal technology for achieving both high-quality and high-quantity yields.

The experiment was laid out according to the randomized block design, with four replications. (Figure 1).

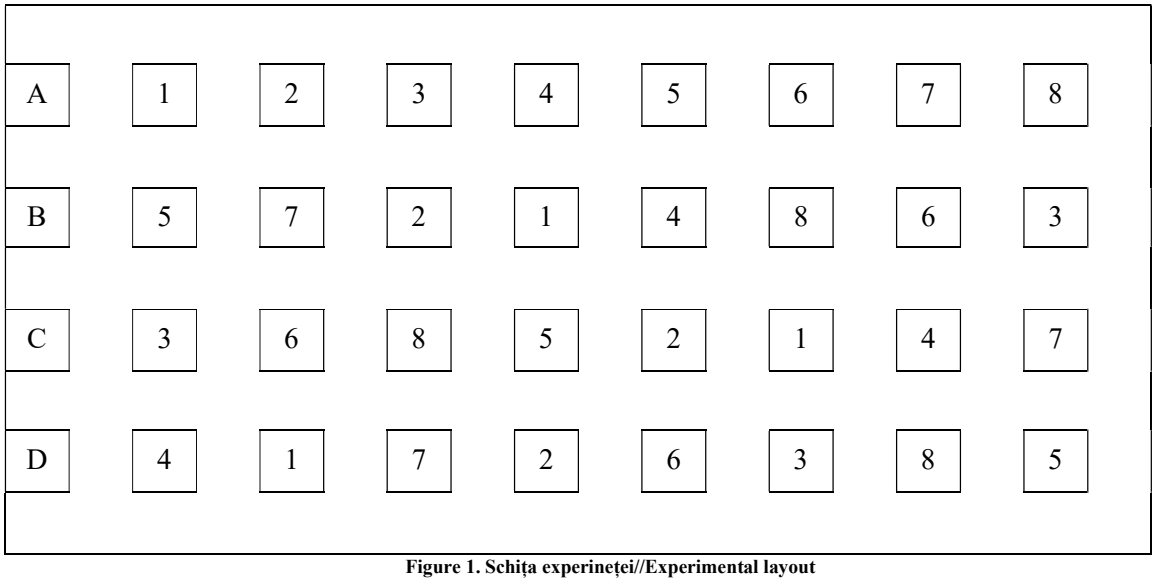


Figure 1. Schița experinței//Experimental layout

The temperatures during the experimental period were characterized by higher values compared to the multiannual average, with the most significant deviations recorded in February (+7.4°C) and March (+4.6°C). These variations indicate a trend of climatic warming, particularly during the winter months and mid-summer. (Figure 2).

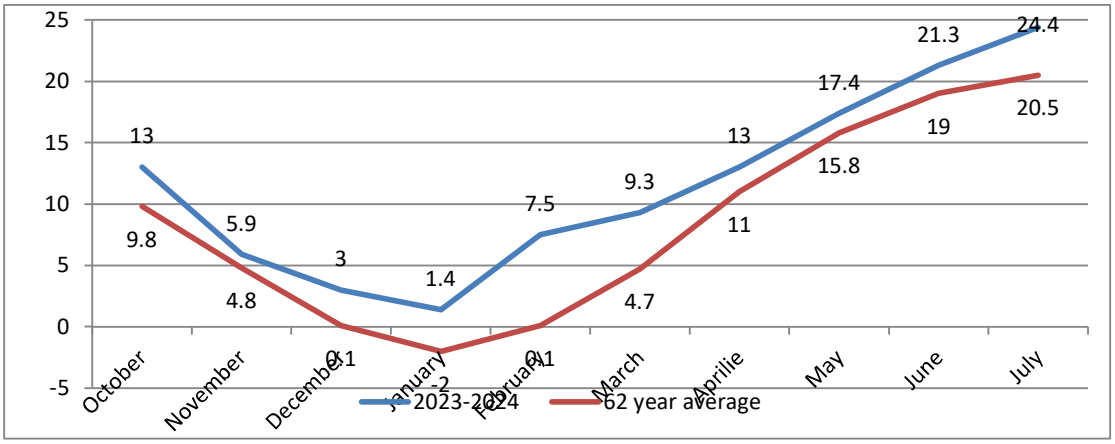


Figure 2. Temperatures in the vegetation period of wheat//Temperaturile lunare din perioda de vegetație a grâului

The distribution of precipitation during the wheat growing season was highly uneven, with amounts significantly exceeding the multiannual average in November and December, and an exceptionally high quantity (147 mm) recorded in June. In contrast, all other months within this period registered significantly lower amounts of precipitation compared to the multiannual average. (Figure 3).

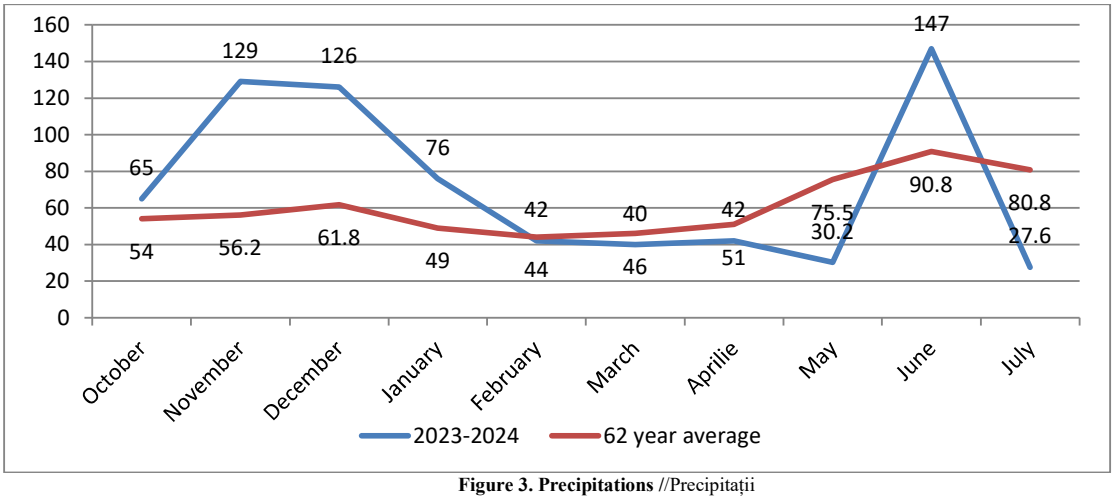


Figure 3. Precipitations //Precipitații

RESULTS AND DISCUSSIONS

Prior to the fungicide treatments, the phytosanitary status of the wheat crop was assessed. It was observed that, before the treatments, the disease commonly known as powdery mildew, caused by the fungus *Erysiphe graminis*, was not present in the wheat crop.

Septoria, caused by the pathogen *Septoria graminis*, was present on the basal leaves, with a frequency of 83.3% on the fourth leaf and 54.1% below the fourth leaf, exhibiting an attack severity of 5% and 51.5%, respectively, and a desiccation percentage of 100%. (Table 2).

Table 2. Pathogen complex manifestation in BBCH 29-30//Manifestarea complexului patogen foliar în fenofaza BBCH 29-30

Leaf order from top to bottom	Disease (Pathogen)				Dryness (%)
	Powdery mildew (Erysiphae graminis)		Leaf blotch (Septoria graminis)		
	Frequency (%)	Degree of attack (%)	Frequency (%)	Degree of attack (%)	
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	20,0	2,0	0
4	0	0	83,3	5,0	100
Sub 4	0	0	54,1	51,5	100

After only 15 days, the severity of attack by the pathogens *Erysiphe graminis* (commonly known as powdery mildew) and *Septoria tritici* (also known as septoria leaf blotch) was significantly reduced in the variants treated during the tillering phenophase, compared to the other variants that were scheduled for treatment at a later time. The statistical analysis of pathogen occurrence and attack severity on leaves 1–3 and the leaf below the third is presented in the table below. (Table 3).

Table 3. The foliar pathogen complex în BBCH 39-40 phenophase, 15 days after the first treatment // Manifestarea complexului patogen foliar în fenofaza BBCH 39-40 după 15 zile de la primul tratament

Nr. crt.	Experimental variants			Pathogens												
	Fungicide	Dose (l/ha)	Application phenophas	Powdery mildew D.A.%						Leaf blotch D.A.%					
				Leaf1-3			Sub 3			Leaf 1-3			Sub 3		
				m	d	s	m	d	s	m	d	s	M	d	s
1	Ortiva	0,5	tillering (BBCH 29-30)	0,0	-0,9	OOO	0,8	-3,4	OOO	0,0	-3,0	OOO	13,7	-8,8	-
2	Ortiva	0,5	tillering+booting	0,0	-0,9	OOO	0,5	-3,7	OOO	0,0	-3,0	OOO	12,0	-10,5	O

Nr. crt.	Experimental variants			Pathogens											
	Fungicide	Dose (l/ha)	Application phenophas	Powdery mildew D.A.%						Leaf blotch D.A.%					
				Leaf1-3			Sub 3			Leaf 1-3			Sub 3		
				m	d	s	m	d	s	m	d	s	M	d	s
3	Ortiva	0,5	tillering+heading	0,0	-0,9	OOO	1,5	-2,7	OOO	0,0	-3,0	OOO	10,0	-12,5	O
4	Ortiva	0,5	tillering+booting+heading	0,0	-0,9	OOO	0,6	-3,6	OOO	0,0	-3,0	OOO	10,8	-11,7	O
5	Ortiva	0,5	booting (BBCH 39-40)	0,6	-0,3	-	3,2	-1,0	O	1,9	-1,1	O	17,5	-5,0	-
6	Ortiva	0,5	burduf+heading	0,8	-0,1	-	3,3	-0,9	-	2,4	-0,6	-	22,5	0	-
7	Ortiva	0,5	heading (BBCH 49-50)	0,6	-0,3	-	3,6	-0,6	-	2,1	-0,9	O	18,8	-3,7	-
8	Control	-	-	0,9		-	4,2	-	-	-3,0	-	-	22,5	-	-
			LSD 5%			0,4%			0,9%			0,8%			9,3%
			LSD 1%			0,5%			1,3%			1,1%			12,7%
			LSD 0,1%			0,7%			1,7%			1,4%			17,2%

Similarly, the pathogen *Puccinia* (rust) and the level of leaf desiccation showed comparable results in the same phenophase as the others. (Table 4).

The chart shows that the lowest *Septoria* attack levels were recorded in treatments applied at the tillering stage and its combinations (especially tillering and tillering+booting+heading), with values under 7% on leaves 1+2 and around 40–46% on leaf 3. In contrast, late treatments (heading) and the untreated control showed significantly higher infection levels, with up to 29.2% and 70.8% respectively. (Figure 4).

Table 4. The foliar pathogen complex în BBCH 39-40 phenophase, 15 days after the first treatment // Manifestarea complexului patogen foliar în fenofaza BBCH 39-40 după 15 zile de la primul tratament

Nr. crt.	Experimental variants			Pathogens				Dryness %		
	Fungicide	Dose (l/ha)	Application phenophas	D.A.%						
				Leaf 1-3			Sub 3	Sub 3		
				m	d	s	m	m	d	s
1	Ortiva	0,5	tillering (BBCH 29-30)	0	-1,9	OOO	0,0	28,1	-6,9	O
2	Ortiva	0,5	tillering+booting	0	-1,9	OOO	0,0	25,6	-9,4	OO
3	Ortiva	0,5	tillering+heading	0	-1,9	OOO	0,0	25,0	-10,0	OO
4	Ortiva	0,5	tillering+booting+heading	0	-1,9	OOO	0,0	25,5	-9,5	OO
5	Ortiva	0,5	booting (BBCH 39-40)	1,7	-0,2	-	0,0	29,1	-5,9	-
6	Ortiva	0,5	booting+heading	1,8	-0,1	-	0,0	35,4	0,4	-
7	Ortiva	0,5	heading (BBCH 49-50)	1,8	-0,1	-	0,0	33,3	-1,7	-
8	Control (fără tratament)	-	-	1,9	-	-	0,0	35,0	-	-
			LSD 5%			0,8%				6,5%
			LSD 1%			1,1%				8,9%
			LSD 0,1%			1,4%				12,0%

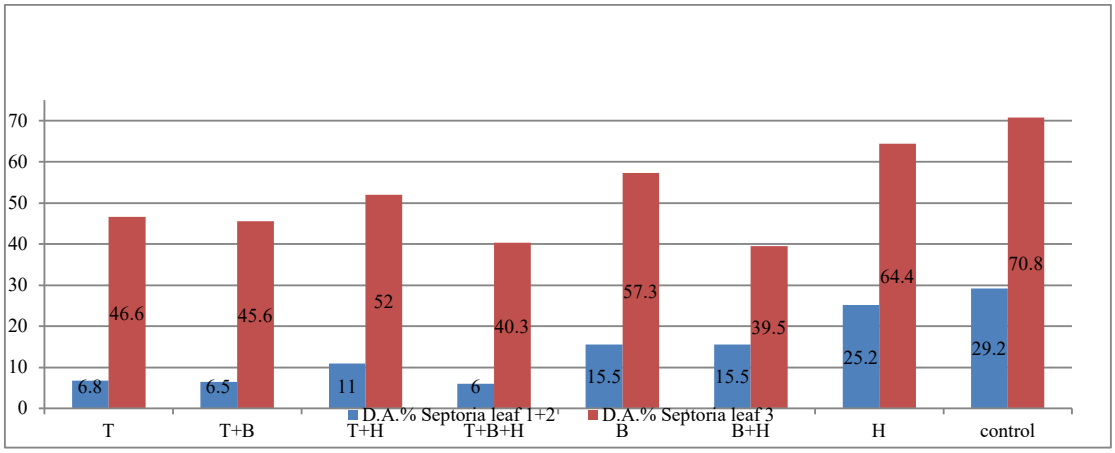
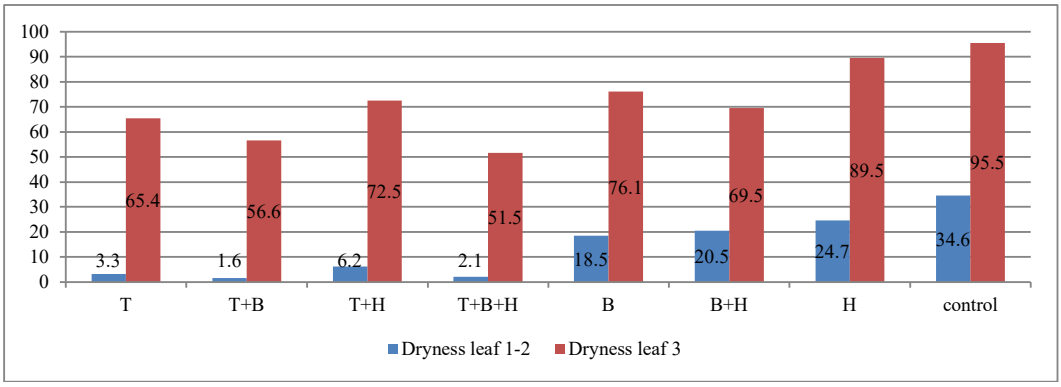


Figure 4. The foliar pathogen complex în BBCH 60 phenophase, 20 days after the second treatment // Manifestarea complexului patogen foliar în fenofaza BBCH 60 după 20 zile de la al doilea tratament

The lowest leaf desiccation was observed in early treatments, especially tillering and tillering+booting, with minimal damage on leaves 1–2 and moderate one on leaf 3. Late treatments (heading) and the untreated control showed the highest desiccation levels, with up to 34.6% on leaves 1–2 and 95.5% on leaf 3. (Figure 5).



F

Figure 5. The foliar pathogen complex în BBCH 60 phenophase, 20 days after the second treatment //Manifestarea complexului patogen foliar în fenofaza BBCH 60 după 20 zile de la al doilea tratament

Very low levels of powdery mildew and rust were recorded in all treated variants, especially those applied early. Only the heading treatment and untreated control showed high rust severity (14.3–14.5%) and slight powdery mildew presence. (Figure 6).

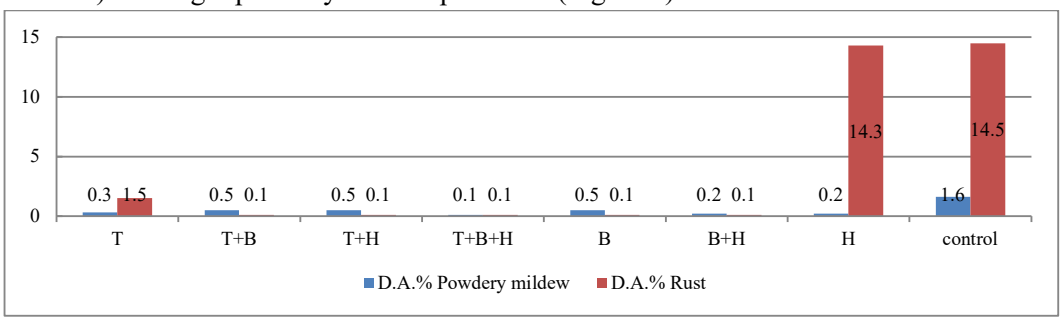


Figure 6. The foliar pathogen complex în BBCH 70 phenophase, 15 days after the third treatment // Manifestarea complexului patogen foliar în fenofaza BBCH 70 după 15 zile de la al treilea tratament

The lowest values for both *Septoria* attack and leaf desiccation were observed in tillering, tillering.+B+S, and booting, confirming the effectiveness of early and combined treatments. The highest severity was recorded in heading and untreated variants, with *Septoria* attack over 37% and desiccation reaching 71.1% and 58.3%, respectively. (Figure 7).

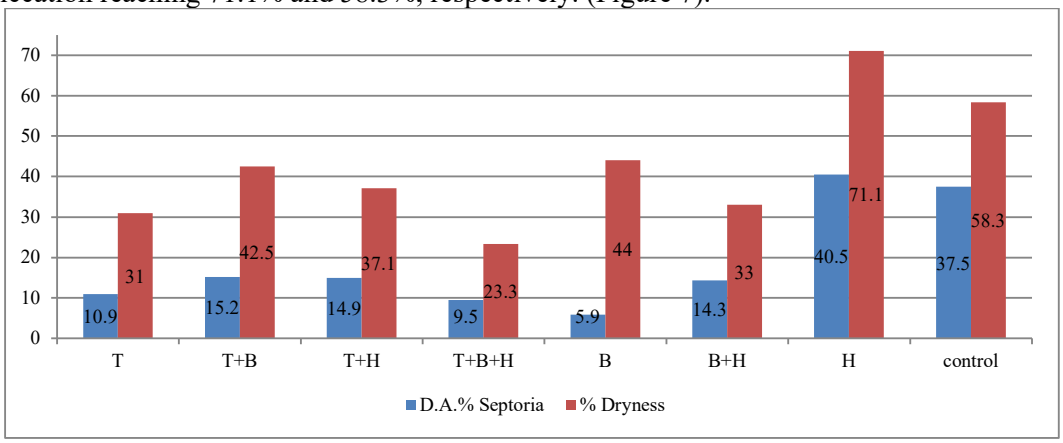


Figure 7. The foliar pathogen complex în BBCH 70 phenophase, 15 days after the third treatment // Manifestarea complexului patogen foliar în fenofaza BBCH 70 după 15 zile de la al treilea tratament

The analysis of variance (Table 5) revealed significant differences ($F = 3.0$, $p < 0.05$) between the treatment variants applied at different phenophases. This result indicates that the timing of fungicide application has a significant influence on wheat yield. The low mean square value for replications suggests that the use of experimental blocks was effective in standardizing conditions.

These findings demonstrate that the phenophase at which the treatment is applied plays a crucial role in both disease control efficacy and yield improvement.

Table 5. ANOVA calculation over the wheat yield//Calculul ANOVA asupra producției de grâu

Source of variance	Sum square	Degree of freedom	Means square	F
Variant	4334392	7	619198,8	3,0*
Replication	306531,3	3	102177,1	
Variant error	4334642	21	206411,5	
T o t a l	8975564	31		

The influence of fungicide treatment timing on yield, as determined by the analysis of variance of differences, shows that the booting + tillering and booting + tillering + heading phenophases are the most important, resulting in statistically significant yield differences. Under conditions where climatic factors had a limited impact on spike diseases (such as fusarium head blight), treatments applied only at heading or in the tillering + heading variant did not lead to significant yield differences. (Table 6).

Table 6. The influence of treatments on the wheat yield//Influența tratamentelor asupre producției de grâu

Variant	Yield (kg/ha)	%	Difference	Signification
Control	5500,25	100,0	0,00	Ct.
tillering	6250,25	113,6	750,00	*
tillering+booting	6547,25	119,0	1047,00	**
tillering+heading	6125,25	111,4	625,00	-
Tillering+booting+heading	6469,25	117,6	969,00	**
booting	6359,75	115,6	859,50	*
booting+heading	6344,00	115,3	843,75	*
heading	5593,75	101,7	93,50	-

LSD (p 5%) 668,21 // LSD (p 1%) 909,16// LSD (p 0.1%)1227,20

Multiple comparisons (Duncan's test) grouped the treatment variants into three classes. The control variant was placed in Class A, with the lowest yield of 5,500 kg/ha. The highest yields, ranging between 6,344 and 6,547 kg/ha, were assigned to Class C, corresponding to treatments applied at booting and all combinations that included the booting phenophase. (Table 7).

Table 7. Duncan test on the wheat yield//Testul Duncan asupra producției

Nr. Crt.	Varinat	Yield (kg/ha)	Classification
1	**Control**	5500,25	A
2	**heading**	5593,75	AB
3	**tillering+heading**	6125,25	ABC
4	**tillering**	6250,25	BC
5	**booting+heading**	6344,00	C
6	**booting**	6359,75	C
7	**tillering+booting+heading**	6469,25	C
8	**tillering+booting**	6547,25	C

In addition to the yield increases obtained through treatment application, the influence is also reflected in the quality traits of the grain.

Significantly positive differences in protein content were recorded in the tillering and tillering + booting variants, and statistically significant differences were observed in the tillering + heading variant.

Table 8. The influence of treatments on the protein content//Influența tratamentelor asupre conținutului de proteine

Variant	Proteine (%)	%	Difference	Signification
Control	13,00	100,0	0,00	Ct.
tillering	14,55	111,9	1,55	**

Variant	Proteine (%)	%	Difference	Signification
tillering+booting	14,63	112,5	1,63	**
tillering+heading	14,45	111,2	1,45	*
Tillering+booting+heading	13,53	104,0	0,52	-
booting	14,00	107,7	1,00	-
booting+heading	13,38	102,9	0,38	-
heading	13,48	103,7	0,47	-

LSD (p 5%) 1,13// LSD (p 1%)1,54 // LSD (p 0.1%)2,08

Table 9. The influence of treatments on the gluten content//Influența tratamentelor asupre conținutului de gluten

Variant	Gluten (%)	%	Difference	Signification
Control	22,45	100,0	0,00	Ct.
tillering	26,43	117,7	3,97	**
tillering+booting	26,68	118,8	4,23	***
tillering+heading	26,58	118,4	4,12	**
Tillering+booting+heading	23,90	106,5	1,45	-
booting	26,28	117,0	3,82	**
booting+heading	23,43	104,3	0,98	-
heading	23,58	105,0	1,13	-

LSD (p 5%) 2,28 // LSD (p 1%) 3,10 // LSD (p 0.1%) 4,18

The gluten content was positively and highly significantly influenced in the tillering + booting variant, and significantly influenced in the tillering, tillering + heading, and booting variants. In the untreated control, the recorded gluten percentage was 22.45%.

According to the multiple comparison analysis, this indicator was divided into two classes, with Class A including the lowest gluten content, ranging from 22.45% to 23.9% and with class B from 26,27 to 26,68%.

Table 10. Duncans test on the gluten content//Testul Duncan asupra conținutului de gluten

Nr. crt.	Variant	Gluten (%)	Classification
1	**Control**	22,45	A
2	**booting+heading**	23,43	A
3	**heading**	23,58	A
4	**tillering+burd+heading**	23,90	A
5	**booting**	26,27	B
6	**tillering**	26,42	B
7	**tillering+heading**	26,57	B
8	**tillering+booting**	26,68	B

The sedimentation index reached its highest value of 49.78 in the tillering + booting variant, with significant values also recorded in the tillering and tillering + heading variants.

Table 11. The influence of treatments on the sedimentation index//Influența tratamentelor asupra indicelui de sedimentare

Variant	Sedimentation index	%	Difference	Signification
Control	37,65	100,0	0,00	Ct.
tillering	47,05	125,0	9,40	*
tillering+booting	49,78	132,2	12,13	**
tillering+heading	46,65	123,9	9,00	*
Tillering+booting+heading	38,78	103,0	1,13	-
booting	44,35	117,8	6,70	-
booting+heading	37,78	100,3	0,13	-
heading	40,20	106,8	2,55	-

LSD (p 5%) 8,33// LSD (p 1%) 11,34 // LSD (p 0.1%) 15,30

The hectoliter weight ranged from 72.63 kg in the untreated control to 76.23 kg in the variant with three treatments.

Highly significant differences were recorded in the variants with three treatments, and distinctly significant differences in the booting + heading variant.

According to the multiple comparison test, treatment timing differentiated the variants into three classes based on hectoliter weight:

- Class A: 72.63–73.70 kg
- Class AB: 74.2 kg (*tillering* variant)
- Class BC: 75.2 kg (*booting* + *heading* variant)
- Class C: 76.23 kg (*three treatments* variant)

Table 12. The influence of treatments on the hectoliter weight//Influența tratamentelor asupra masei hectolitrice

Variant	Hectoliter weight	%	Difference	Signification
Control	72,63	100,0	0,00	Ct.
tillering	74,20	102,2	1,57	-
tillering+booting	73,10	100,7	0,47	-
tillering+heading	73,70	101,5	1,07	-

Variant	Hectoliter weight	%	Difference	Signification
Tillering+booting+heading	76,23	105,0	3,60	***
booting	73,33	101,0	0,70	-
booting+heading	75,50	104,0	2,88	**
heading	73,05	100,6	0,43	-

LSD (p 5%) 1,69 // LSD (p 1%) 2,30 // LSD (p 0.1%) 3,11

Table 13. Duncans test on the hectoliter weight//Testul Duncan asupra masei hectolitrice

Nr. crt.	Varianta	HL weight	Classification
1	**Control**	72,63	A
2	**heading**	73,05	A
3	**Tillering+booting**	73,10	A
4	**booting**	73,32	A
5	**tillering+heading**	73,70	A
6	**tillering**	74,20	AB
7	**booting+heading**	75,50	BC
8	**tillering+burd+heading**	76,22	C

From the perspective of economic efficiency, the calculation of income differences based on the costs incurred by fungicide application and the yield increases achieved per treatment variant shows that, in the experimental year, all treatment variants except the one applied only at heading (heading) recorded a positive profit.

The highest profit, amounting to 719.4 RON/ha, was obtained with a single treatment at booting, followed by the tillering + booting variant with a profit of 632.4 RON/ha.

In contrast, the variant with a single treatment applied only at heading (heading) did not result in a profit; instead, it led to a reduction in income of -199.8 RON/ha.

It is important to note that this outcome occurred under climatic conditions less favorable for spike diseases.

However, in years when precipitation is abundant during heading, fusarium head blight can cause significant qualitative and quantitative yield losses, especially in seed production systems. (Table 14).

Table 14. Economic efficiency//Eficiența economică

Nr.	Variant	Yield increase over the control (kg/ha)	Value (lei)	Expenses/ha (lei)	Profit/ha (lei)
1	tillering	750	900	312	588
2	tillering+booting	1047	1256,4	624	632,4
3	tillering+heading	625	750	624	126
4	Tillering+booting+heading	969	1162,8	936	226,8
5	booting	860	1031,4	312	719,4
6	booting+heading	844	1012,5	624	388,5
7	heading	94	112,2	312	-199,8
	Whweat cost - Prețul grâului din 2024 (lei/kg)			1,2	RON
	Treatement expences - Cheltuieli cu aplicarea tratamentelor (lei/ha)			120	RON
	Fungicide - Fungicid Ortiva Top 0,6l/ha (lei/ha)			192	RON

CONCLUSION

Timing of fungicide application has a decisive impact on both disease control and yield performance in wheat. The application of treatments in multiple phenological stages, particularly tillering + booting + heading, significantly reduced the severity of foliar pathogens such as *Septoria tritici*, *Puccinia spp.*, and *Erysiphe graminis*.

The tillering + booting treatment combination proved to be the most economically efficient, offering both high disease suppression and a 19% yield increase over the untreated control. It generated a profit of 632.4 RON/ha, second only to the booting-only treatment (719.4 RON/ha).

Treatments applied only at heading were the least effective in disease control and did not result in a profitable yield under the climatic conditions of the 2023–2024 growing season, showing a negative economic return.

Regarding grain quality, early and combined applications positively influenced key traits:

- Protein content was significantly increased in the tillering and tillering + booting variants.
- Gluten content reached its highest levels in the tillering + booting combination (26.68%), with other early treatments also showing strong improvements.
- The sedimentation index peaked in the tillering + booting variant (49.78), confirming improved baking quality.

- Hectoliter weight was highest (76.23 kg/hl) in the variant with three treatments, indicating improved grain density.

Statistical analyses (ANOVA and Duncan's test) confirmed the significance of treatment timing, with booting-inclusive variants forming a distinct group with the highest yields and quality parameters.

In years with low fusarium pressure, treatments at heading alone are not justified economically. However, in wetter years, especially during heading, Fusarium head blight risks may shift the optimal timing, necessitating site- and season-specific strategies.

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COMPARATIVE ANALYSIS OF SOME CORN HYBRIDS BASED ON PROTEIN AND OIL CONTENT

ANALIZA COMPARATIVĂ A UNOR HIBRIZI DE PORUMB PE BAZA CONȚINUTULUI DE PROTEINĂ ȘI ULEI

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Abstract
Fifteen corn hybrids, created within NARDI Fundulea, were tested in comparative crops, under specific climate and soil conditions within ARDS Lovrin, agricultural year 2023 - 2024. Based on a common technology, fertilization was differentiated into a fertilized and an unfertilized version. The quality indices represented by protein (Pro, %) and oil (Oil, %) were analyzed comparatively. In the unfertilized version, the protein content varied between 7.93% (hybrid 8029) and 10.63% (hybrid 8034), and the oil content varied between 5.10% (hybrid 8021) and 6.10% (hybrid 8031). On the fertilized variant, protein values between 7.63% (hybrid 8032) and 10.13% (hybrid 8034) were recorded, and oil content values between 4.93% (hybrid 8026) and 6.37% (hybrid 8023). Hybrids were identified that showed statistically significant differences in protein and oil content within each fertilization variant. The average values of protein content ($\overline{\text{Pro}}$*) between the two variants (fertilized / unfertilized) showed statistically significant differences (p = 0.0126). The average oil content*
($\overline{\text{Oil}}$*) between the two fertilization options did not shown statistical significance (p = 0.651). Cluster analysis facilitated the grouping of hybrids based on similarity in protein and oil content, for each fertilized variant.*

Abstract
Cincisprezece hibrizi de porumb, creați în cadrul INCDA Fundulea, au fost testați in culturi comparative, în condițiile specifice de climă și sol in cadrul SCDA Lovrin, anul agricol 2023 – 2024. Pe fondul unei tehnologii comune, a fost diferentiata fertilizarea, în variantă fertilizată și variantă nefertilizată. Indicii de calitate reprezentați de proteină (Pro, %) și ulei (Oil, %) au fost analizati comparativ. Pe varianta nefertilizată, conținutul de proteină a variat intre 7.93% (hibridul 8029) și 10.63% (hibridul 8034), iar conținutul de ulei a variat între 5.10% (hibridul 8021) și 6.10% (hibridul 8031). Pe varianta fertilizată, s-au inregistrat valori ale proteinei între 7.63% (hibridul 8032) și 10.13% (hibridul 8034), și valori ale conținutul de ulei între 4.93% (hibridul 8026) și 6.37% (hibridul 8023). Au fost identificați hibrizi care au prezentat diferențe ăn conditii de siguranța statistică pentru conținutul de proteină și ulei, in cadrul fiecărei variante de fertilizare. Valorile medii ale conținutului de proteina ($\overline{\text{Pro}}$*) dintre cele două variante (fertilizat / nefertilizat) au prezentat diferențe cu siguranță statistică (p = 0.0126). Media conținutului de ulei (*$\overline{\text{Oil}}$*) dintre cele două variante de fertilizare, nu a prezentat siguranță statistică (p = 0.651). Analiza clusterială a facilitat gruparea hibrizilor pe bază de similaritate pentru conținutul de proteină și ulei, pe fiecare variantă de fertilizare.*

Keywords*: corn hybrids, protein, oil, comparative analysis, fertilization*
Cuvinte cheie*: hibrizi porumb, proteină, ulei, analiză comparativă, fertilizare*

INTRODUCTION

Agricultural crops provide various resources for human food, animal feed, and raw materials for various industries (Fuglie et al., 2021; Feng et al., 2023; Knorr and Augustin, 2025). Among agricultural crops, corn is a plant with a high share globally, of agronomic, ecological, economic and social importance (Tiammee and Likasiri, 2020; Wang and Hu, 2021; Afshar and Dekamin, 2022). Corn grains have a series of physical, chemical, biochemical indices, with food and feed value, or for industrialization (Paulsen et al., 2019; Song et al., 2023; Sun et al., 2023).

Yield and quality indices in corn varied in relation to genotype, environmental factors (e.g. soil, climate), and crop technology (Jahangirlou et al., 2021; Liu et al., 2023; Liu et al., 2025). Protein content is a relevant indicator for the quality of corn production, in order to support the increasing

demand for protein for human consumption and animal feed (Rouf Shah et al., 2016; Maqbool et al., 2021). Protein is also an important indicator for genetic selection in breeding programs (Maqbool et al., 2021; Amegbor et al., 2022; Lu et al., 2022). Oil content is also an important index for the quality and value of corn production and also in breeding programs (Fang et al., 2021; Zhang et al., 2023). Variation in protein and oil content in corn kernels has been studied in relation to different types of fertilizers and fertilization rates (Wang et al., 2023; Lu et al., 2024; Yin et al., 2025).
This research evaluated the protein and oil content of fifteen corn hybrids grown in fertilized and unfertilized systems, and comparatively analyzed the performance of the hybrids based on the two quality indices.

MATERIAL AND METHODS

The study and field research were carried out within ARDS Lovrin. Fifteen corn hybrids were cultivated, originating from NARDI Fundulea. The field experiments were carried out during 2023 – 2024 agricultural year. The values of the climatic parameters are presented in figure 1.

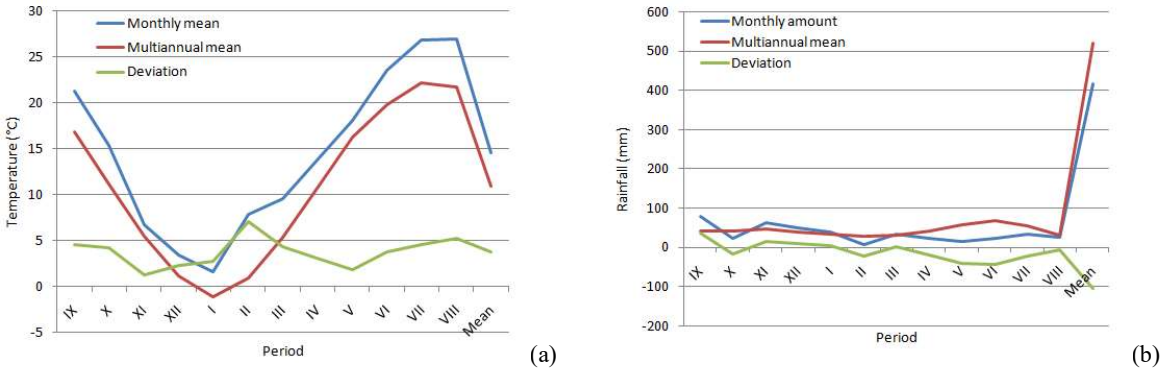


Figure 1. Climatic parameter values during the study period; (a) – temperature, (b) – rainfall
Figura 1. Valorile parametrilor climatici pe perioada de studiu; (a) – temperatura, (b) – precipitatii

The comparative crop of corn hybrids was carried out under non-irrigated conditions, on a cambic chernozem soil. The land was prepared in a conventional system. The corn hybrids were cultivated in a fertilized (F) and unfertilized (UF) system. Complex fertilizers (N/P/K - 15/15/15; 300 kg ha^{-1}) and ammonium nitrate (200 kg ha^{-1}) were applied. Sowing was done in early April, at a distance of 70 cm between rows. Pre-emergence herbicide was applied and mechanical and manual weeding (as necessary) was done in the vegetation. According to the source of the biological material, the corn hybrids were assigned numerical codes (8021 to 8035). Each corn hybrid was cultivated in replicates. Grain samples were harvested at physiological maturity (Meier, 2001), and protein (Pro, %) and oil (Oil, %) were analyzed. Statistical analysis of the data was performed in the EXCEL mathematical module and PAST software (Hammer et al., 2001).

RESULTS AND DISCUSSION

The protein (Pro, %) and oil (Oil, %) content were determined in the 15 corn hybrids, in fertilized and unfertilized systems. The recorded values for each corn hybrid in the two fertilization systems, the mean calculated value for each quality index, and the standard error are presented in table 1. The experimental data presented statistical reliability, according to the Anova Test, table 2. The series of mean values presented normal distribution, with r = 0.903 for $Pro_{(F)}$, r = 0.981 for $Oil_{(F)}$, r = 0.933 for $Pro_{(UF)}$, r = 0.981 for $Oil_{(UF)}$, figure 2.

Table 1. Values of protein and oil content in grains of corn hybrids in fertilized and unfertilized systems // *alori ale continutului de proteina si ulei in boabe la hibrizi de porumb in system fertilizat si nefertilizat*

Corn Hybrid	Fertilized		Unfertilized	
	Pro	Oil	Pro	Oil
	(%)			
8021	8.07	5.17	8.40	5.10
8022	8.63	5.10	8.37	5.43
8023	8.77	6.37	8.93	6.10
8024	8.47	5.50	9.40	5.63
8025	8.07	5.13	8.40	5.23
8026	8.67	4.93	9.07	5.13
8027	8.70	5.20	9.33	5.37
8028	8.10	5.33	8.37	5.40
8029	7.83	5.77	7.93	5.80
8030	8.13	5.87	8.33	5.70
8031	8.60	5.60	9.07	5.80
8032	7.63	5.60	8.23	5.67
8033	8.33	5.93	9.17	5.63
8034	10.13	5.80	10.63	6.10
8035	8.37	5.70	9.20	5.63
Mean	8.43	5.53	8.86	5.58
SE	±0.15	±0.10	±0.17	±0.08

Table 2. ANOVA Test results // *Rezultatle Testului ANOVA*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	432.8029	3	144.2676	402.2870	1.64E-78	5.6639
Within Groups	63.1169	176	0.3586			
Total	495.9198	179				

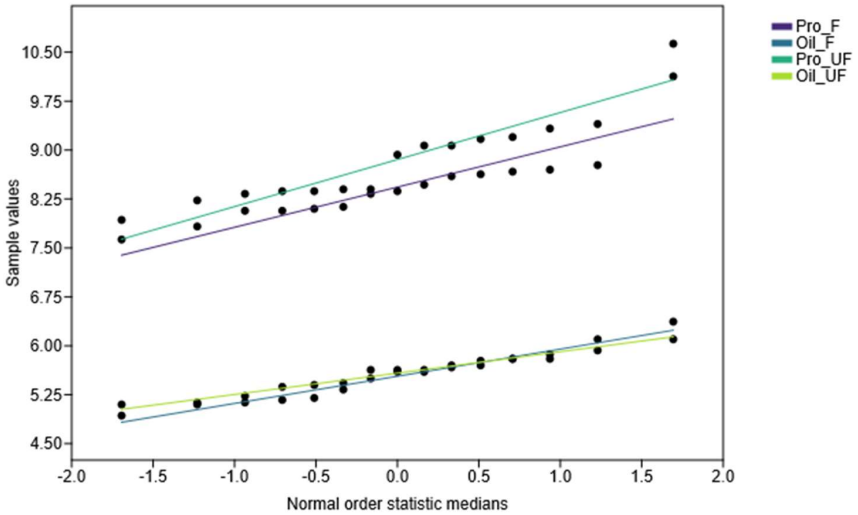


Figure 2. Probability plot for data series // *Probabilitatea de distributie a seriilor de date*

The mean values of the quality indices, in the fertilized / unfertilized system, were analyzed comparatively. The mean protein content in the fertilized system was $\overline{Pro}_{(F)}$ = 8.43±0.15%, and in the unfertilized system was $\overline{Pro}_{(UF)}$ = 8.86±0.17%, with a statistically significant difference at the p = 0.0126

level. The mean oil content in the fertilized system was $\overline{Oil}_{(F)}$ = 5.53±0.10%, and in the unfertilized system was $\overline{Oil}_{(UF)}$ = 5.58±0.08%, with a difference without statistical significance, p = 0.651. Within each fertilization system, each hybrid was analyzed in comparison to the mean of the hybrid group. The results for protein are presented in table 3, and the results for oil content are presented in table 4.

Table 3. Differences in protein content in corn hybrids compared to the mean value of the fertilization system // *Diferentele continutului de proteina la hibrizii de porumb fata de valoarea medie a sistemului de fertilizare*

Corn hybrid	Protein (%)		Statistical Parameters				
	Given mean	Sample mean:	95% conf. interval:	Difference:	95% conf. interval:	t:	p (same mean):
	Fertilized System						
8021	8.07	8.43	(8.1132 8.7535)	-0.36^{O}	(0.043215 0.68345)	2.4343	0.029
8022	8.63			0.20^{ns}	(-0.12345 0.51678)	-1.3177	0.209
8023	8.77			0.34*	(0.016548 0.65678)	-2.2557	0.041
8024	8.47			0.04^{ns}	(-0.28345 0.35678)	-0.2457	0.810
8025	8.07			-0.36^{O}	(0.043215 0.68345)	2.4343	0.029
8026	8.67			0.24^{ns}	(-0.083452 0.55678)	-1.5857	0.135
8027	8.70			0.27^{ns}	(-0.053452 0.58678)	-1.7867	0.096
8028	8.10			-0.33^{O}	(0.013215 0.65345)	2.2333	0.042
8029	7.83			-0.60^{OO}	(0.28322 0.92345)	4.0423	0.001
8030	8.13			-0.30^{O}	(-0.016785 0.62345)	2.0323	0.062
8031	8.60			0.17^{ns}	(-0.15345 0.48678)	-1.1167	0.283
8032	7.63			-0.80^{OOO}	(0.48322 1.1235)	5.3823	<0.001
8033	8.33			-0.10^{ns}	(-0.21678 0.42345)	0.6923	0.500
8034	10.13			1.70***	(1.3765 2.0168)	-11.3680	<0.001
8035	8.37			-0.06^{ns}	(-0.25678 0.38345)	0.4243	0.678
	Unfertilized System						
8021	8.40	8.86	(8.481 9.2296)	-0.46^{O}	(0.081027 0.82964)	2.6091	0.021
8022	8.37			-0.49^{O}	(0.11103 0.85964)	2.7810	0.015
8023	8.93			0.07^{ns}	(-0.29964 0.44897)	-0.4278	0.675
8024	9.40			0.54**	(0.17036 0.91897)	-3.1210	0.008
8025	8.40			-0.46^{O}	(0.081027 0.82964)	2.6091	0.021
8026	9.07			0.21^{ns}	(-0.15964 0.58897)	-1.2300	0.239
8027	9.33			0.47*	(0.10036 0.84897)	-2.7199	0.017
8028	8.37			-0.49^{O}	(0.11103 0.85964)	2.7810	0.015
8029	7.93			-0.93^{OOO}	(0.55103 1.2996)	5.3022	<0.001
8030	8.33			-0.53^{OO}	(0.15103 0.89964)	3.0102	0.009
8031	9.07			0.21^{ns}	(-0.15964 0.58897)	-1.2300	0.239
8032	8.23			-0.63^{OO}	(0.25103 0.99964)	3.5832	0.003
8033	9.17			0.31^{ns}	(-0.05964 0.68897)	-1.8030	0.093
8034	10.63			1.77***	(1.4004 2.149)	-10.1690	<0.001
8035	9.20			0.34^{ns}	(-0.02964 0.71897)	-1.9749	0.068

Table 4. Differences in oil content in corn hybrids compared to the average value of the fertilization system // *Diferentele continutului de ulei la hibrizii de porumb fata de valoarea medie a sistemului de fertilizare*

Corn hybrid	Oil (%)		Statistical Parameters				
	Given mean:	Sample mean:	95% conf. interval:	Difference:	95% conf. interval:	t:	p (same mean):
	Fertilized System						
8021	5.17	5.53	(5.3171 5.7496)	-0.36^{OO}	(0.1471 0.57957)	3.6038	0.003
8022	5.10			-0.43^{OO}	(0.2171 0.64957)	4.2981	0.001
8023	6.37			0.84***	(0.62043 1.0529)	-8.2987	<0.001

Corn hybrid	Oil (%)		Statistical Parameters				
	Given mean:	Sample mean:	95% conf. interval:	Difference:	95% conf. interval:	t:	p (same mean):
8024	5.50			-0.03ns	(-0.1829 0.24957)	0.3306	0.746
8025	5.13			-0.40^{OO}	(0.1871 0.61957)	4.0006	0.001
8026	4.93			-0.60^{OOO}	(0.3871 0.81957)	5.9843	<0.001
8027	5.20			-0.33^{OO}	(0.1171 0.54957)	3.3063	0.005
8028	5.33			-0.20^{ns}	(-0.012901 0.41957)	2.0168	0.063
8029	5.77			0.24*	(0.020432 0.4529)	-2.3474	0.034
8030	5.87			0.34**	(0.12043 0.5529)	-3.3393	0.005
8031	5.60			0.07^{ns}	(-0.14957 0.2829)	-0.6613	0.519
8032	5.60			0.07^{ns}	(-0.14957 0.2829)	-0.6613	0.519
8033	5.93			0.40**	(0.18043 0.6129)	-3.9344	0.001
8034	5.80			0.27*	(0.050432 0.4829)	-2.6450	0.019
8035	5.70			0.17^{ns}	(-0.049568 0.3829)	-1.6531	0.121
	Unfertilized System						
8021	5.10			-0.48^{OOO}	(0.31116 0.6515)	6.0666	<0.001
8022	5.43			-0.15^{ns}	(-0.018838 0.3215)	1.9074	0.077
8023	6.10			0.52***	(0.3485 0.68884)	-6.5371	<0.001
8024	5.63			0.05^{ns}	(-0.1215 0.21884)	-0.6134	0.549
8025	5.23			-0.35^{OO}	(0.18116 0.5215)	4.4281	0.001
8026	5.13			-0.45^{OOO}	(0.28116 0.6215)	5.6885	<0.001
8027	5.37			-0.21^{O}	(0.041162 0.3815)	2.6636	0.019
8028	5.40	5.58	(5.4112 5.7515)	-0.18^{O}	(0.011162 0.3515)	2.2855	0.038
8029	5.80			0.22*	(0.048496 0.38884)	-2.7560	0.015
8030	5.70			0.12^{ns}	(-0.051504 0.28884)	-1.4956	0.157
8031	5.80			0.22*	(0.048496 0.38884)	-2.7560	0.015
8032	5.67			0.09^{ns}	(-0.081504 0.25884)	-1.1175	0.283
8033	5.63			0.05^{ns}	(-0.1215 0.21884)	-0.6134	0.549
8034	6.10			0.52***	(0.3485 0.68884)	-6.5371	<0.001
8035	5.63			0.05^{ns}	(-0.1215 0.21884)	-0.6134	0.549

For protein content (Pro, %), in the case of the fertilized system, two corn hybrids presented valuesabove the mean, in conditions of statistical safety, respectively hybrid 8023 (p<0.05, *) and hybrid 8034 p<0.001, ***). Six hybrids presented negative differences from the mean value, in conditions of statistical safety, respectively hybrids 8021, 8025, 8028 and 8030 (p<0.05, ^{O}), hybrid 8029 (p<0.01, ^{OO}), and hybrid 8032 (p<0.001, ^{OOO}). The other hybrids presented differences (positive or negative) from the mean, without statistical safety. Within the unfertilized system, three hybrids presented positive differences from the mean value, in conditions of statistical safety, respectively hybrid 8027 (p<0.05, *), hybrid 8024 (p<0.01, **) and hybrid 8034 (p<0.001, ***). Seven hybrids presented negative differences from the mean value, in conditions of statistical safety, namely hybrids 8021, 8022, 8025, 8028 (p<0.05, ^{O}), hybrids 8030 and 8032 (p<0.01, ^{OO}), and hybrid 8029 (p<0.001, ^{OOO}). The other hybrids showed differences from the mean value, without statistical safety. The graphic presentation of the protein content values in relation to the mean values on fertilization systems is given in figure 3.

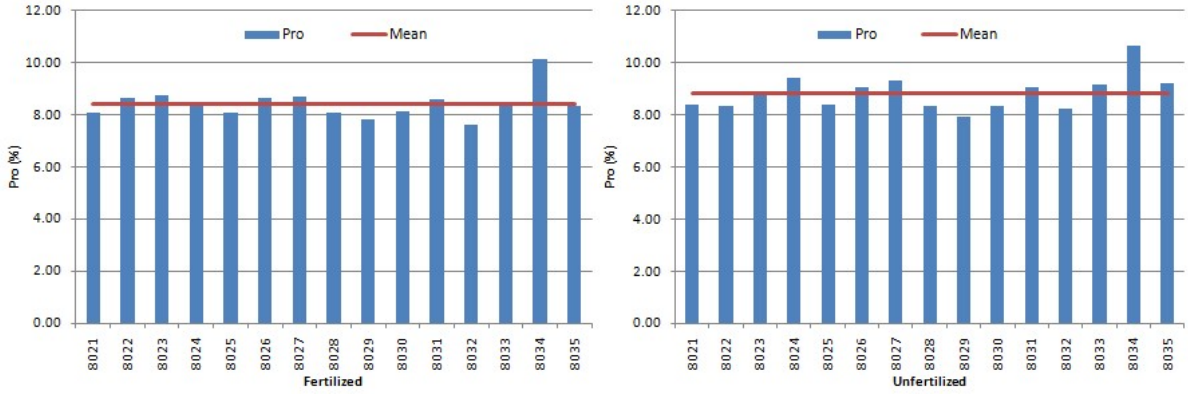


Figure 3. Graphical representation of protein content compared to the mean value, by fertilization systems // *Reprezentarea grafică a conținutului de proteină față de valoarea medie, pe sisteme de fertilizare*

For oil content (Oil, %), in the case of the fertilized system, five hybrids showed positive differences from the mean value with statistical safety, respectively hybrids 8029 and 8034 ($p<0.05$, *), hybrids 8030 and 8033 ($p<0.01$, **), respectively hybrid 8023 ($p<0.001$, ***). Five hybrids showed negative differences, respectively hybrids 8021, 8022, 8025 and 8027 ($p<0.01$, ^{OO}) and hybrid 8026 ($p<0.001$, ^{OOO}). The other hybrids showed differences without statistical safety.

In the case of the unfertilized system, five hybrids showed positive differences, with statistical safety, respectively hybrids 8029 and 8031 ($p<0.05$, *), and hybrids 8023 and 8034 ($p<0.001$, ***). Five hybrids showed negative differences with statistical safety, respectively hybrids 8027 and 8028 ($p<0.05$, ^{O}), hybrid 8025 ($p<0.01$, ^{OO}), and the hybrids 8021 and 8026 ($p<0.001$, ^{OOO}). The other hybrids showed differences without statistical safety. The graphic presentation of the oil content values in relation to the mean values on fertilization systems is given in figure 4.

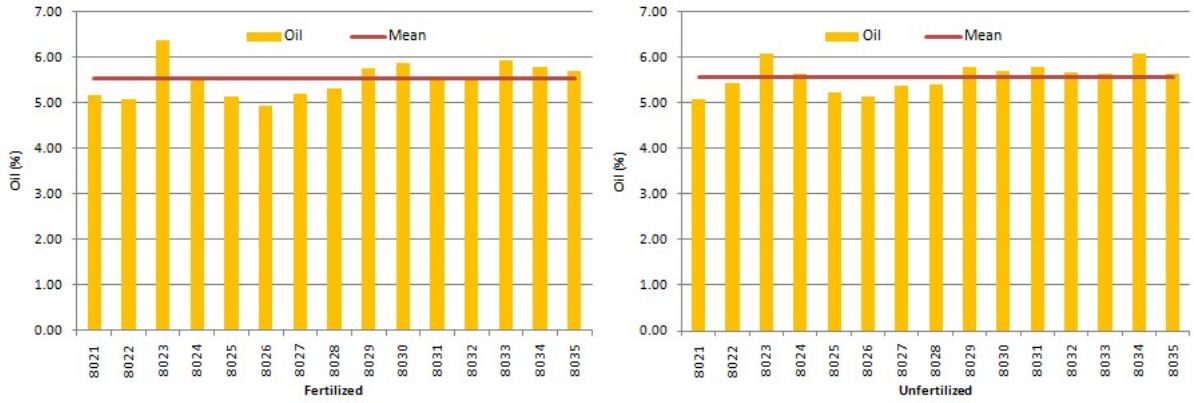


Figure 4. Graphical representation of oil content compared to the mean value, on fertilization systems // *Reprezentarea grafică a conținutului de ulei față de valoarea medie, pe sisteme de fertilizare*

The multivariate analysis led to the PCA diagram, figure 5, in which the corn hybrids were distributed differently in relation to quality parameters. There were corn hybrids positioned correlated with protein, or with oil, and hybrids with independent positioning. The cluster analysis led to the dendrogram in figure 6 (Coph.corr. = 0.865). Two distinct clusters were found that include the corn hybrids.

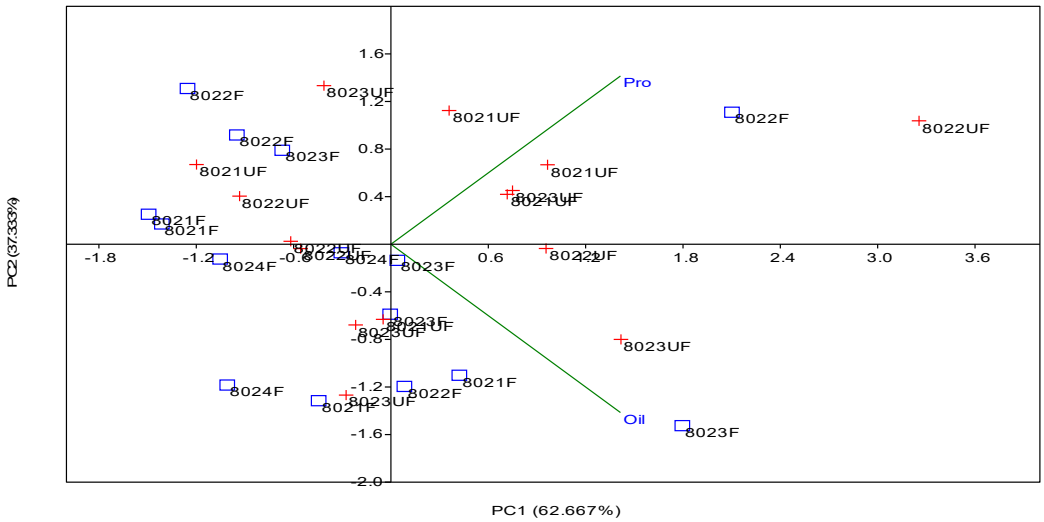


Figure 5. PCA diagram with the distribution of corn hybrids in relation to quality parameters (blue color – fertilized system; red color – unfertilized system) // *Figura 5. Diagrama PCA cu distributia hibrizilor de porumb în raport cu parametri de calitate (culoare albastra – sistem fertilizat; culoare roșie – sistem nefertilizat)*

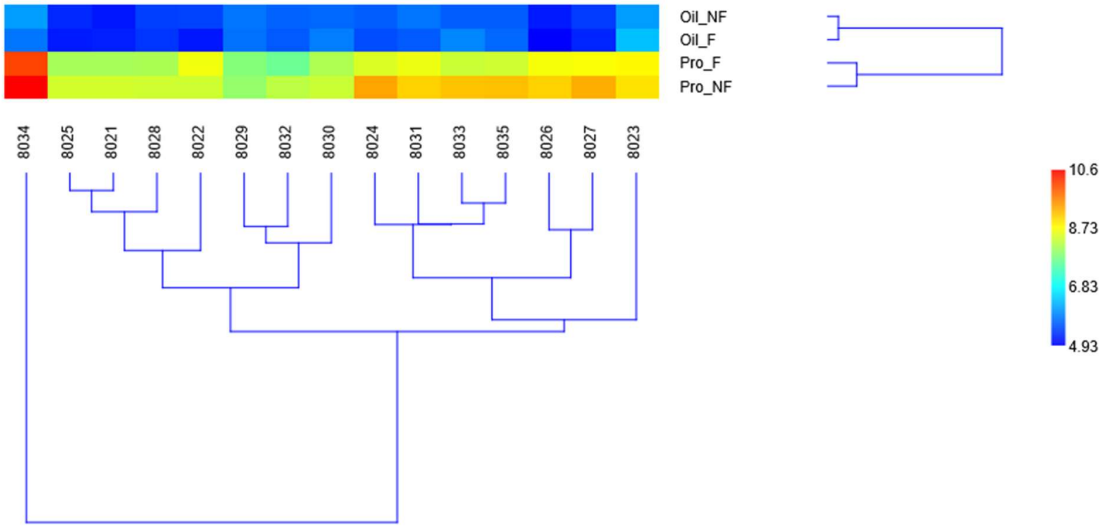


Figure 6. Cluster diagram based on Euclidean distances // *Diagrama cluster pe baza distantelor Euclidiene*

Cluster C1 included hybrid 8034 which showed advantage for protein content in both crop systems (fertilized/unfertilized). The other hybrids were positioned in cluster C2, with several subclusters, in which hybrids were associated based on similarity.

The highest level of similarity was recorded between hybrids 8021 and 8025 (SDI = 0.136). The SDI values for all maize hybrids tested in the two cropping systems (fertilized / unfertilized) in relation to determined quality parameters are presented in Table 5.

Table 5. SDI values for corn hybrids // *Valorile SDI pentru hibrizii de porumb*

	8021	8022	8023	8024	8025	8026	8027	8028	8029	8030	8031	8032	8033	8034	8035
8021		0.654	1.792	1.245	0.136	0.931	1.156	0.343	1.062	0.927	1.185	0.856	1.232	3.258	1.137
8022	0.654		1.548	1.134	0.596	0.781	0.970	0.579	1.191	0.958	0.937	1.152	1.208	2.880	1.075
8023	1.792	1.548		1.135	1.751	1.745	1.438	1.528	1.528	1.086	0.855	1.602	0.816	2.250	0.950
8024	1.245	1.134	1.135		1.207	0.851	0.464	1.131	1.635	1.184	0.406	1.444	0.507	2.140	0.300
8025	0.136	0.596	1.751	1.207		0.927	1.134	0.266	1.006	0.881	1.129	0.798	1.209	3.228	1.102
8026	0.931	0.781	1.745	0.851	0.927		0.446	1.024	1.778	1.431	0.950	1.590	1.173	2.503	0.975

	8021	8022	8023	8024	8025	8026	8027	8028	8029	8030	8031	8032	8033	8034	8035
8027	1.156	0.970	1.438	0.464	1.134	0.446		1.140	1.796	1.372	0.650	1.614	0.873	2.151	0.666
8028	0.343	0.579	1.528	1.131	0.266	1.024	1.140		0.787	0.620	0.986	0.622	1.052	3.153	0.975
8029	1.062	1.191	1.528	1.635	1.006	1.778	1.796	0.787		0.520	1.386	0.419	1.357	3.560	1.392
8030	0.927	0.958	1.086	1.184	0.881	1.431	1.372	0.620	0.520		0.923	0.578	0.868	3.075	0.921
8031	1.185	0.937	0.855	0.406	1.129	0.950	0.650	0.986	1.386	0.923		1.290	0.470	2.215	0.330
8032	0.856	1.152	1.602	1.444	0.798	1.590	1.614	0.622	0.419	0.578	1.290		1.218	3.498	1.225
8033	1.232	1.208	0.816	0.507	1.209	1.173	0.873	1.052	1.357	0.868	0.470	1.218		2.368	0.235
8034	3.258	2.880	2.250	2.140	3.228	2.503	2.151	3.153	3.560	3.075	2.215	3.498	2.368		2.318
8035	1.137	1.075	0.950	0.300	1.102	0.975	0.666	0.975	1.392	0.921	0.330	1.225	0.235	2.318	

A ranking of corn hybrids was generated based on protein and oil content values, in a fertilized system, figure 7 (a) and in an unfertilized system, figure 7 (b).

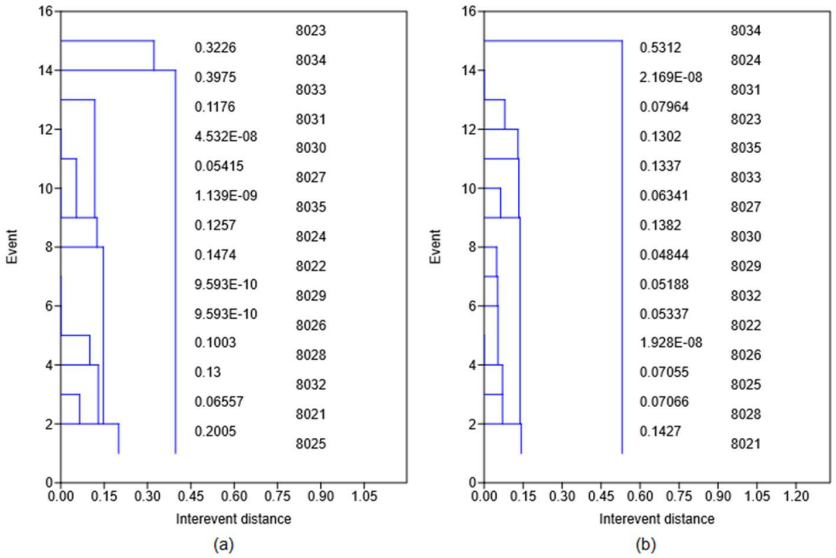


Figura 7. Ranking of corn hybrids// *Ierarhizarea hibrizilor de porumb*

The variation of yield and quality indices in corn were analyzed in relation to different fertilizers, to improve crop technologies and optimize yields (Boldea and Sala, 2011; Abdo et al., 2022; Lu et al., 2024; Ojeniyi et al., 2024).

The genetic potential of corn hybrids for protein and oil was analyzed under different technology and environmental factors to select and promote high-performing genotypes for breeding programs and specific crop conditions (Amegbor et al., 2022; Liu et al., 2023; Zhang et al., 2023).

Under the specific conditions of the present study, valuable data were recorded regarding protein and oil production in corn hybrids in comparative crops.

CONCLUSIONS

1. Corn hybrids grown in a fertilized system (N-15/P-15/K-15 complex fertilizer, ammonium nitrate) and in an unfertilized system responded differently in the accumulation of protein and oil in corn kernels, in relation to the genetic potential and the complex response to the "genotype x environment x technology" interaction.
2. Under the study conditions, the mean protein content in the fertilized system was $\overline{Pro}_{(F)}$ = 8.43±0.15%, and in the unfertilized system was $\overline{Pro}_{(UF)}$ = 8.86±0.17%, with a statistically significant difference, at the p = 0.0126 level. The mean oil content in the fertilized system was $\overline{Oil}_{(F)}$ = 5.53±0.10%, and in the unfertilized system was $\overline{Oil}_{(UF)}$ = 5.58±0.08%, without

statistically significance, p = 0.651.

3. For protein content (Pro, %), in the fertilized system, two corn hybrids presented values above the mean, in statistical safety conditions, respectively hybrid 8023 ($p<0.05$, *) and hybrid 8034 $p<0.001$, ***). In the unfertilized system, three hybrids presented positive differences from the mean value, in statistical safety conditions, respectively hybrid 8027 ($p<0.05$, *), hybrid 8024 ($p<0.01$, **) and hybrid 8034 ($p<0.001$, ***).
4. For oil content (Oil, %), in the fertilized system, five hybrids showed positive differences from the mean value, with statistical safety, respectively hybrids 8029 and 8034 ($p<0.05$, *), hybrids 8030 and 8033 ($p<0.01$, **), respectively hybrid 8023 ($p<0.001$, ***). In the unfertilized system, five hybrids showed positive differences, with statistical safety, respectively hybrids 8029 and 8031 ($p<0.05$, *), and hybrids 8023 and 8034 ($p<0.001$, ***).
5. The 8034 corn hybrid was noted for its high protein content and statistically significant differences in both, fertilized and unfertilized systems. The 8023 corn hybrid was noted for its high oil content and statistically significant differences in both, fertilized and unfertilized systems. These hybrids showed high genetic stability for the two quality indices in the study conditions.

ACKNOWLEDGMENTS

The present data were generated following the implementation of the ADER 1.3.1. Project.

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THE IMPACT OF YELLOW RUST ATTACK (*PUCCINIA STRIIFORMIS* F. SP.*TRITICI*) ON THE QUALITY INDICES OF TRITICALE GRAINS (*TRITICOSECALE* WITTMACK)

IMPACTUL ATACULUI DE RUGINĂ GALBENĂ (PUCCINIA STRIIFORMIS F. SP.TRITICI) ASUPRA INDICILOR DE CALITATE A BOABELOR DE TRITICALE (*TRITICOSECALE* WITTMACK)

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Abstract

Triticale is a nearlyy creted genus as the result of human crossbreeding between the species Triticum aestivum and Secale cereale. It has inherited the high production capacity and high protein content from wheat, and from rye it has a high lysine content and high resistance to biotic and abiotic stress factors. Due to these properties, it is used in a variety of fields such as: the baking and biscuit industries, beer production, fish nutrition, the pulp industry, human medicine, animal feed in different forms (concentrated feed, straw, silage, grazing), bioethanol production, and in the manufacture of food packaging. Through the improvement of this species, the dominant genes are those of wheat, and thus it has started to show a reduced resistance to pathogen attacks, including the attack of yellow rust.

At A.R.D.S. Livada, during complex ecological testing on triticale, 15 triticale varieties were evaluated in comparative crops during the 2023-2024 period, with an emphasis on the degree of attack on the quality indices of the grains.

In 2021 and 2022, the attack of yellow rust was nonexistent or almost nonexistent, but as the temperatures during the winter period increased, with positive monthly temperature averages, yellow rust showed a strong attack early in the spring, almost completely compromising the crop. The degree of attack by yellow rust ranged between 10% and 75%. The most resistant triticale variety was Zaraza, which, even under climatic conditions that favored the intense manifestation of yellow rust, demonstrated good genetic resistance (GA 10%), leading to a hectoliter weight of 70 kg/hl and a thousand grain weight of 46.8 g. The hectoliter weight for the other 14 varieties ranged between 43 and 61 kg/hl, and the thousand grain weight varied between 24 and 34 g.

Rezumat

Triticalele sunt rezultatul încrucișării de către om dintre genurile Triticum aestivum și Secale cereale. Acestea au preluat de la grâu capacitatea ridicată de producție și conținutul ridicat în proteină, iar de la secară conținut ridicat în lizină și o rezistență ridicată la factorii de stres biotici și abiotici. Datorită acestor proprietăți se utilizează într-o multitudine de domenii precum: industria de panificație și a biscuiților, a berii, in nutriția peștilor, industria celulozei, în medicină umană, hrana animalelor sub diferite forme (furaj concentrat, paie, siloz, pășunat), industria bioetanolului și în fabricarea ambalajelor alimentare. Prin ameliorarea acestei specii genele dominante sunt cele ale grâului și astfel au început să manifeste o rezistență mai mică la atacul agenților patogeni, inclusiv la atacul de rugină galbenă.

La S.C.D.A. Livada, în cadrul testărilor ecologice complexe la triticale, s-au luat în evidență 15 soiuri de triticale testate în culturi comparative în perioada 2023-2024 și evidențierea gradului de atac asupra indicilor de calitate a boabelor.

In 2021 și 2022, atacul de rugină galbenă a fost inexistent sau aproape inexistent, dar odată cu creșterea temperaturilor din perioada iernii, când media temperaturilor lunare a fost pozitivă, rugina galbenă a manifestat un atac puternic primăvara devreme, ajungând să compromită aproape în totalitate această cultură. Gradul de atac la rugina galbenă a fost între 10 și 76%. Cel mai rezistent soi de triticale a fost soiul Zaraza, care și în conditiile climatice care au favorizat manifestarea intensă a atacului de rugină galbenă, a confirmat o bună rezistență genetică (GA 10%) rezistență care a dus la înregistrare unei mase hectolitrice de 70 kg/hl și masa a o mie de boabe de 46,8 g. Masa hectolitrica la cele 14 soiuri a fost cuprinsă între 41 si 61 kg/hl iar masa a o mie de boabe între 24 și 34 g.

Key words: *attack, yellow rust, hectoliter weight, resistence, triticale.*
Cuvinte cheie: *atac, rugină galben, masă hectolitrică, rezistență, triticale.*

INTRODUCTION

Triticale was first developed in 1875 through artificial hybridization between wheat (*Triticum aestivum*) and rye (*Secale cereale*), with the objective of combining the most advantageous agronomic traits of both parental species (McGoverin et al., 2011), namely high productivity and enhanced resistance to extreme environmental conditions (Zhu, 2018). Due to its favorable agronomic characteristics, triticale can be successfully cultivated on marginal lands characterized by low fertility and high acidity (Çiftci et al., 2003).

From a chemical composition standpoint, triticale grains present intermediate values between those of wheat and rye. A limiting factor for its use in the baking industry is the relatively low content of the essential amino acid lysine. Reported protein contents in triticale grains vary considerably among studies, ranging from 12% to 22% (Lorenz and Pomeranz, 1974). Other sources report an average protein content of 11.7%, with 66.5% starch and 4.6% crude sugars (Petterson and Aman, 1987), while additional studies indicate protein contents between 11.4% and 14.0%, and lysine levels between 0.33% and 0.71% (Fras et al., 2016). According to Sehgal (1983), who conducted comparative analyses on several triticale and wheat cultivars, the mean protein content was found to be 13.1%.

Given these compositional attributes, triticale flour is suitable for certain applications in the baking industry, particularly in the production of biscuits (Perez et al., 2003; Leon et al., 1996). Although its primary historical use has been as feed for livestock—especially poultry and swine—this application remains predominant today, with triticale being recognized as a valuable feed grain (Gaviley et al., 2024). Furthermore, triticale has been integrated into various industrial sectors, including brewing (Glatthar et al., 2003; Ambriez-Vidal et al., 2019), pulp and paper production (Tarres et al., 2017), and bioethanol manufacturing (Pejin et al., 2011; García-Aparicio et al., 2010).

Recent investigations have also demonstrated the potential of triticale in human health applications, highlighting its anti-inflammatory properties and cholesterol-lowering effects, attributed to its high content of lunasin (Nakurte et al., 2012), as well as its immunostimulatory activity (Jańczak-Pieniążek et al., 2023). Additionally, when it is cultivated in mixtures with other forage species, triticale can serve as a valuable pasture component (Baron et al., 2015). Due to its compositional similarity to wheat, it is also utilized in aquaculture nutrition (Dawood et al., 2020; Hughes, 1990).

Given its broad spectrum of uses, the global cultivated area of triticale in 2023 exceeded 3.5 million hectares, according to data from the Food and Agriculture Organization (FAO). Poland was the leading producer with 1.2 million hectares, followed by Belarus (442 thousand ha), France (326 thousand ha), and Germany (311 thousand ha). In Romania, the cultivated area reached 59,460 hectares in the same year (FAO, 2023).

Triticale has historically been recognized for its resistance to a wide range of phytopathogens, a trait conferred by resistance genes inherited from rye. Initially, the species was intended to act as a genetic bridge for the transfer of rye resistance traits into wheat. Although its reputed resistance has been reevaluated in recent years, triticale generally exhibit a healthier status when compared to their wheat and rye counterparts.

The fungal pathogen *Puccinia striiformis* f. sp. *tritici* was first reported in Australia and became the initial disease to reach epidemic levels in triticale crops (Arseniuk, 1996), particularly affecting regions with colder climates (Zhao et al., 2022). In recent years, several triticale cultivars have also exhibited susceptibility to Blumeria graminis, another pathogen reaching epidemic proportions (Arseniuk and Goral, 2015). Among foliar diseases, yellow rust remains the most significant for triticale, with resistance being largely associated with the rye-derived genomic component (Skowrońska et al., 2020).

MATERAL AND METHOD

The present study was conducted at the Livada Agricultural Research-Development Station and it is part of the complex ecological testing carried at the national level. Fifteen triticale varieties were tested using the randomized block method, in experimental plots of 10 m², with three replications. The varieties used in the experiment were developed by the National Agricultural Research and Development Institute, Fundulea.

The soil type where the experiment was located is a typical preluvosol, characterized by an acidic reaction, with a pH ranging from 5.82 in the arable layer to 6.65 deeper in the soil profile. This type of soil has a low humus content in the arable layer, of 2.82% (Table 1).

Table 1. Soil characteristics from the experimental field in Livada//Caracteristicile de sol din câmpul experimental de la Livada

Specification	UM cm	Ap 0-18	Ao 18–40		AB 40–55	Bt_1w 55–70	Bt_2w 70–110
			20-30	30-40		55–70	80–95
Humus (C x 1.72)	%	2.82	1.44	0.90	0.90	0.84	3.24
N total	%	0.168	0.102	0.072	0.068	0.064	-
C:N	-	8.21	9.15	10.14	10.34	10.57	-
pH in water	-	5.19	6.24	6.65	6.53	5.62	5.28
Clay (<0.002 mm)	%g/g	20.9	21.1	23.1	27.0	32.4	33.1
Apparent density	g/cm^3	1.35	1.54	1.49	1.48	-	1.48
Hydraulic Conductivity	mm/h	31.27	5.87	3.11	0.35	-	1.04

In the experiment, no phytosanitary treatments were applied in order to highlight the most resistant and adapted triticale varieties in the north-western part of Romania.

The preceding crop was fodder pea (variety MagistraLiv). After harvesting it in July, a pass was made with a disc harrow to interrupt capillarity and water evaporation from the soil. In September, plowing was performed at a depth of 22-25 cm, followed by another pass with the disc harrow in October. The base fertilization of the soil was carried out using a complex fertilizer of the DAP 18:46:0 type at a dose of 150 kg/ha. Sowing took place on October 9th using the experimental seeder from Wintersteiger (Figure 1).



Figure 1. Sowing with Wintersteiger seeder//Semănatul cu semănătoare Wintersteiger

In the spring, in March, after the elongation of the stem, additional fertilization was carried out with a nitrogen-based fertilizer, Calcium Ammonium Nitrate 27% nitrogen active substance, at a dose of 300 kg/ha. Weed control was performed with the herbicide Sekator Progress OD at a dose of

0.15 l/ha. During the vegetation period, the phytosanitary status of the experimental plots was recorded using the FAO scale from 1 to 9. Harvesting took place in July using the experimental combine from Wintersteiger (Figure 3).



Figure 2. Harvesting with Wintersteiger combine//Recoltarea cu combina Wintersteiger

RESULTS AND DISCUSSIONS

The greatest influence on the attack of yellow rust is the climatic conditions. The increase in average temperatures and the rise in precipitation levels are the predominant factors that favor the establishment of the phytopathogenic agent.

The average temperature during the vegetation period of triticale in the 2023-2024 season was 11.6°C, which is 3.2°C higher than the multi-year average temperature for the same period. The largest difference between the average temperature and the multi-year average was recorded in February, with a 7.4°C difference, where the average temperature in the winter month was 7.5°C. A significant temperature difference was mainly recorded during the winter months, where temperatures were positive: January showed a 3.4°C difference compared to the multi-year average, December had a 2.9°C difference, March had a 4.6°C difference, and July had a 3.9°C difference (Figure 3).

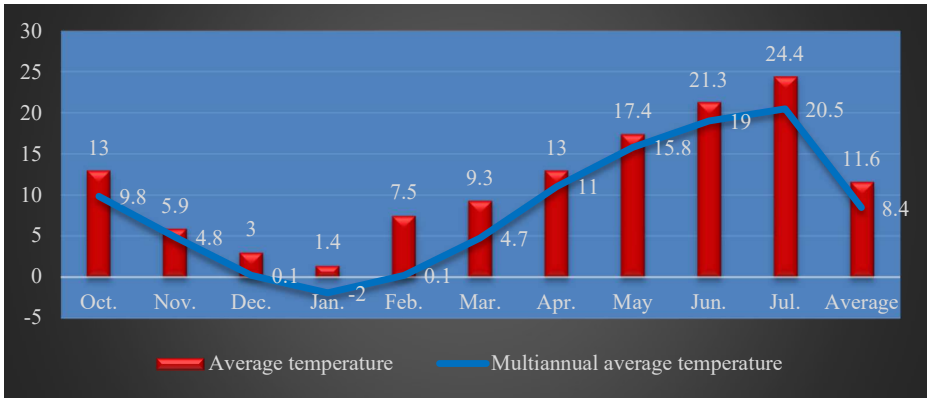


Figure 3. Average temperatures during the vegetation period, 2023-2024//Temperaturi medii în perioada de vegetație, 2023-2024

The total precipitation during the vegetation period of the triticale recorded an excess of 115.7 mm/m² compared to the multi-year average (Figure 4).

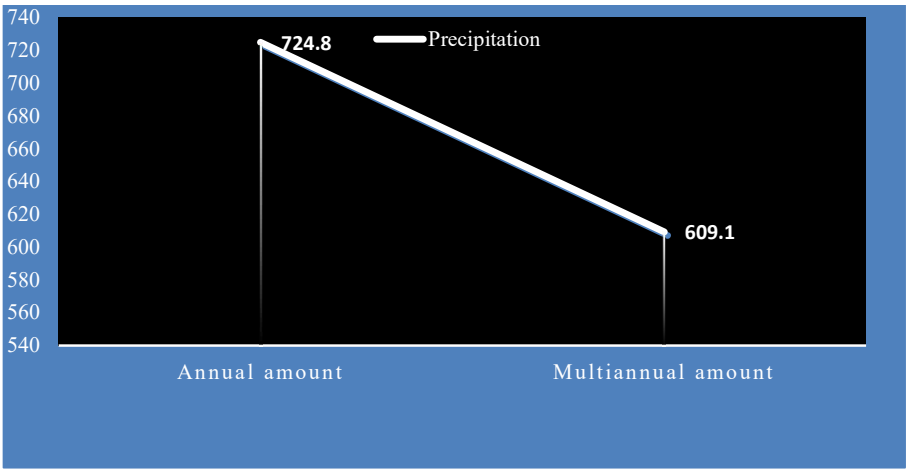


Figure 4. Annual and multi-annual precipitation sum for 2023-2024//Suma anuală și multianuală a precipitațiilor din 2023-2024

The highest precipitation levels were recorded in July (147 mm/m²), November (129 mm/m²), and December (126 mm/m²). In the spring, the values were close to the multi-year averages. The largest deficit occurred in July, with 53.2 mm/m², and in May, with 45.3 mm/m² (Figure5).

The high temperatures during winter, which did not lead to the destruction of pathogens, along with the precipitation and high temperatures in the spring, contributed to the favoring of yellow rust attacks, even on the most resistant plant species.

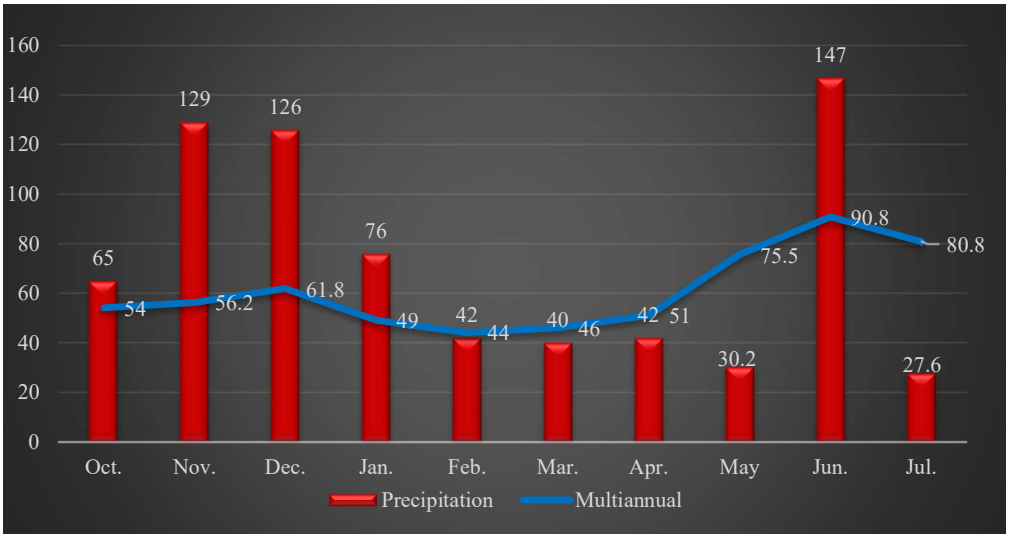


Figure 5. The amount of precipitation during the vegetation period, 2023-2024// Suma de precipitații în perioada de vegetație, 2023-

The results obtained are primarily due to the high level of yellow rust attack on triticale plants (Figure 6). Climatic conditions are the main factor that favored the appearance and massive attack of yellow rust.

In this study, 15 triticale varieties from I.N.C.D.A. Fundulea were tested, and they were cultivated according to the growing technology used in the north-western part of the country. The experiment was set up without phytosanitary treatments to highlight the most resistant and adapted varieties for the reference area. To assess the degree of attack by the main phytopathogenic agents, observations were made during the vegetation period on a scale from 1 to 9, according to FAO standards.

The degree of yellow rust attack ranged from 10% to 76%. Most of the varieties recorded a yellow rust attack level of 76%, meaning that 76% of the total plant area was affected by this pathogen. The control variety (Plai) recorded a 54% attack level, and Negoiu showed a similar level. FDL Ascendent, Pisc, and Tulnic recorded an attack level of 63%. The most resistant to this pathogen attack was the variety Zaraza, which has consistently proven to be the most resistant variety over the years, with an attack level of 10% (Figure 6, 7).

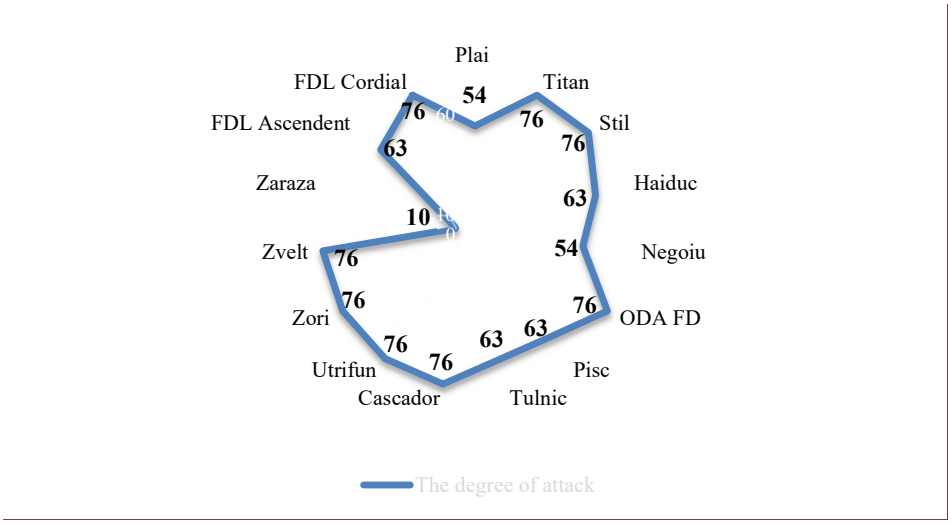


Figure 6. The degree of attack recorded on triticale plants//Gradul de atac înregistrat la nivelul plantelor de triticale



Figure 7. The experiement field//Câmpul experimental

The massive attack of yellow rust was evident in the quality index results. The control variety, which recorded an attack level of 54%, had a TGW (thousand grain weight) of 34.4 g. The varieties that recorded the highest attack levels, such as Titan, Stil, and Haiduc, had a TGW ranging between 25 and 27 g. The lowest TGW value was recorded by Zori, at 24 g. Zaraza, the most resistant variety, recorded a thousand-grain weight of 46.8 g, which is a normal value for cereals where the attack is present to a small extent or when phytosanitary treatments have been applied (Figure 7).

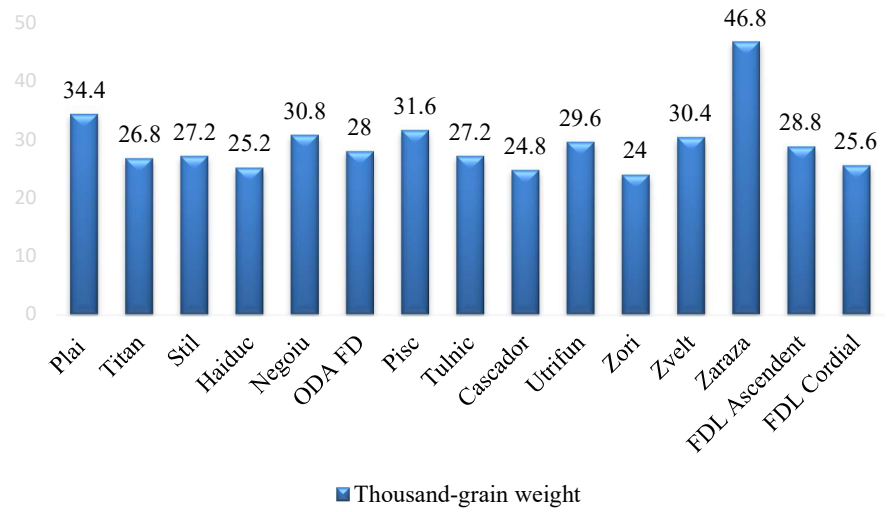


Figure 7. Thousand-grain weight recorder by the 15 varieties//Masa a o mie de boabe înregistrată de cele 15 soiuri

In the experiment, the hectolitric weight ranged between 41 and 70 kg/hl. The control variety, which had 54% of its leaf area attacked by yellow rust, recorded a hectolitric weight of 61.9 kg/hl. For the varieties with the highest attack levels (76%), the hectolitric weight did not exceed 51 kg/hl. The lowest value was recorded by the variety Cascador, with 41.4 kg/hl. Similarly to the thousand-grain weight, the Zaraza variety also recorded the highest hectolitric weight, further demonstrating its resistance (70 kg/hl).

As observed, the degree of attack recorded by the 15 triticale varieties directly influenced the quality index values. Thus, the varieties with the highest degree of attack recorded the lowest values for thousand-grain weight and hectolitric weight (Figure 8).

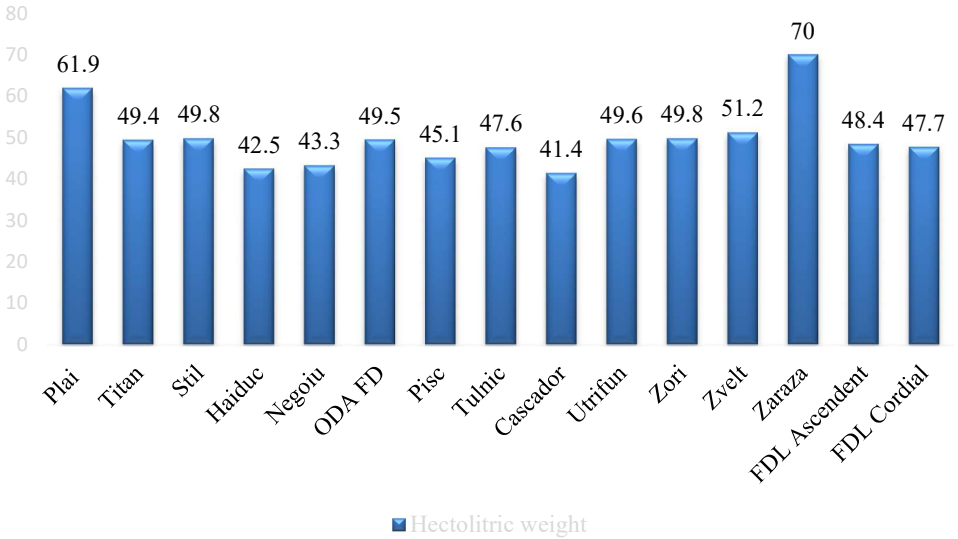


Figure 8. Hectolitric weight recorder by the 15 varieties//Masa hectolitrică înregistrată de cele 15 soiuri

CONCLUSIONS

1. The experiment was part of the national testing plant of varieties and lines aimed at adapting and identifying the most productive varieties in the reference area. No treatments were applied against foliar diseases during these tests. The experiment was conducted on a typical

preluvosol, which has a humus content of 2.82% and an acidic reaction with a pH ranging from 5.59 to 6.65.

2. Out of the 15 varieties tested, 8 recorded an attack level of 76%. The control variety (Plai) along with Negoiu recorded an attack level of 54%. The most resistant variety proved to be Zaraza, with an attack level of only 10%.
3. Of the 15 varieties, 10 recorded a thousand-grain weight (TGW) of less than 30 g. The control variety Plai recorded 34.4 g. Zaraza, the most resistant variety to this pathogen attack, recorded 46.8 g.
4. The hectoliter weight values ranged from 41 to 70 kg/hl. The lowest value was recorded by Cascador, with 41.4 kg/hl, while 13 of the 15 varieties recorded values below 50 kg/hl. The highest value was recorded by Zaraza at 70 kg/hl.
5. The results confirm that recent climate changes, the increase in average monthly temperatures, and the unevenly distributed high levels of precipitation are predominant factors in the installation of the yellow rust pathogen and its the manifestation. This study highlights that the Zaraza variety exhibits high resistance to this pathogen attack, but this could be confirmed in future studies under different climatic conditions.

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SUNFLOWER PRODUCTION IN RELATION TO MORPHOLOGICAL PARAMETERS AND PLANT DENSITY

PRODUCȚIA DE FLOARE-SOARELUI ÎN RAPORT CU PARAMETRII MORFOLOGICI ȘI DENSITATEA PLANTELOR

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Abstract

*This study analyzed the variation of several morphological parameters and yield in sunflower hybrids grown at three planting densities under non-irrigated conditions. The field experiment was conducted at ARDS Lovrin during the 2023–2024 agricultural year. The sunflower hybrids - ES Celion SU (G1), LID 1046H SU (G2), and LID 5053L SU (G3) were cultivated at planting densities of 40,000 (A), 60,000 (B), and 80,000 (C) plants/ha. Morphological parameters, including plant height (PH), stem diameter (SD), and calatidium diameter (CD), along with yield (P), were measured. Polynomial models were used to describe the variation in yield in relation to morphological traits and planting density. Among the morphological parameters, SD and PH showed the highest accuracy in yield estimation (RMSE = 193.2755). When plant density (PD) was included, the combination of PD and SD provided even greater precision (RMSE = 94.0254). Compared to the average yield ($\overline{P}$), certain hybrid × density combinations showed statistically significant yield differences, either positive (e.g., G1C**, $p<0.01$; G2C*, $p<0.05$) or negative ($G2A^{OOO}$, $p<0.001$; $G3A^{OO}$, $p<0.01$). Principal component analysis (PCA) explained the total variance and illustrated the relationship between biometric parameters, yield, and experimental variants. Cluster analysis grouped variants based on their similarity in achieved yield (Cophenetic corr. = 0.875). These results support the selection of optimal sowing strategies for tested hybrids under non-irrigated conditions to achieve maximum yield.*

Abstract

*Studiul a analizat variația unor parametri morfologici și a producției la hibrizi de floarea-soarelui cultivați la trei densități de semănat, în condiții de neirigare. Experimentul de câmp a fost realizat la SCDA Lovrin, în anul agricol 2023–2024. Hibrizii de floarea-soarelui — ES Celion SU (G1), LID 1046H SU (G2) și LID 5053L SU (G3) — au fost cultivați la densități de plantare de 40.000 (A), 60.000 (B) și 80.000 (C) plante/ha. S-au determinat parametrii morfologici, inclusiv înălțimea plantei (PH), diametrul tulpinii (SD) și diametrul calatidiului (CD), precum și producția (P). Modelele polinomiale au fost utilizate pentru a descrie variația producției în funcție de caracterele morfologice și densitatea plantelor. Dintre parametrii morfologici, SD și PH au oferit cea mai mare precizie în estimarea producției (RMSE = 193.2755). Atunci când a fost inclusă și densitatea plantelor (PD), combinația PD și SD a furnizat o precizie și mai mare (RMSE = 94.0254). Comparativ cu producția medie ($\overline{P}$), anumite combinații hibrid × densitate au prezentat diferențe semnificative statistic, fie pozitive (de exemplu, G1C**, $p<0.01$; G2C*, $p<0.05$), fie negative ($G2A^{OOO}$, $p<0.001$; $G3A^{OO}$, $p<0.01$). Analiza componentelor principale (PCA) a explicat variația totală și a ilustrat relația dintre parametrii biometrici, producție și variantele experimentale. Analiza cluster a grupat variantele în funcție de similitudinea valorilor producției obținute (Coeficientul cofenetic = 0.875). Rezultatele susțin alegerea strategiilor optime de semănat pentru hibrizii testați, în sistem neirigat, pentru a obține producții maxime.*

Keywords*: plant density, sunflower, models, morphological parameters, production*
Cuvinte cheie*: densitate plante, floarea soarelui, modele, parametri morfologici, producție*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is a crop of major global importance due to the nutritional value of its seeds and, particularly, as a source of oil (Adeleke et al., 2020; Puttha et al., 2023). Both physiological and agronomic aspects have been studied in relation to sunflower genetics as well as crop management practices (Alberio et al., 2015; Giannini et al., 2022; Lamichhane et al., 2022).
Ecological plasticity in sunflower has been analyzed to understand the response of genotypes to climate and soil conditions, to support hybrid zoning, and to adapt agricultural technologies for achieving high yields (Sadras et al., 2009; Saucă et al., 2018; Debaeke et al., 2021; Bustos-Korts et al., 2022; Aboye and Edo, 2024).

Sunflower yield has been evaluated in relation to cultivated genotypes, environmental factors, and technological inputs (Li et al., 2019; Modanlo et al., 2021; Nagy et al., 2024; Li and Liu, 2025). In the context of climate change, various aspects of sunflower cultivation have been analyzed with regard to their impact on seed yield and quality (Gurkan et al., 2021; Beteri et al., 2024).
Plant density is a key factor influencing sunflower yield and has recently been the focus of intensive study, particularly in the context of technological optimization (Li et al., 2019; Nagy et al., 2024; Olson et al., 2024; Tomasi et al., 2024).

The present study analyzed agronomic parameters and yield variation in three sunflower hybrids under different planting densities. It explored the correlations between yield and agronomic traits and identified yield variation patterns in relation to both planting density and plant morphology.

MATERIAL AND METHODS

The study was conducted in ARDS Lovrin, Timis County, Romania. Field experiments, in comparative culture, were carried out in the agricultural year 2023 - 2024. The values of the climatic conditions are presented in figure 1.

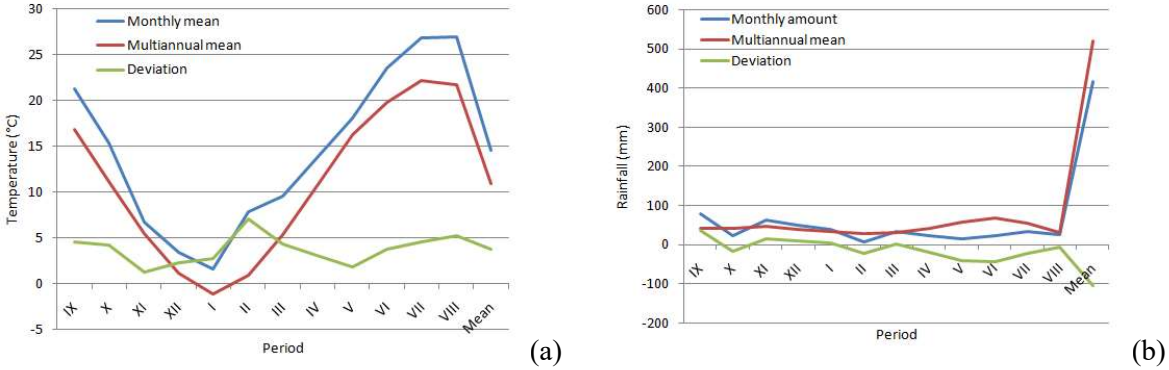


Figure 1. Climatic conditions during the study period; (a) – temperatue, (b) – rainfall *// Condițiile climatice în perioada de studiu; (a) – temperatura, (b) – precipitatii*

The comparative sunflower trial was conducted under non-irrigated conditions. Appropriate crop technology for sunflower was applied throughout the experiment. The hybrids ES Celion SU (G1 – experimental code), LID 1046H SU (G2), and LID 5053L SU (G3) were cultivated. Each hybrid was grown at three planting densities: 40,000 plants ha^{-1} (A – experimental code), 60,000 plants ha^{-1} (B), and 80,000 plants ha^{-1} (C). The resulting experimental variants were: G1A, G1B, G1C; G2A, G2B, G2C; and G3A, G3B, G3C. Each variant was replicated three times. Agronomic parameters were measured for each variant, including stem diameter (SD, cm), plant height (PH, cm), and calatidium diameter (CD, cm). All experimental variants were harvested mechanically at physiological maturity (Meier, 2001). Yield (Y, kg ha^{-1}) was determined for each variant and replicate. Statistical analysis was conducted using the Excel analysis tools and the PAST software package (Hammer et al., 2001).

RESULTS AND DISCUSSIONS

The values of the agronomic parameters and yield of the sunflower hybrids cultivated at three planting densities are presented in Table 1. The stem diameter (SD) ranged from 7.02 ± 0.32 cm (G3C) to 10.38 ± 0.32 cm (G3A). Plant height (PH) ranged from 116.40 ± 2.72 cm (G3A) to 140.60 ± 2.72 cm (G1C). Calatidium diameter (CD) varied between 13.32 ± 0.77 cm (G3C) and 20.85 ± 0.77 cm (G2A, G2B). Yield (Y) ranged from 1354.29 ± 116.00 kg ha^{-1} (G2A) to 2371.43 ± 116.00 kg ha^{-1} (G1C).
The experimental data demonstrated statistical reliability, and the presence of variance in the recorded values was confirmed (Table 2). The level of correlation between agronomic parameters,

yield, and plant density is illustrated in Figure 2.

Table 1. Values of agronomic parameters and yield of sunflower hybrids// V*alori ale parametrilor agronomici şi randamentului la hibrizii de floarea soarelui*

Sunflower hybrid	Trial	PD	Plant parameters			Y
			SD	PH	CD	
		(no)	(cm)	(cm)	(cm)	(kg ha^{-1})
ES Celion SU	G1A	40000	8.74	133.60	17.00	1954.29
ES Celion SU	G1B	60000	8.98	129.60	16.75	2160.00
ES Celion SU	G1C	80000	8.84	140.60	17.10	2371.43
LID 1046H SU	G2A	40000	9.72	131.20	20.85	1354.29
LID 1046H SU	G2B	60000	9.74	132.20	20.85	2051.43
LID 1046H SU	G2C	80000	8.60	130.20	18.60	2262.86
LID 5053L SU	G3A	40000	10.38	116.40	17.90	1508.57
LID 5053L SU	G3B	60000	9.30	117.20	16.65	1851.43
LID 5053L SU	G3C	80000	7.02	137.20	13.32	2240.00

Table 2. ANOVA Test results// *Rezultatele Testului ANOVA*

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	2.55E+10	4	6.37E+09	106.1534	9.65E-21	2.6060
Within Groups	2.4E+09	40	60024235			
Total	2.79E+10	44				

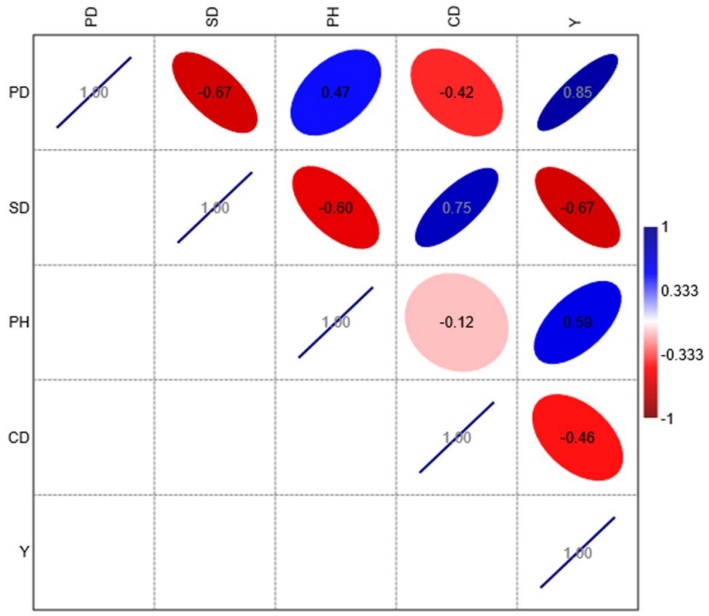


Figura 2. The level of correlation between agronomic parameters, yield and plant density in sunflower // *Nivelul de corelaţie între parametri agronomici, randament şi densitatea plantelor la floarea soarelui*

The yield for each experimental variant was analyzed compared to the average yield value at the experiment level. The differences recorded, and the values of the statistical parameters are presented in Table 3, with a graphical representation in Figure 3.

Table 3. Differences in sunflower yield compared to the average value of the experiment// *Diferentele randamentului la floarea soarelui in*

raport cu valoarea medie a experimentului

Trial code	Yield (kg ha^{-1})		Statistical parameters					
	Given mean:	Sample mean:	95% conf. interval:	Difference:	95% conf. interval:	t:	p (same mean):	Significance of differences
G1A	1954.29	1972.7	(1705.2 2240.2)	-18.41	(-249.08 285.9)	0.1587	0.878	ns
G1B	2160.00			187.30	(-80.189 454.79)	-1.6147	0.145	ns
G1C	2371.43			398.73	(131.24 666.22)	-3.4374	0.009	**
G2A	1354.29			-618.41	(350.92 885.9)	5.3312	<0.001	ooo
G2B	2051.43			78.73	(-188.76 346.22)	-0.6787	0.516	ns
G2C	2262.86			290.16	(22.671 557.65)	-2.5014	0.037	*
G3A	1508.57			-464.13	(196.64 731.62)	4.0012	0.004	oo
G3B	1851.43			-121.27	(-146.22 388.76)	1.0454	0.326	ns
G3C	2240.00			267.30	(-0.18924 534.79)	-2.3043	0.050	ns

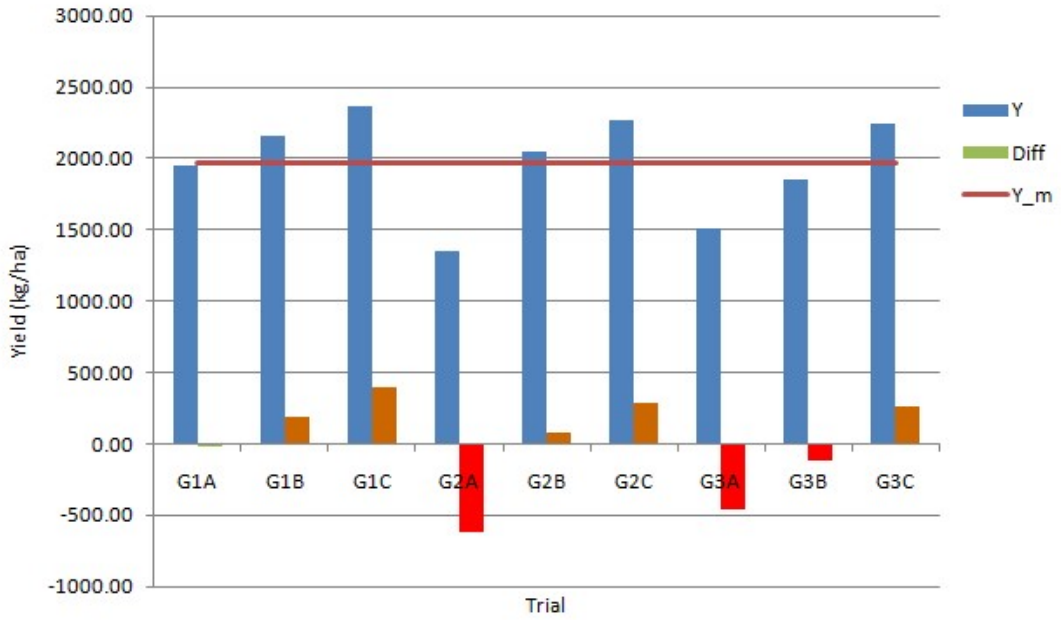


Figura 3. Graphical representation of yield and differences from the experiment average in sunflower hybrids in relation to plant density // *Reprezentarea grafică a randamentului și a diferențelor față de media experimentului la hibrizii de floarea soarelui în raport cu densitatea plantelor*

Under the planting density of 40,000 plants ha^{-1} (experimental code A), all three tested hybrids produced yields below the experimental average (Table 3, Figure 3). At a density of 60,000 plants ha^{-1} (experimental code B), an improvement in yield was recorded. At a density of 80,000 plants ha^{-1} (experimental code C), all variants recorded yields above the experimental average, with statistically significant differences observed for G1C and G2C.

Multivariate analysis explained 84.343% of the total variance based on the first two principal components (Figure 4). A correlation between yield and plant density (Y, PD) was observed, along with the associated positioning of variants G1C, G2C, and G3C in relation to these two parameters, as shown in the biplot.

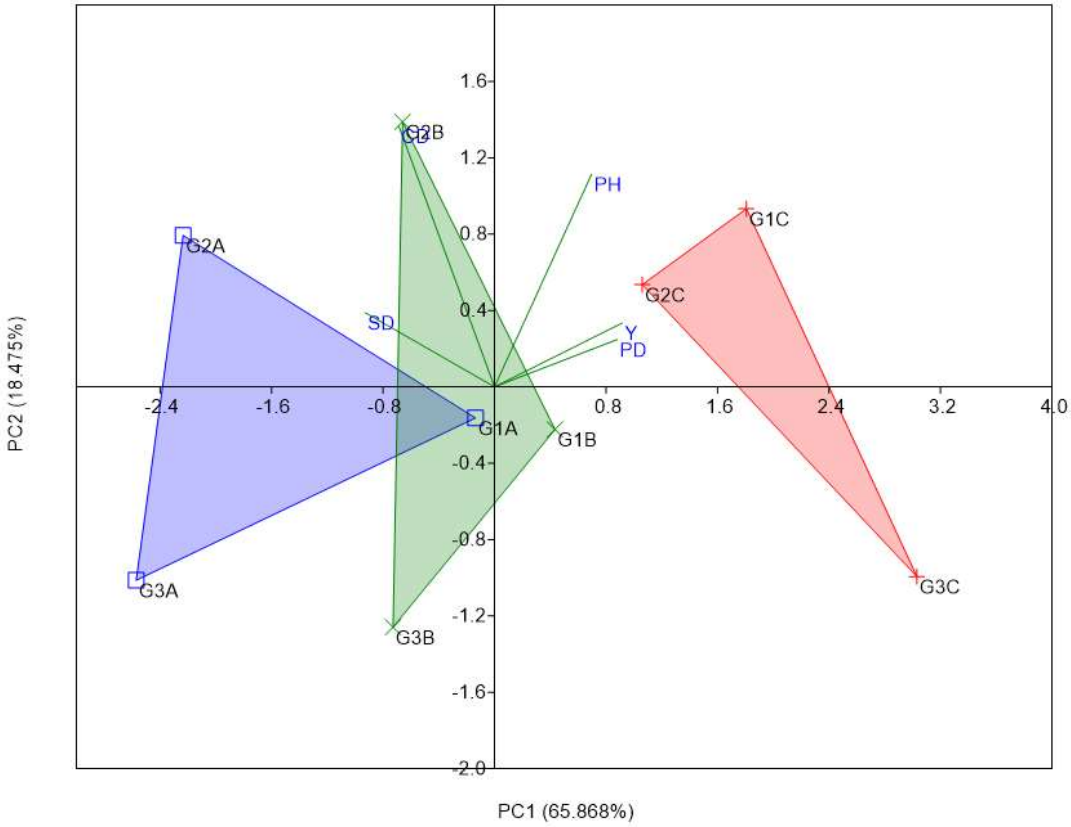


Figure 4. PCA diagram with the distribution of variants in sunflower (blue color – density 40000 plt ha^{-1}; green color – density 60000 plt ha^{-1}; red color – density 80000 plt ha^{-1}) // *Diagrama PCA cu distribuția variantelor la floarea soarelui (culoare albastra – densitate 40000 plt ha^{-1}; culoare verde – densitate 60000 plt ha^{-1}; culoare roșie – densitate 80000 plt ha^{-1})*

Cluster analysis resulted in the dendrogram shown in Figure 5, where the experimental variants were grouped based on similarity (Cophenetic correlation = 0.875). Three distinct subclusters were identified. A high degree of similarity was observed between variants G2C and G3C (SDI = 24.54). The SDI values for all experimental variants are presented in Table 4.

The variation in sunflower yield was further analyzed in relation to plant morphological parameters, using different combinations. Regression analysis produced a general equation, presented as Equation (1), with coefficient values varying depending on the parameters considered (Table 5). Table 5 also includes the statistical significance values for each combination of parameters used in yield estimation.

The graphical representation of yield (Y, kg ha^{-1}) in relation to plant morphological parameters and planting density (*x, y* variables) is shown in Figures 6 to 10: (a) 3D model; (b) isoquants model.

$$Y = a\,x^2 + b\,y^2 + c\,x + d\,y + e\,xy + f \qquad (1)$$

where: a, b, c, d, e, f – parameters of equation (1), with values in table 5

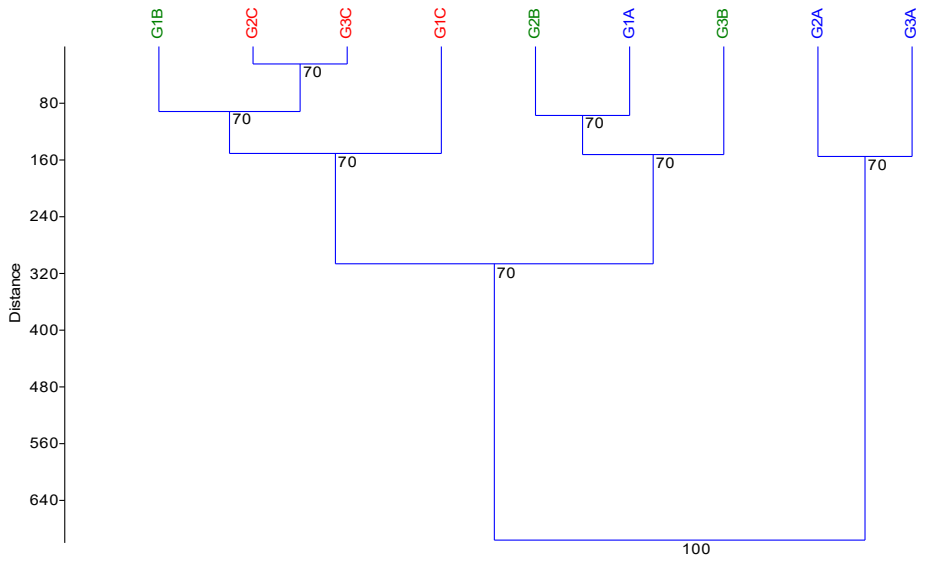


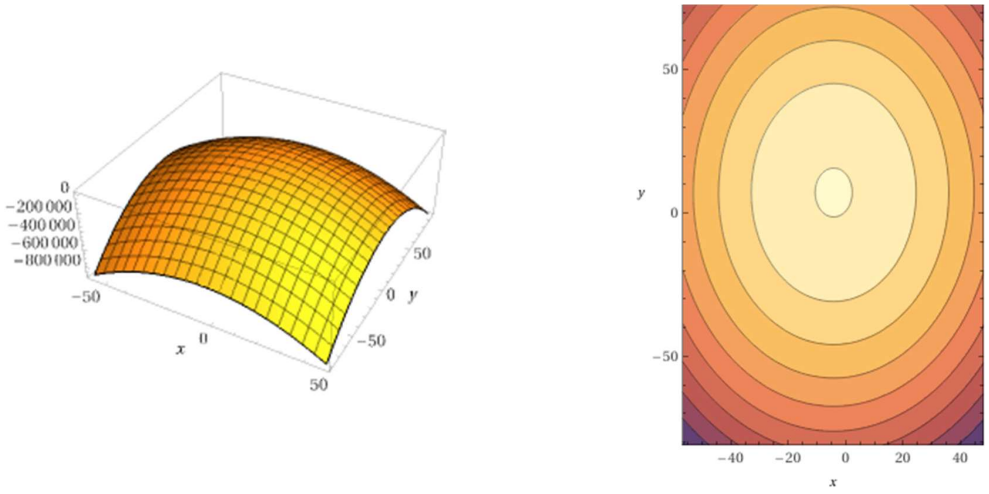
Figure 5. Cluster diagram of experimental variants in sunflower, based on Euclidean distances// *Diagrama cluster a variantelor experimentale la floarea soarelui, pe baza distantelor Euclidiene*

Table 4. SDI values for experimental variants in sunflower// *Valorile SDI pentru variantele experimentale la floarea soarelui*

	G1A	G2A	G3A	G1B	G2B	G3B	G1C	G2C	G3C
G1A		600.02	446.06	205.75	97.23	104.16	417.20	308.59	285.76
G2A	600.02		155.02	805.72	697.14	497.35	1017.20	908.57	885.77
G3A	446.06	155.02		651.57	543.10	342.86	863.20	754.42	731.75
G1B	205.75	805.72	651.57		108.68	308.82	211.72	102.88	80.46
G2B	97.23	697.14	543.10	108.68		200.61	320.13	211.45	188.81
G3B	104.16	497.35	342.86	308.82	200.61		520.53	411.64	389.11
G1C	417.20	1017.20	863.20	211.72	320.13	520.53		109.08	131.54
G2C	308.59	908.57	754.42	102.88	211.45	411.64	109.08		24.54
G3C	285.76	885.77	731.75	80.46	188.81	389.11	131.54	24.54	

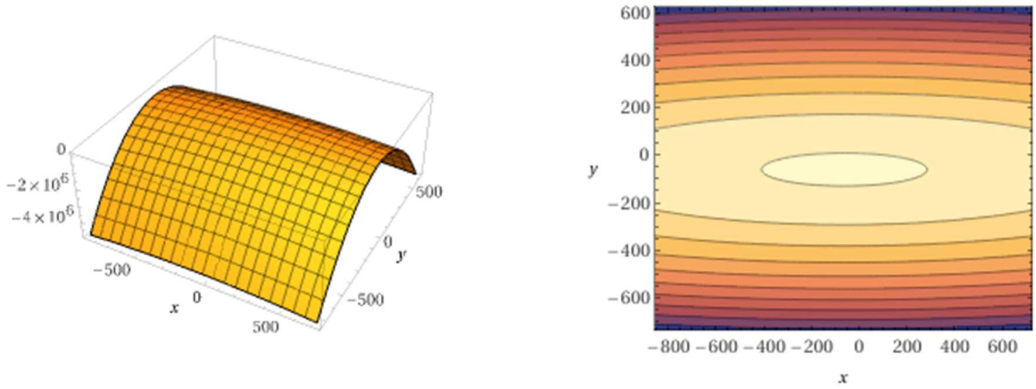
Table 5. The values of the coefficients and statistical parameters of equation (1) // *Valorile coeficienților si a parametrilor statistici ai ecuației (1)*

Model number	Variables of equation (1)	Coefficients of equation (1) values						Statistical parameters		
		a	b	c	d	e	f	R^2	F	p
M1	x = SD, y = PH	-171.7249549	0.7257931	4437.3845253	-60.7351286	-12.5793965	-13649.9799882	0.653	4.5159	0.0151
M2	x = SD, y = CD	-127.6688472	-72.7780473	-1115.1080848	1071.8852059	173.2190554	-1206.9146649	0.633	4.1409	0.0203
M3	x = PH, y = CD	-0.4071720	-10.1069213	-53.6670659	-1147.9488432	10.7657830	14640.6698529	0.629	4.0675	0.0215
M4	x = PD, y = SD	3.11728E-07	230.2261192	-0.2718578	-5832.9303904	0.0279159	35939.4467831	0.918	26.8222	0.000001
M5	x = PD, y = PH	-3.3865E-07	0.4222055	0.0834655	-80.9544904	-0.0002208	3376.3355903	0.811	10.2958	0.00051
M6	x = PD, y = CD	5.90441E-08	18.6883058	-0.1057039	-1087.8853163	0.0064065	14689.4310102	0.872	16.3886	0.000001



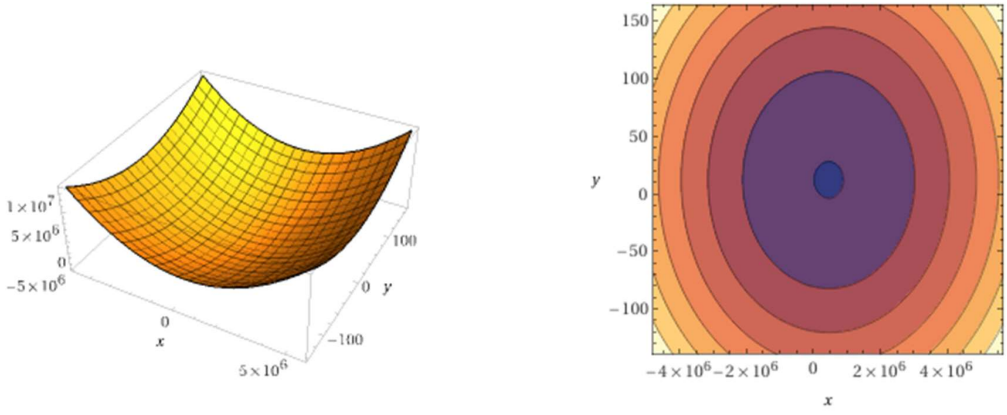
(a) (b)

Figure 6. Yield variation in relation to SD (*x*-axis), and CD (*y*-axis) parameters // *Variatia randamentului in relatie cu parametri SD (axa x), si CD (axa y)*



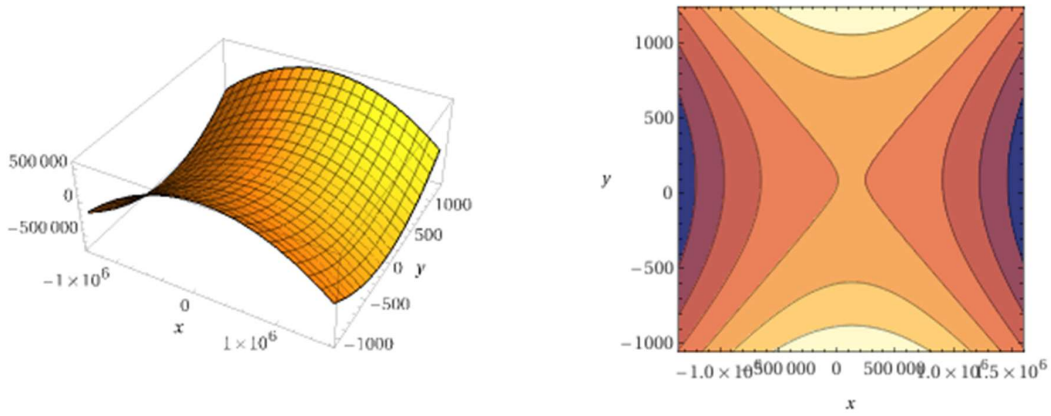
(a) (b)

Figure 7. Yield variation in relation to PH (*x*-axis), and CD (*y*-axis) parameters // *Variatia randamentului în relație cu parametri PH (axa x), si CD (axa y)*



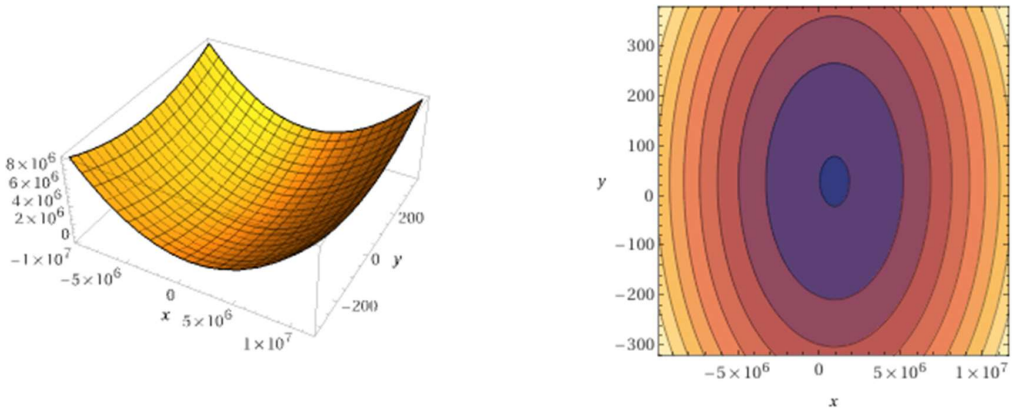
(a) (b)

Figure 8. Yield variation in relation to PD (*x*-axis), and SD (*y*-axis) parameters// *Variația randamentului in relatie cu parametri PD (axa x), si SD (axa y)*



(a) (b)

Figure 9. Yield variation in relation to PD (*x*-axis), and PH (*y*-axis) parameters// *Variația randamentului în relatie cu parametri PD (axa x), si PH (axa y)*



(a) (b)

Figure 10. Yield variation in relation to PD (*x*-axis), and CD (*y*-axis) parameters // *Figura 10. Variația randamentului în relatie cu parametri PD (axa x), si CD (axa y)*

The RMSEP parameter was calculated for each yield prediction model. The RMSEP parameter values are graphically presented in Figure 11.

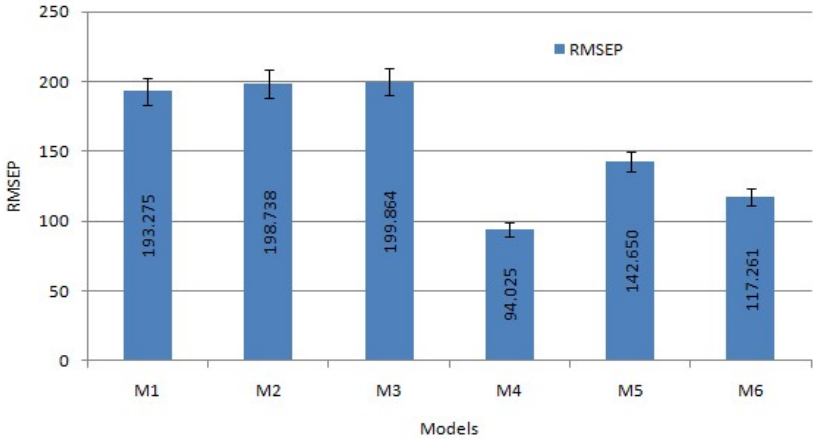


Figure 11. Graphical representation of RMSEP values, associated with yield estimation models// *Reprezentarea grafica a valorilor RMSEP, associate modelelor de estimare a randamentului*

Sunflower cultivation has been analyzed in relation to various soil and plant interventions, incorporating different inputs and cropping systems, to improve agricultural technologies in line with the requirements of cultivated genotypes (Pîrvulescu et al., 2015; Hamzei and Seyyedi, 2016;

Khalifani et al., 2023; Centorame et al., 2024).

Interest in modeling agricultural crops has been reflected in numerous studies aimed at describing responses to vegetation and technological factors, nutritional status, biomass production, and more (Constantinescu et al., 2018; Herbei et al., 2023; Centorame et al., 2024).

In relation to plant density, this study recorded varying values for agronomic parameters and yield in the sunflower hybrids analyzed.

Yield estimation models based on different combinations of agronomic parameters and plant density revealed the distinct contributions of these variables within the models. A convergent contribution was observed in most models, though with varying levels of precision. However, when plant density (PD) and plant height (PH) were used as variables, a divergent behavior between the two was recorded (Table 5, Figure 9).

The results indicate that some elements used as variables in the yield optimization process can be approached uniformly, while others require a differentiated approach.

CONCLUSIONS

6. Differential response of sunflower hybrids under experimental conditions was recorded, based on agronomic parameters and yield.
7. Plant density influenced the yield of the tested sunflower hybrids. The density of 40,000 plants ha^{-1} led to yields below the average of the experiment, except for the G1A variant which presented close values. The density of 60,000 plants ha^{-1} led to yields above the average for the G1B and G2B variants (without statistical certainty). The density of 80,000 plants ha^{-1} led to yields above the average for all three tested hybrids, with statistical certainty for the G1C ($p<0.01$, **) and G2C variants ($p<0.05$, *).
8. PCA analysis showed the correlation of yield with plant density, and the G1C and G2C hybrids were positioned in association.
9. Mathematical and graphical models described the variation of yield in relation to agronomic parameters and plant density. Plant density (PD) and stem diameter (SD) facilitated the estimation of yield with the highest level of certainty (RMSEP = 94.025 kg ha^{-1}).
10. The analysis method and the obtained models can be extended to other sunflower hybrids, and can also be adapted for other agronomic, physiological parameters, or technology elements, as variables that influence sunflower crop yield.

ACKNOWLEDGMENTS

The authors thank the ARDS Lovrin for facilitating this study.

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THE INFLUENCE OF CLIMATE CHANGES ON MAIZE GRAIN YIELD IN CENTRAL TRANSILVANIA IN EARLY SOWING CONDITIONS

INFLUENȚA SCHIMBĂRILOR CLIMATICE ASUPRA PRODUCȚIEI DE BOABE LA PORUMB ÎN CENTRUL TRANSILVANIEI ÎN CONDIȚIILE SEMĂNATULUI TIMPURIU

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ABSTRACT

The main climatic factors that have changed in recent years have had a negative influence primarily on maize production.

Scientists in the field are making efforts to obtain maize biotypes capable of overcoming these changes, but until these results are obtained, intervention in crop technology remains a possibility.

In solving this problem for the time being, we have developed earlier sowing without waiting for the minimum germination threshold to be achieved in the soil. Finding hybrids with resistance to temperatures lower than 10 °C at germination has been a constant concern.

In this study, we present the evolution of the average daily temperature and the amount of precipitation compared to the multiannual average and the maize production achieved at R.D.S.C.B. Târgu Mureș.

REZUMAT

Principali factori climatici care au cunoscut modificări în ultimii ani, au avut o influență negativă privind în primul rând producția la cultura porumbului.

Oamenii de știință din domeniu depun eforturi în obținerea de biotipuri de porumb capabile să treacă peste aceste schimbări, dar până la obținerea acestor rezultate intervenția în tehnologia de cultură rămâne o posibilitate.

În rezolvarea pe moment a acestui deziderat am elaborat semănatul mai timpuriu fără a aștepta realizarea în sol a pragului minim de germinare. Găsirea unor hibrizi cu rezistență la temperaturi mai mici de 10 °C la încolțire a fost o preocupare constantă.

În acest studiu prezentăm evoluția temperaturii medii zilnice și cantitatea de precipitații în comparație cu media mutianuală și producția de porumb boabe realizată la SCDCB Târgu Mureș.

Keywords: *maize, climate conditions, grain production, early sowing*
Cuvinte cheie: *porumb, condiții climatice, producția de boabe, semănat timpuriu*

INTRODUCTION

Maize (*Zea mays L.*) is an important food for world population and as such emphasis is placed on achieving the higest yield, but this also depends on the agricultural tehnologies which are applied (Yusuf D.D., 2006). In Romania maize is the most extensive crop, with a use in human and animal nutrition, that is why it is necessary to consider the achievement of a balance between the production capacity in the choice of corn hybrids and grain quality indicators (Scott et all., 2006).

Climat changes has become the bigest global challenge, causing problems in crop development and yield, leadind to threats the food security for the growing population (Bennetzen et al., 2016).

Global warming is recognized as an extremely worrying environmental problem by both the scientific community and the world population in general. A total increase in temperature of 3.2 °C is expected by the end of the current century. Also, the increase in CO2 concentration in the atmosphere is in itself a worrying factor if we think that sustainable agricultural production and food security are one of the major challenges both at present and in the future. Assessing the effects of climate change on agriculture is a major concern especially in countries where extreme climatic conditions (tempera-ture, precipitation) are registered (Harison P. A., at all, 1996; Tatar Ö. 2016).

Drought is a particularly significant phenomenon which limits plant production in all countries of the world. The negative effects of the drought depend, in particular, on its severity and the period of its occurrence, which causes a state of stress that influences production. The state of stress to which plants are subjected may vary from year to year depending on the amount of rainfall and the temperature of the air (Soltani A. At all, 2001).

According to predictions, global cereal demand for water will increase. And this is because climate change is expected to increase global temperature by 4-6 °C (Casaretto J.A, at all, 2016). There is currently extensive research sugesting alternatives to secure water supplies for soil and plants such as corn, a plant that plays a crucial role in human and animal nutrition (İştipliler D. et all 2016). Total world corn production was, according to FAO data, in 2014, for example, of about 1 billion tons and for this reason, the addition of irrigated water supply and the cultivation of drought-tolerant corn hybrids are among the most appropriate solutions (İştipliler D., et all 2016).

Temperature influences growth processes through cardinal points, minimum, optimum, maximum, which are differentiated according to the species and the stage of development of the plant (Şumălan, 2000). Regarding growth, it generally follows the curve of biochemical processes (Gâdea, 2013).

The period that a plant needs to complete a certain stage of growth is directly related to the temperatures and especially to the sums of the daily temperatures in that stage, but also those before. Research conducted on temperatures and stages of development of corn has shown that corn can survive between 0ºC and 43ºC, with slow growth below 10ºC and above 30ºC (Lehenbauer, 1914).

Domokos et al. (2022) observed that early sowing did not reduce the percentage of plants that emerged compared to the optimal sowing date, but influenced the emergence dynamics, as the number of days with suboptimal temperatures increased.

Corn growth takes place under good conditions when average temperatures do not fall below 13°C in May and below 18°C in July and August (Vâtcă et al., 2021). The highest growth rate is recorded at temperatures between 24 and 30°C. In the flowering phase, in June-August, the optimal temperature is between 18 and 23°C (Rogojanu and Chisel, 1952).

Maize has good drought resistance, especially in the first part of the vegetation period, due to the low transpiration coefficient (between 246 and 589), the strongly developed root system and the possibility of reducing the transpiration surface by twisting the leaves in case of drought, it is considered a drought-resistant plant, but it responds differently to water deficit depending on the stages of development (Cakir, 2004).

The critical period for water is between 10-20 June and 10-20 August, respectively before the appearance of panicles and until milk maturity. During this period, the soil should have 60-80% water of the field capacity. In a study by Udomprasert et al., (2005), the anthesis-silk interval increased by 2-3 days when moisture stress occurred in the early stages of silking, and the anthesis-silk interval increased by 0.75-1.25 days when water was deficient during the anthesis period. In the grain filling phase, the lack of moisture causes the grain to shrink.

For our country, the optimal distribution of precipitation during the vegetation period is as follows: 60-80 mm in May, 100-120 mm in June, 100-120 mm in July and 20-60 mm in August (Bâlteanu, 1998). Corn finds the best vegetation conditions when annual precipitation is over 500 mm, precipitation between January 1 and August 31 is over 350 mm or precipitation between May 1 and August 31 is over 250 mm (Bâlteanu, 1998).

MATERIALS AND METHODS

A ten-year experiment (2015-2024) was conducted at the Research and Development Station for Cattle Breeding Tg. Mures (R.D.S.C.B. Tg. Mures) at Sangeorgiu de Mures, Romania (46°33ʾ N, 24°33ʾ E).

The soil on which the experiment was located, was a brown forest soil, weakly podzolic, clay loam texture (representative of most of the Mures county area), with a humus content of 1.9, pH of

5.8, P_2O_2 supply is 17.4 mg/100g soil, K_2O 21.7 mg/100 g soil and an N index of 1.4. The predecessor plant was soybean.

The sowing rate was 65,000 plants/ha, with a distance of 70 cm between rows, and the plot size of each experimental plot was of 2,8m x 7m. Sowing date was an early sowing (ES), conducted when the soil temperature reached 6°C Fieldwork was done by harrowing in August, deep plowing in the autumn, leveling discs in the spring, and a work with the cultivator to prepare the germinative bed. The application of the whole dose of fertilizers was done after the disk, and their incorporation into the soil was done with the combinator. The amount and type of fertilizer used were $N_{16}P_{16}K_{16}$ 500 kg/ha, and every three years 4 t amendments per ha were applied.

The results of the studies were analyzed according to the climatic conditions of the area, recorded at the weather station in Sângeorgiu de Mureș and presented in Table 1.

Table 1. Mean monthly (T_m) air temperatures and total monthly rainfall at Sangeorgiu de Mures, during the experiment (from 2015 to 2024). Long-term (60 years) mean annual temperature and rainfall values at Sangeorgiu de Mures// Temperaturile medii lunare (Tm) ale aerului și precipitațiile totale lunare la Sângeorgiu de Mureș, în timpul experimentului (2015 - 2024). Temperatura medie anuală și valorile precipitațiilor pe termen lung (60 de ani) la Sângeorgiu de Mureș

														Avg.
	10 Years	**X**	**XI**	**XII**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**sum**
mm	**2014-2015**	23,0	36,0	30,0	24,0	20,0	20,0	23,0	0,0	132,0	27,0	90,0	73,0	**498,0**
	2015-2016	46,0	47,8	19,8	25,6	35,6	46,7	75,6	89,9	127,9	59,0	59,6	15,3	**648,8**
	2016-2017	77,3	90,8	19,3	9,1	32,1	26,0	51,0	111,5	51,8	50,0	26,5	53,0	**598,4**
	2017-2018	60,7	52,6	43,2	32,5	41,2	64,9	12,7	53,8	187,2	58,5	8,7	58,8	**674,8**
	2018-2019	32,2	30,5	60,1	36,2	27,7	16,6	30,3	134,5	49,8	59,9	78,6	50,1	**606,5**
	2019-2020	31,6	28,2	41,7	7,9	57,8	36,3	20,1	61,4	120,9	99,0	40,9	62,2	**608,0**
	2020-2021	74,2	34,9	50,6	9,0	37,2	66,4	93,9	95,0	70,4	98,2	77,5	59,7	**767,0**
	2021-2022	12,5	34,0	87,7	27,4	11,2	3,6	118,9	49,0	9,2	56,3	100,5	176,4	**686,7**
	2022-2023	45,7	71,5	47,5	28,2	55,8	31,6	74,0	39,0	151,5	84,8	67,0	19,0	**715,6**
	2023-2024	28,0	112,0	29,5	22,0	40,0	50,0	66,0	61,0	91,0	59,0	35,0	77,0	**670,5**
	Avg. rain fall	**43,1**	**53,8**	**42,9**	**22,2**	**35,9**	**36,2**	**56,6**	**69,5**	**99,2**	**65,2**	**58,4**	**64,5**	**647**
	10 Years	**X**	**XI**	**XII**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**Avg.**
t °C	**2014-2015**	10,8	4,8	2,4	2,4	2,1	5,0	9,2	15,8	18,9	21,7	21,5	17,3	**10,98**
	2015-2016	9,9	5,7	1,9	-2,0	5,3	6,4	13,1	14,5	20,8	21,4	20,4	17,2	**11,21**
	2016-2017	8,8	3,3	-3,1	-6,6	1,9	8,3	10,2	16,7	20,5	21,1	24,0	16,2	**10,12**
	2017-2018	10,1	5,4	1,7	0,9	0,2	4,3	15,6	18,7	20,1	20,7	22,8	16,2	**11,40**
	2018-2019	12,1	5,7	-0,1	-1,5	1,7	6,9	12,3	15,3	22,1	20,7	21,8	17,0	**11,17**
	2019-2020	11,6	10,0	1,2	-2,5	2,9	6,6	10,3	14,3	19,7	21,0	22,0	18,3	**11,28**
	2020-2021	12,5	4,4	4,3	2,6	2,2	2,7	8,6	14,9	20,0	23,8	20,6	17,0	**11,13**
	2021-2022	8,7	4,9	2,0	-4,2	2,6	3,7	9,3	16,6	21,7	23,0	22,5	14,8	**10,47**
	2022-2023	12,1	6,2	2,5	3,9	1,4	6,4	9,6	16,1	19,1	19,1	22,5	19,5	**11,53**
	2023-2024	13,3	5,8	2,4	0,7	7,3	9,0	13,4	16,1	22,1	24,0	22,6	18,2	**12,90**
	Avg. temp.	**11,0**	**5,6**	**1,5**	**-0,6**	**2,8**	**5,9**	**11,2**	**15,9**	**20,5**	**21,7**	**22,1**	**17,2**	**11,2**

The analysis of the two climatic factors over the entire agricultural year revealed a yearly variation in precipitation distribution, with an increasing trend observed in recent years within the station's area of influence.

However, in terms of thermal input, there has been a clear rise in the annual average temperature from 2014–2015 to 2023–2024, with an increase of 1.92 °C over ten years (from 10.9 °C to 12.90 °C).

Factor 1 is represented by years and factor 2 by the 6 tested maize hybrids. Six hybrids from the company KWS Semences, KWS 2370 (FAO 290), Karpatis (FAO 340), Kashmir (FAO 370), Durango (FAO 450), Smaragd (FAO 350), Kapitolis (FAO 410).



Fig. 1 Aspects from the experimental field // Aspecte din câmpul experimental

RESULTS AND DISCUSSIONS

Climate change, increasingly pronounced in recent years, has substantially affected the production capacity of cultivated plants. The crop that has suffered the most was corn. In this paper, we attempted to identify the degree of influence of the main climatic factors, temperature and precipitation on grain production, in our region, Central Transylvania.

From the statistical analysis, it results that the greatest share of influence on production over 80% was determined by the effect of the year. The hybrid accounted for 3.1%, and the interaction between year and hybrid accounted for 5.68%.

Table 2. Weigh of factors// Ponderea factorilor

Variation source	*SPA*	*DF*	*S^2*		*F*	*Weight of factors*
A	97071330,00	9	107857000,00		47,904	85,4
H	34892650,00	5	6978531,00		33,951	3,1
AH	64603970,00	45	1435644,00		6,985	5,68
R	5433570,00	2	2716785,00			0,47
AR	40527360,00	18	2251520,00			3,56
HR	1506894,00	10	150689,40			0,13
AHR	19047640,00	90	211640,50			1,67
Eroarea A	40527360,00	18	2251520,00			
Eroarea H	20554540,00	100	205545,40			
Total	1136726000,00					100

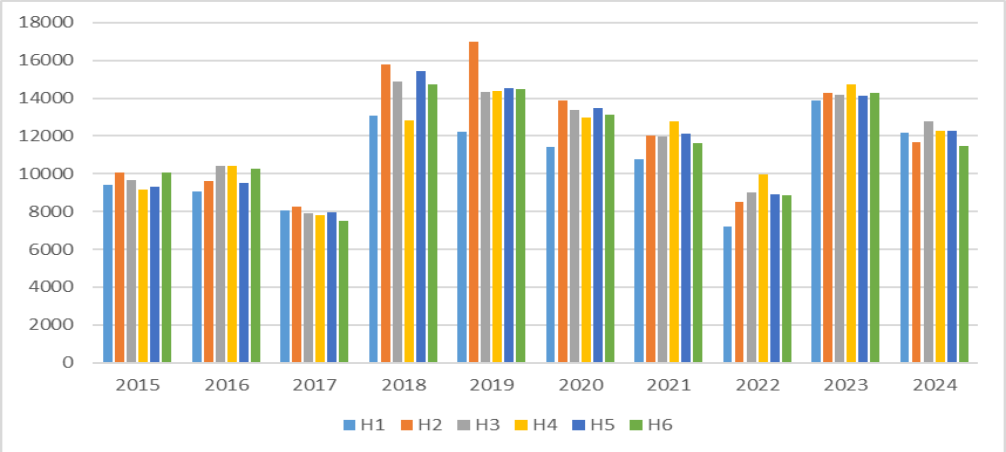
An analysis of the study years highlights that in 2015-2024 time period, 2017, and 2022 had a very significant negative impact on production, while 2016 had a significant negative impact. The years 2018, 2019, and 2023 had a very significant positive influence, and the year 2020 had a significant positive influence. From the analysis of production achieved by hybrids compared to the control, a genetic progress of 10.2% is observed. The results for the hybrids are very significantly positive compared to the control.

Table 3. The influence of the year and maize hybrids on grain yield//Influența anului și hibridului asupra producției de boabe

Factors	*Grain yield kg/ha*	*% Control*	*Difference (%)*	*Significance*
		Year		
Years average	11651,12	100	0,00	Control
2015	9602,22	82,4	-2048,90	000
2016	9894,33	84,9	-1756,78	00
2017	8012,89	68,8	-3638,23	000
2018	14464,39	124,1	2813,27	***
2019	14488,00	124,3	2836,88	***
2020	13032,67	111,9	1381,55	*
2021	11886,39	102,0	235,27	-
2022	8733,78	75	-2917,34	000

Factors	*Grain yield kg/ha*	*% Control*	*Difference (%)*	*Significance*
2023	14243,00	122,2	2591,88	***
2024	12153,50	104,3	502,38	-
DL /LSD p 5% = 1050,36; DL/ LSD p1%=1440,49; DL/LSD p 0,1% = 1960,66				
Hybrids				
KWS 2370	10726,23	100,0	0,00	Control
KWS KARPATIS	12129,33	113,1	1403,10	***
KWS KASHMIR	11851,00	110,5	1124,77	***
KWS DURANGO	11733,23	109,4	1007,00	***
KWS SMARAGD	11830,23	110,3	1104,00	***
KWS KAPITOLIS	11636,37	108,5	910,43	***
DL /LSD p 5% = 231,78; DL /LSD p 1% = 307,87; DL LSD p 0,1% = 396,83				

Fig. 2 Grain yield at 6 maize hybid during the experiment (from 2015 to 2024)// Producția de boabe la 6 hibrizi ecperimentați (între 2015-2024)



An analysis of the number of thermally unfavorable days during the crop vegetation period highlighted both the number of days with temperatures below the optimum and those above it.

The highest number of unfavorable days was recorded in the years 2015, 2017, and 2022 years in which yields also reached their lowest levels.

The greatest number of unfavorable days were those with temperatures above 30°C, starting in June and peaking in July and August.

The number of days with temperatures exceeding 30°C ranged between 32 and 55 days in 2022.

It is worth noting that during the April–August interval, the total number of unfavorable days for the growth and development of maize plants ranged between 58 and 67 days in the years analyzed.

Table 4. Number of thermally unfavorable days from the growing season of maize// Număr zile nefavorabile din punct de vedere termic din perioada de vegetație a porumbului

Year		**IV <10^{0}C**	**V <13^{0}C**	**VI <18^{0}C**	**VII <18^{0}C**	**VIII <18^{0}C**	**Total**
2015	Number of days below the optimal germination threshold	6	0	0	0	0	**6**
	Number of days below the optimal development threshold	0	2	9	3	3	**17**
	Number of days with temperatures above 30 °C	0	0	5	14	19	**38**
Total		**6**	**5**	**14**	**17**	**22**	**61**
2016	Number of days below the optimal germination threshold	4	0	0	0	0	**4**
	Number of days below the optimal development threshold	0	9	10	0	3	**22**
	Number of days with temperatures above 30 °C	0	0	9	15	8	**32**
Total		**4**	**9**	**19**	**15**	**11**	**58**

Year		IV <10^{0}C	V <13^{0}C	VI <18^{0}C	VII <18^{0}C	VIII <18^{0}C	Total
2017	Number of days below the optimal germination threshold	10	0	0	0	0	**10**
	Number of days below the optimal development threshold	0	3	4	4	4	**15**
	Number of days with temperatures above 30 °C	0	0	6	10	19	**35**
Total		**10**	**3**	**10**	**14**	**23**	**60**
2022	Number of days below the optimal germination threshold	10	0	0	0	0	**10**
	Number of days below the optimal development threshold	**0**	**0**	1	1	0	**2**
	Number of days with temperatures above 30 °C	0	0	**12**	**19**	**24**	**55**
Total		**10**	**0**	**13**	**20**	**24**	**67**

Another element studied that helped us identify the disturbing factors affecting maize plants was the hydrothermal index for the June–August period (the ratio between the sum of temperatures and precipitation).

Table 5. Hydrothermal index // Indici hidrotermici

Year/Month	VI	VII	VIII	Avg.	Defination
2015	2,30	0,40	1,35	1,30	Moderate deficit
2016	2,05	0,88	0,94	1,29	Dry
2017	0,84	0,76	0,38	0,66	Dry
2018	3,10	0,90	0,12	1,38	Optimal
2019	0,74	0,91	1,10	0,92	Dry
2020	2,05	1,52	0,60	1,39	Optimal
2021	1,17	1,37	1,20	1,25	Moderate deficit
2022	0,14	0,79	1,44	0,79	Dry
2023	2,64	1,25	1,27	1,72	Slight surplus
2024	1,15	0,90	0,49	0,85	Dry

<1 = dry; 1,01-1,30 = moderate deficit; 1,31-1,70 = optimal; 1,71-2,00 = slight surplus; >2,01 = Surplus

Based on the compiled data, we concluded that in the years with the lowest yields, the lowest hydrothermal index was also recorded—0.66 in 2017 and 0.79 in 2022—indicating that these periods were characterized by drought.

For the two climatic factors (temperature and precipitation), the correlation coefficient with yield was also calculated.

In the case of precipitation, a negative correlation between grain yield and rain fall was observed in the months of April and August for all analyzed hybrids, and a positive correlation in May, June, and July. The value of the correlation coefficient varied from one hybrid to another and from one month to the next.

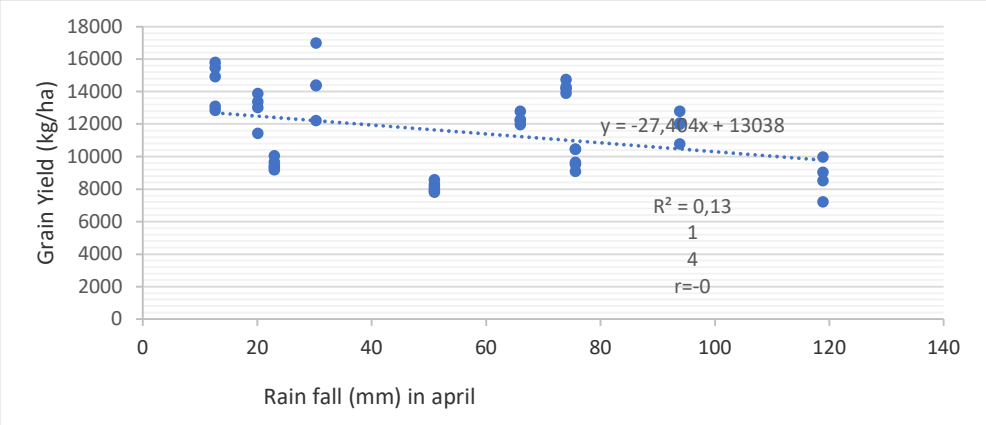


Fig. 3 Coralation between grain yield and rain fall in April // Analiza de corelație între producția de boabe și precipitațiile în luna aprilie

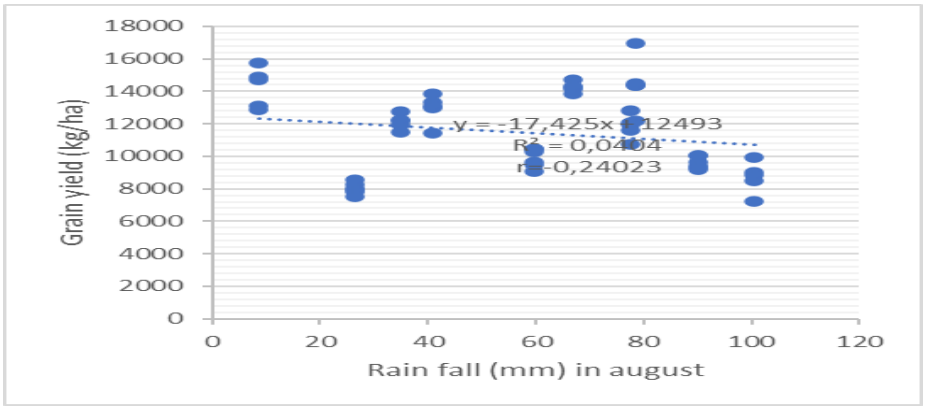


Fig. 4 Coralation between grain yield and rain fall in August// Analiza de colerație între producția de boabe și precipitațiile în luna august

The analysis of the correlation coefficient between production and average monthly temperature revealed that in the months of April and May, a positive correlation was recorded. However, starting from July and August, the correlations were negative.

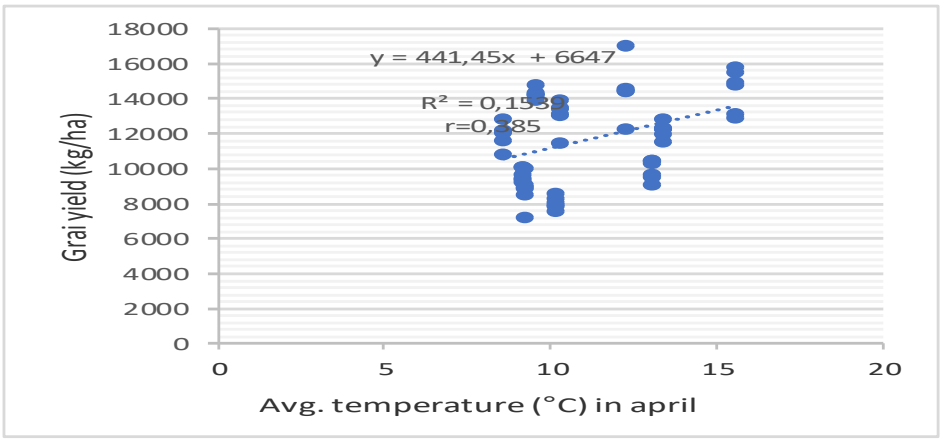


Fig. 5 Coralation between grain yield and average temperature// Analiza de colerație între producția de boabe și temperature medie în luna august

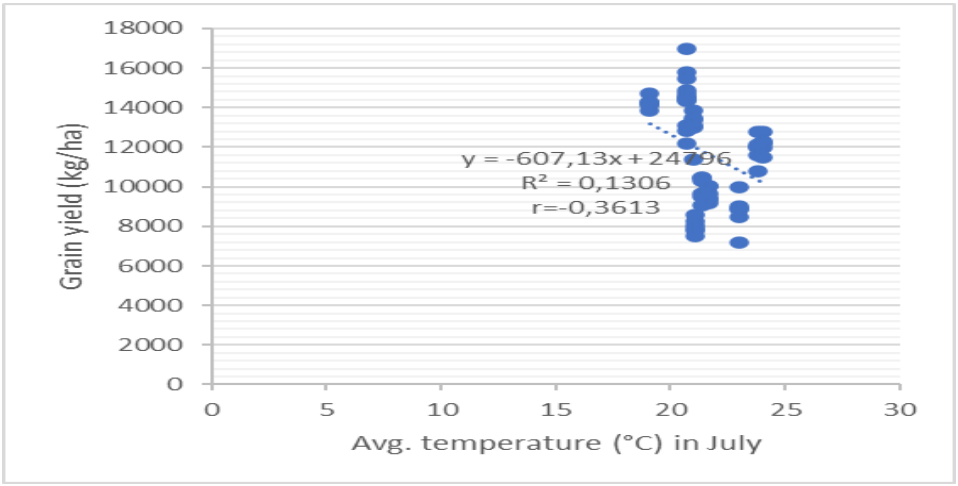


Fig. 6 Coralation between grain yield and average temperature in July // Analiza de colerație între producția de boabe și temperature medie în luna iulie

In August, however, the hybrids reacted differently to the amount of precipitation. The hybrids KWS 2370, Karpatis, Kashmir, and Kapitolis recorded lower values, while the hybrids Durango and Kapitolis showed a more pronounced negative response.

We believe that this reaction is due to the different vegetation periods of the hybrids. In general, it can be concluded that each hybrid reacted differently to the two factors, but there was a tendency for yield to decrease in the years when the two factors were not harmonized, either there were periods with high temperatures and no rain, or there were periods with more precipitation but lower temperatures.

CONCLUSIONS

1. Under the conditions in the area where the experiments were conducted, the evolution of the two analyzed factors varied during the studied years, which also led to differences in yields.
2. The least favorable years for corn were 2017 and 2022, years in which the production potential was significantly reduced.
3. The best years climatically for maize cultivation in the Transylvania region were 2018, 2019, and 2023, years in which the production potential recorded the highest values.
4. For zoning new maize hybrids, testing over at least 3 years is necessary in order to verify their response to climate changes.

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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.*

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

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.

MATERIAL AND METHOD

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:

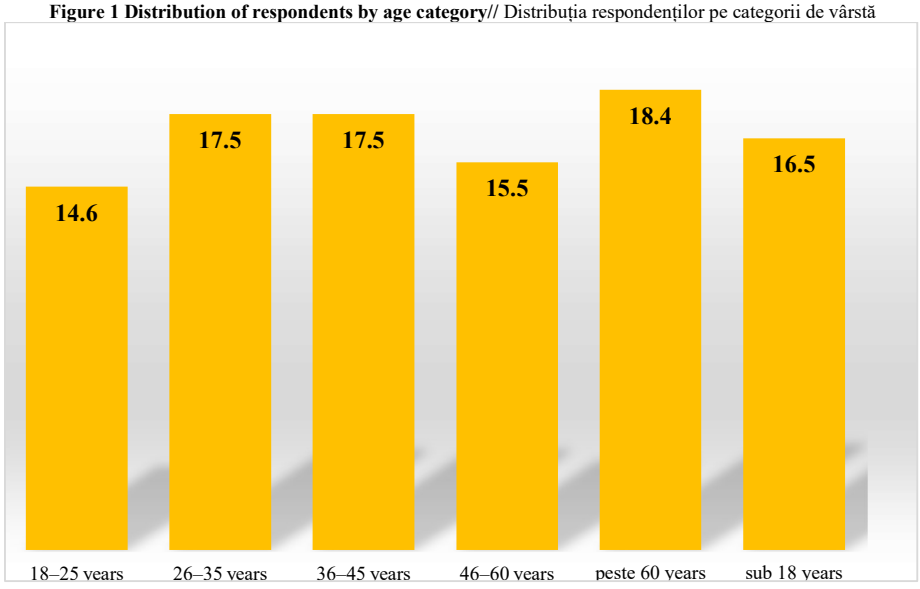
1. Socio-demographic data;
2. Knowledge about biotechnologies and GMOs
3. Behaviors regarding product labeling;
4. Opinions regarding the consumption of genetically modified products.

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

RESULTS AND DISCUSSION

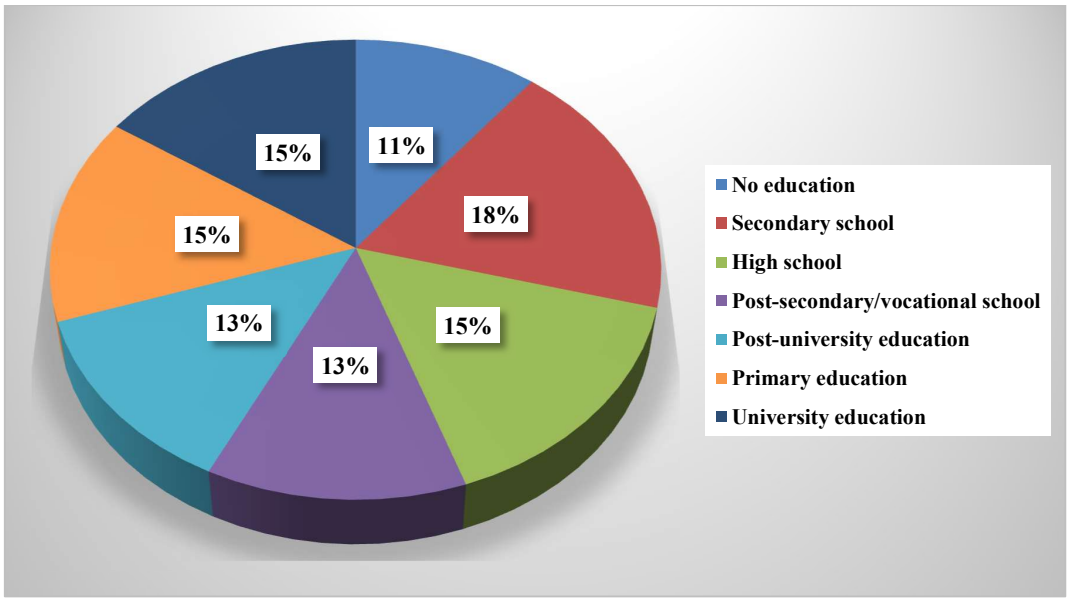
Regarding the profile of the respondents, the socio-demographic analysis showed that the majority of respondents are between 18 and 35 years old (over 60%), which suggests a relatively young and active population. The gender distribution was balanced, with a slight female predominance. The level of education 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

Figure 2 Distribution of respondents by level of education// Distribuția respondenților pe nivelul de studii

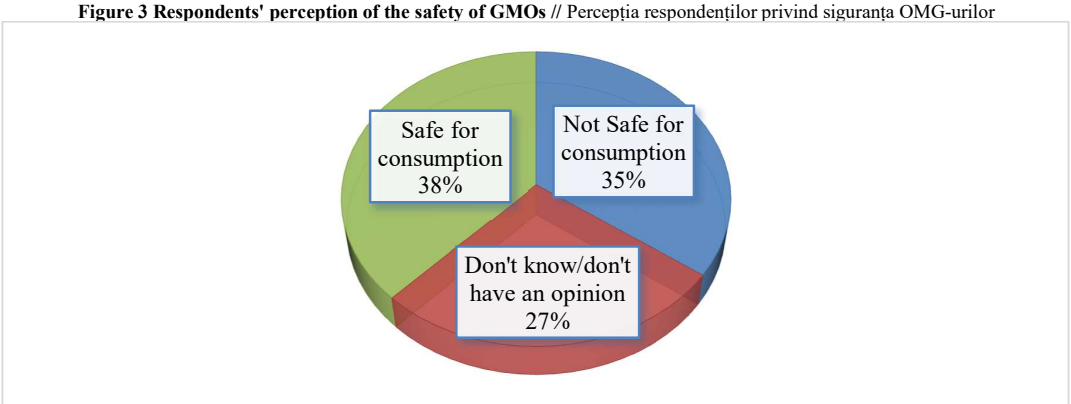


Source: processing based on questionnaire data

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

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

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

Source: processing based on questionnaire data

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.

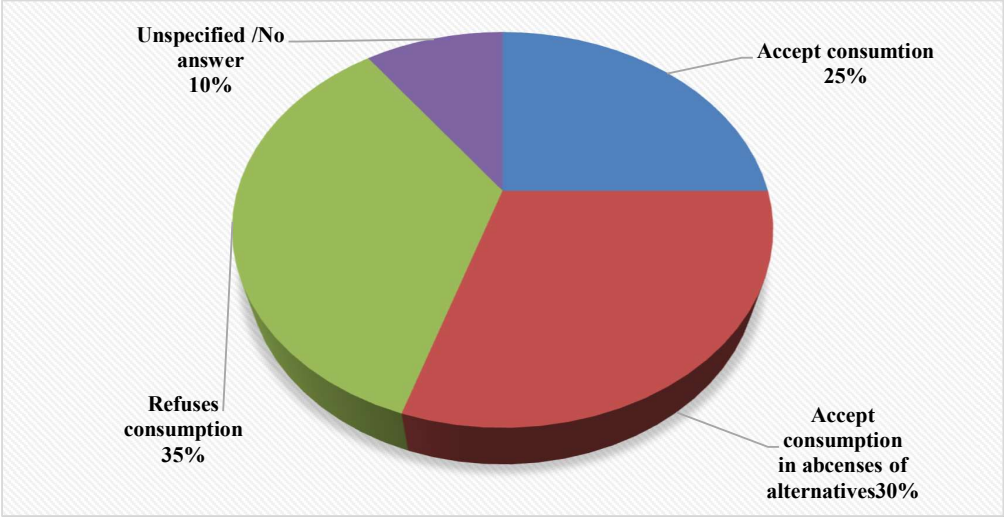
In contrast, 30% of respondents would accept genetically modified products, but only in the absence of safer and more affordable alternatives. They are therefore more flexible, being willing to accept these products only for economic reasons or in conditions where there are no other viable options on 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

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.

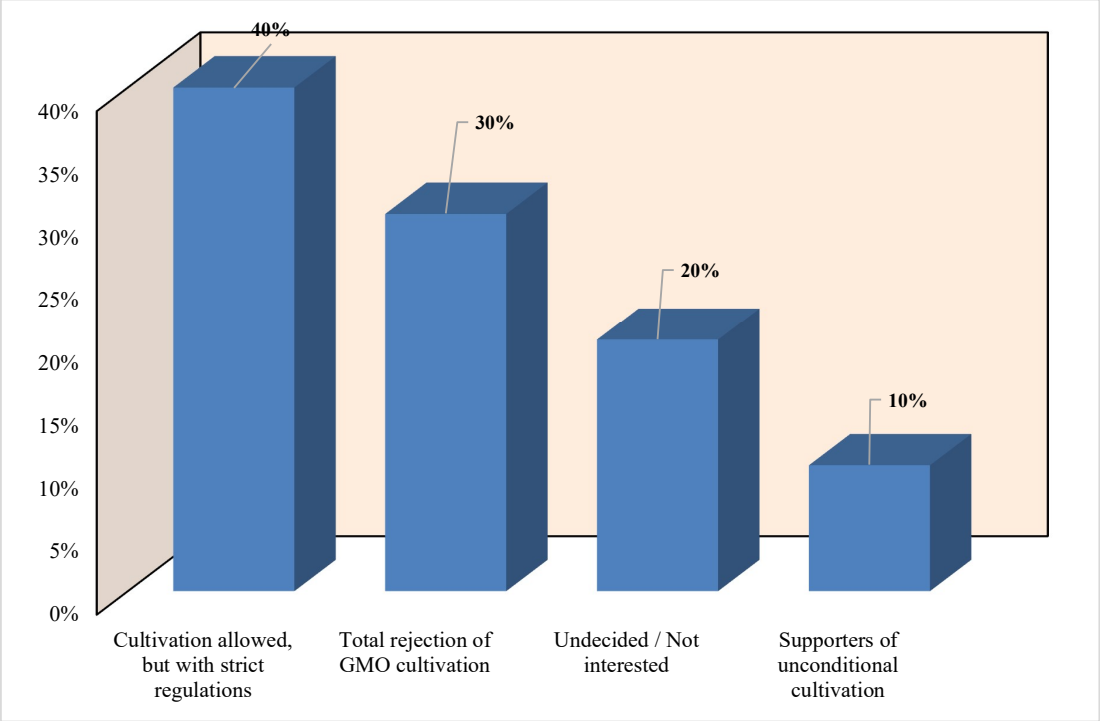
Regarding the cultivation of genetically modified organisms (GMOs) in Romania, the study revealed a diversity of opinions, reflecting a cautious and sometimes skeptical attitude of the public towards this practice. Approximately 40% of respondents believe that GMOs could be cultivated in the country, 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

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 factindicates 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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UTILIZATION OF THE OLD MAIZE POPULATIONS IN THE BREEDING PROGRAM FOR EXTRA-EARLY HYBRIDS

ACCESAREA POPULAȚIILOR VECHI ÎN PROGRAMUL DE AMELIORARE A HIBRIZILOR EXTRATIMPURII DE PORUMB

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Abstract

This study explores the potential of old maize populations from Suceava County in northern Romania for inclusion in breeding programs aimed at developing extra-early hybrids. Sixteen local maize populations were evaluated in both field and laboratory conditions to assess their morphological and physiological traits related to earliness, cold tolerance, and productivity. The results revealed significant genetic variability among the populations, with several ones showing promising characteristics for early development and stress resistance. Particular attention was given to traits such as early spring emergence, seedling vigor, flowering time, and grain yield. Populations Rodna 16, Vama 31 and Ciocănești stood out due to their early vegetation onset, uniform development, and resilience to unfavorable environmental conditions. These populations also demonstrated stable productivity and phenotypic traits that make them suitable candidates for hybridization in breeding programs targeting short vegetation cycles. Overall, the study confirms that certain old maize populations in Bucovina possess valuable genetic traits that can contribute to the creation of extra-early maize hybrids. These findings highlight the importance of conserving and utilizing traditional germplasm in modern crop improvement strategies, especially in regions with short growing seasons and climatic constraints.

Rezumat

Acest studiu explorează o parte din potențialul populațiilor de porumb vechi din județul Suceava, din nordul României, pentru includerea acestora în programe de ameliorare care vizează dezvoltarea de hibrizi extra-timpurii si timpurii. Saișprezece populații locale de porumb au fost evaluate atât în condiții de câmp, cât și in laborator pentru a evalua trăsăturile lor morfologice și fiziologice legate de timpurietate, toleranță la frig și productivitate. Rezultatele au relevat o variabilitate genetică semnificativă în rândul populațiilor, câteva prezentând caracteristici promițătoare pentru dezvoltarea timpurie și rezistența la stress termic. O atenție deosebită a fost acordată unor trăsături precum răsărirea, vigoarea, perioada de înflorire și producția de boabe. Populațiile Rodna 16, Vama 31 și Ciocănești s-au remarcat datorită debutului vegetativ timpuriu, dezvoltării uniforme și rezistenței la condiții de mediu nefavorabile. Aceste populații au demonstrat, de asemenea, o productivitate stabilă și trăsături fenotipice care le fac candidate potrivite pentru hibridizare în programele de ameliorare care vizează cicluri de vegetație scurte. Per total, studiul confirmă faptul că anumite populații de porumb vechi din Bucovina posedă trăsături genetice valoroase care pot contribui la crearea de hibrizi de porumb extra-timpurii si timpurii. Aceste constatări subliniază importanța conservării și utilizării germoplasmei tradiționale în strategiile moderne de ameliorare a culturilor, în special în regiunile cu sezoane de creștere scurte și constrângeri climatice.

Keywords: old maize population; extra-early hybrids; breeding program
Cuvinte cheie: populații vechi de porumb; hibrizi extra-timpurii și timpurii; program de ameliorare

INTRODUCTION

Accessing ancient maize populations is essential for genetic diversification and improvement of specific traits of modern crops. Traditional populations present higher genetic variability compared to modern hybrids, which allows the identification of useful genes for traits such as drought tolerance, disease resistance, nutrient use efficiency, production size and quality, etc. By combining traditional selection methods with advanced genetic technologies, these resources can significantly contribute to the creation of new, resistant, more productive and more sustainable varieties. Bucovina is a region with a special agricultural tradition and presents significant diversity in terms of maize crop zoning.

Ancient maize populations in this area represent an essential component of the country's agricultural heritage and an example of important genetic diversity for crop adaptation to local conditions and climate change. Their characterization involves studying various aspects related to genesis, morphology, behavior in the face of environmental factors and their use in agriculture.

The morphological characterization descriptors of plant architecture, cobs, grains and those of physiological characterization regarding plant resistance to breakage and falling, to diseases and sterility, highlight local populations from those studied, as sources of real interest for improvement. Also, earlier populations up to the silking phase were identified, which may constitute a valuable initial material for improving corn earliness.

MATERIAL AND METHOD

Characterization of local maize populations based on physical descriptors involves the evaluation and description of plant morphological traits, which can be used for identification, selection and conservation of genetic diversity. These descriptors provide information about the appearance and structure of plants, being essential for the management and improvement of local maize populations. A number of 16 local maize populations from Bucovina were analyzed.

The morphology of old maize populations is an essential aspect in understanding their characteristics and their adaptability to different environmental conditions. Compared to modern hybrids, old maize populations are distinguished by a greater diversity in morphological traits, due to natural selection and traditional cultivation practices. These traits are directly influenced by the ecological and climatic characteristics of the region in which they are cultivated. We present some key aspects of the morphology of these populations: plant height, main ear insertion height, stem diameter (min./max.), total number of leaves, panicle length, ear length, ear diameter (max./min.), number of rows of grains per ear, number of grains/row, grain weight per ear and 1000-grain weight. These descriptors are used to differentiate local populations, to select material for breeding and conservation programs, as well as to understand their adaptation to local environmental conditions. In addition, the use of a standard set of physical descriptors allows for the objective comparison and assessment of genetic diversity between maize populations.

Among the physiological descriptors studied were: plant resistance to low temperatures, plant vigor, sum of useful temperatures until stigma appearance, plant resistance to breakage and fall, and resistance to fusarium. The observations were carried out directly in the field and in the laboratory before measurements.

In the laboratory, the resistance of corn plants to low temperatures was determined, the assessment being carried out by the Debbert method. The Debbert method is a technique used to evaluate the resistance of plants to low temperatures, especially in corn. This method is used to determine the ability of plants to withstand stress caused by low temperatures or frost, which can negatively affect their development and yield. The purpose of this method is to identify corn genotypes that are more resistant to low temperatures, thus allowing to establish populations that have a greater chance of surviving in cold or frost conditions. There are also more advanced laboratory techniques that can be used to complement the Debbert method, such as genotype analysis or physiological tests that measure plant responses to low temperatures.

In the Debbert method, several parameters are monitored, such as:

- visual assessment of cold stress symptoms: after exposing plants to low temperatures, their general appearance is observed. Symptoms usually include spots or abnormal coloration on the leaves, yellowing, necrosis or stunting.
- measurement of freezing temperature: the temperature at which tissue damage due to freezing begins to occur is determined. This can be done by measuring the temperature at which corn plants begin to show signs of visible damage or by evaluating the electrical conductivity of a leaf extract, which can indicate cellular damage.
- plant vigor testing: plants exposed to low temperature conditions are then monitored as they recover. Measuring the speed of plant recovery is another indicator of their resistance to cold.

According to this method, each variant was made up of two samples: the low temperature test sample and the control sample. For this, each type of genetic resource was sown in two pots (30 seeds each). After germination, 25 plants were left to grow under optimal artificial laboratory conditions: temperature= 25 °C, light 10-15 thousand lux, day (14 hours) and night (10 hours), for 14-15 days until the third leaf developed. At this stage, one pot of each genetic resource was transferred to the LabTech growth chamber available at the unit. (Murariu M. et all., 2010)

Here, the genetic resources matched to those in the laboratory were subjected to 7 days under the same conditions, but with two temperature regimes: night 4-5 °C, day 8-9 °C. The growth chamber performs successive day-night cycles in an automated system, with the achievement of programmed parameters of light, temperature and humidity. After the 7 days of low temperature treatment, the growth chamber casseroles were transferred back to the laboratory, for another 6-7 days next to the paired pots.

Finally, 20 seedlings from the experiment (seedlings subjected to low temperatures in the growth chamber) and 20 seedlings from the control variant (grown under optimal laboratory conditions) were cut. All samples were dried in ovens until constant weight. The coldtest index was assessed after the accumulation of dry mass calculated with Ki index = the ratio of the dry mass of the experimental sample to the control sample, according to the following scale:

- resistant genotypes > 80%
- semi-resistant genotypes 60 - 79%
- weakly resistant genotypes 40 - 59%
- sensitive genotypes < 40%.

The cold-test index with high values coincides in most cases with the higher grades (7,8,9) awarded in the field for plant resistance to low temperatures.

The following estimators were calculated for a number of 13 morphological descriptors and 7 physiological descriptors: arithmetic mean (x), amplitude of variation, variance (s2) and coefficient of variation (s%). The dispersion of the results regarding the morphophysiological characterization descriptors of the analyzed local maize populations provides a conclusive picture of the genetic diversity existing within this category of germplasm, insufficiently exploited.

RESULTS AND DISCUSSIONS

Under natural conditions, corn finds in the Suceava Plateau the necessary rainfall (over 500mm) but also average temperatures of over 8.0 ºC. Below this limit, it is necessary to cultivate early and extra-early hybrids, in order to have enough time to reach maturity before the first frosts and early autumn frosts are recorded.

However, the temperatures required for different phenological phases are also important. Thus, the minimum thermal threshold for triggering germination varies depending on the hybrid, between 6 and 12 ºC. The optimal sowing period is May 1-10, a period in which seed germination and sprouting take place under normal conditions, with average daily temperatures being favorable.

The average temperatures of June and July, exceeding 15 – 16 ºC and approaching 20 ºC (sometimes even exceeding this value), are corresponding to the following phenological phases, which require daily averages of 10-20 ºC after sunrise, until the appearance of the leaf 10-14 and 20-22 ºC during the period of panicle appearance, flowering and silking.

The high daily temperatures and durations of sunshine in this interval, favor first abundant flowering and then normal pollination, under normal conditions of atmospheric and pedological humidity. Both abundant precipitation and their lack during the flowering and pollination period of plants are harmful to these processes.

It has been found so far that optimal development conditions are met for corn cultivation in the Suceava Plateau, especially in its southern part, where thermal advantages prevail, while in the northern half, water conditions prevail. In Suceava too, corn cultivation benefited in the 144 days of phenological evolution from a thermal potential of over 3000ºC and a pluviometric one of 374 mm precipitation (table 1).

Table 1 Phenological evolution and main bioclimatic indicators of corn cultivation in Suceava (5 year average 2020-2024)// *Evoluția fenologică și principalii indicatori bioclimatici ai culturii porumbului la Suceava (medie pe 5 ani 2020-2024)*

No. crt .	Phase phenological	inter-phases (days)	ΣT >0 (°C)		ΣP	
			in inter-phase	cumulative from sowing	mm	mm
1	Sowing					
2	Risen	7	132.7	132.7	17.2	17.2
3	appearance the 3rd leaf	4	126.4	259.1	2.2	19.4
4	appearance panicle	52	1379.1	1638.2	123.9	143.3
5	Flourishing panicle	4	104.3	1742.5	0.0	143.3
6	Flourishing cob	3	67.0	1809.5	0.2	143.5
7	Maturity in milk	25	492.2	2301.7	116.4	259.9
8	Maturity in wax	28	454.4	2756.1	98.4	358.3
9	Maturity wholly	21	271.5	3027.6	16.2	374.5
Total		144	3027.6		374.5	

Type of genetic material	Insertion height (cm)
Local populations	60 – 100 cm
Commercial hybrids (semi-early)	80 – 120 cm
Late hybrids, tall	100 – 140 cm
Inbred lines	50 – 90 cm

The second table shows the values of morphological descriptors in maize populations selected based on the coldtest index>78%. Old maize populations have a variable height, usually lower than modern hybrids. Although plant height can vary significantly depending on the region and cultivation conditions, old maize tends to be more resistant to winds and extreme weather conditions due to its shorter stature.

The total plant height records an average value of 195.4 cm, which denotes that the maize populations analyzed generally have a medium to tall height. However, populations with very tall height over 220 cm (Rodna 26, Ciocănești) and populations with low and very low height, below 160 cm (Slatina22, Valea Stânei 203).

The height of the main ear insertion in corn, represents the distance from the base of the plant (soil) to the attachment point (insertion) of the first ear on the stem. It is an important morphological descriptor used in breeding, agronomy and the evaluation of harvesting mechanization. The average values of the cob insertion height differ depending on the type of genetic material (see tab.1).

The insertion height of the main cob is, on average, approximately 60 cm, with very large variations between populations, from 105 cm, in the very tall population, Ciocănești, to 28 cm, in the very short population, Valea Stânei 203.

The maximum and minimum diameter of the stem, as well as the total number of leaves, are naturally higher in tall populations and lower in short-stemmed populations.

Compared to modern hybrids, the stems of old-growth corn are usually thicker and more resistant, having a better ability to withstand difficult environmental conditions, such as strong winds or the weight of the ear.

The corn leaves of old-growth corn populations are generally wider and longer than those of modern hybrids. They help photosynthesis more efficiently and protect the plant from excessive water evaporation, providing additional protection against drought. The leaves are arranged on the stem in a way that maximizes light capture and minimizes water loss, providing better protection under variable environmental conditions.

The panicle length has an average value of about 63 cm, with a small variation within the populations analyzed, ranging from 70 to 53 cm, regardless of the size of the plants

Morphological descriptors for corn cobs — such as length, maximum diameter and minimum diameter — are essential parameters used in research, breeding and selection, as they directly influence the production potential of a variety or population (Murariu D., et all., 1999).

The descriptors, cob length, maximum diameter and minimum diameter, highlight many populations with large cobs: Rodna 16, Pîrteștii de Sus 1, Rodna 26, Ciocănești. The values of the descriptors regarding cob sizes provide an image of the productivity of the local corn populations analyzed.

Table 2 Values of morphological descriptors in selected maize populations based on coldtest index>78%// *Valorile descriptorilor morfologici în populații selectate de porumb pe baza unui indice de test la rece > 78%*

Population name	Plant height cm	Cob insertion height cm	Maximum diameter, mm	Minimum diameter mm	Total number of leaves	Panicle length cm	Cob length, cm	Maximum cob diameter mm	Minimum cob diameter mm	No. of rows of grains	No. of grains per row	Grain/cob weight g	MMB g
Lunca Ilvei 3	179	51	17	7	9	57	14,9	41,0	31,8	16	31	99	240
Rodna 16	211	68	21	10	10	66	17,5	44,7	33,9	14	37	146	332
Pîrteștii de Sus 1	182	50	19	9	9	65	17,1	45,1	29,0	16	36	137	276
Frumosu 32	196	54	19	8	10	66	12,9	42,7	33,0	14	28	87	244
Solca 3	203	68	20	9	10	65	16,9	43,8	36,3	12	34	133	328
Slatina 2	154	50	17	7	9	53	14,8	43,6	35,1	14	32	121	288
Vama 7	208	51	19	9	9	68	13,7	44,1	32,5	14	30	96	216
Rodna 26	226	79	20	9	10	66	17,2	44,5	35,2	14	36	143	356
Vama 31	192	59	18	7	8	62	15,9	41,5	30,4	16	34	104	212
Straja 14	195	54	19	8	10	65	13,5	41,5	24,7	16	30	85	188
Frumosu 12	189	54	18	8	9	63	16,0	44,6	33,7	16	32	111	220
Frumosu 166	174	44	16	6	8	59	13,1	39,0	22,6	14	29	64	184
Ciocănești	246	105	22	8	14	59	21,0	51,4	39,3	16	42	212	360
Brodina 1	189	59	19	8	8	65	13,8	43,5	26,6	16	32	91	200
Valea stânei 203	155	28	14	5	7	60	14,0	38,0	28,7	16	28	76	176
Pojorâta	172	40	15	6	8	58	11,8	43,7	30,9	16	28	82	200

The weight of grains per cob records an average value of 113.5g, with a very large amplitude of variation (212+63g). A series of populations with high values for this descriptor, over 140 g, are noted: Rodna 16, Vama 31, Ciocănești. The highest value of the weight of grains per cob is the local population of Ciocănești, 212 g. The weight of 1000 grains also highlights local corn populations with values over 300g: Rodna 16, Solca 3, Rodna 26, Ciocănești (table 3).

Table 3 Values of morphological descriptor estimators for old local maize populations characterized in Suceava// *Valorile estimatorilor descriptorilor morfologici pentru populații locale vechi de porumb caracterizate în Suceava*

Estimated	Plant height cm	Cob insertion height cm	Maximum diameter, mm	Minimum diameter mm	Total number of leaves	Panicle length cm	Cob length, cm	Maximum cob diameter mm	Minimum cob diameter mm	No. of rows of grains	No. of grains per row	Grain/cob weight g	MMB g
16 local maize populations tested													
X	194.8	60.6	18.5	8.1	9.4	62.9	16.4	42.5	32.1		32.6	114.8	271.4
Maximal	246.0	105.0	25.0	12.0	14.0	71.0	23.7	52.6	39.9	22	43.0	212.0	484.0
Minimum	132.0	27.0	11.0	5.0	6.0	39.0	10.5	32.2	22.2	10	23.0	48.0	108.0
X2	509.9	209.2	5.7	1.5	1.7	22.2	7.5	12.0	10.6		21.1	1116.2	4490.9
S%	11.6	23.9	12.9	15.2	13.8	7.5	16.7	8.1	10.2		14.1	29.1	24.7
7 local maize populations with higher coldtest index values													
X	195.4	61.2	18.7	8.3	9.4	63.2	16.2	42.8	31.9		33.3	113.5	259.2
Maximal	246.0	105.0	23.0	10.0	14.0	70.0	22.4	51.4	39.3	20	42.0	212.0	456.0
Minimum	154.0	28.0	14.0	5.0	7.0	53.0	11.8	35.3	22.6	10	27.0	63.0	108.0
X2	414.5	198.5	3.6	1.3	1.5	12.3	6.1	9.5	11.7		14.9	988.8	4361.1
S%	10.4	23.0	10.2	13.8	13.0	5.5	15.2	7.2	10.7		11.6	27.7	25.5

From the analysis of the coldtest indices determined for the local populations in the entire experimental system, it results that most of the maize populations are semi-resistant to low temperatures, with the values of this index ranging between 60-79%. However, resistant and even very resistant populations (coldtest index > 80%) were detected in this biological material (Murariu D., 2001).

These populations, to which are added only a few with a coldtest index of 78-79%, were the subject of this study (table 4). Among the local maize populations with very high values of the coldtest index, the following can be highlighted: Slatina 22, Vama 7, Vama 31, Straja 14, Frumosu 12.

Many of these populations show good and very good growth vigor and have proven to be resistant, both at the seedling stage and in the field. These populations represent valuable sources of genes for improving maize resistance to low temperatures.

The sum of useful temperatures (0C) from the sowing period to the appearance of stigmas (silk) represents an indirect indicator of the earliness of maize populations. The calculation of the average of this indicator of development processes and earliness, determined in Suceava, highlights a series of earlier populations up to this phase, needing less than 550 0C t.u. up to the silking phase: Frumosu 32, Vama 31, Straja 14, Frumosu 12, Pojorâta. These populations can constitute a valuable initial material for improving the earliness of maize.

The resistance of plants to breakage and falling represents an important technological indicator for maize. From the analysis of the average results obtained in Suceava it results that the vast majority of the studied populations are less resistant with regard to this characteristic, which can be explained by the fact that old local maize populations represent unimproved genetic material. Only a few populations with a proportion of broken and fallen plants of 25 and below 25% are noted: Pîrteștii de Sus 1, Frumosu 32, Solca 3, Vama 31, Ciocănești, Valea Stânei 203.

Table 4 Value of the coldtest index and descriptors of local maize populations// *Valoarea indicelui de temperatură scăzută și descriptorii populațiilor locale de porumb*

Population name	Index coldtest	Growth vigor	Resistance to low temperatures in the field	Sum of useful temperatures up to silkiness	Broken + fallen plants at harvest	Sterile plants	*Fusarium* resistance
	%	notes	notes	0 ^{0}C	%	%	notes
Lunca Ilvei 3	81	8,5	8,5	488,2	29,4	23,5	4
Rodna 16	85	9	7,5	531,5	41,6	5,5	7
Pîrteștii de Sus 1	79	7	7,5	531,5	24,0	16,0	7
Frumosu 32	82	7	7,5	518,7	22,5	25,0	6
Solca 3	84	7,5	7,5	557,1	19,3	3,8	5
Slatina 2	90	6,5	8	570,4	37,0	8,7	4
Vama 7	94	6,5	7	570,4	33,3	27,8	7
Rodna 26	83	7,5	8	570,4	34,1	4,5	4
Vama 31	97	6	5,5	544,5	18,2	36,4	4
Straja 14	85	7,5	6,5	518,7	34,8	13,0	6
Frumosu 12	85	7	6,5	531,5	33,3	23,8	5
Frumosu 166	81	7	6	570,4	27,8	16,7	5
Ciocănești	84	6.5	6	645.5	18.0	8.0	5
Brodina 1	86	7	5	557.1	28.3	7.7	6
Valea stanei 203	80	6	4.5	557.1	17.5	15.0	6
Pojorata	92	4	6	518.7	27.3	18.2	7

CONCLUSIONS

1. Old maize populations in Romania are highly diverse from a genetic standpoint. These populations have traditionally evolved under various climatic, soil, and cultivation conditions, becoming well-adapted to the different regions of the country. Over the decades, Romanian farmers have preserved these varieties through both natural and local selection practices, leading to the development of populations with genetics specific to their respective regions.

2. The morphology of traditional maize populations in Romania reflects a continuous adaptation process to local environmental conditions, resulting in plants with highly variable traits. These morphological characteristics are crucial for the resilience and adaptability of traditional maize to extreme weather events and for ensuring sustainable yields across a wide range of ecological conditions. Moreover, the morphological diversity of these populations makes them extremely valuable for conserving agricultural biodiversity and contributing to future crop improvement programs.

3. In the Suceava region, where maize cultivation approaches the northern limit of its viable growing area in Romania, specific temperature and humidity conditions highlight the ecological need to improve extra-early and very early maize types. The breeding strategy for such areas should focus on developing high-yielding hybrids with strong ecological plasticity, resistance to low spring temperatures, a short growing season, and genetic resistance to diseases and pests.

4. Breeding efforts will continue with the specific goal of genetically mitigating certain antagonistic correlations between biological processes, so that the resulting maize genotypes align as closely as possible with the proposed ideotype.

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IMPACT OF WATER STRESS ON THE GROWTH AND DEVELOPMENT OF *PHASEOLUS VULGARIS* L.: COMPARATIVE STUDIES AND PERSPECTIVES

IMPACTUL STRESULUI HIDRIC ASUPRA CREȘTERII ȘI DEZVOLTĂRII SPECIEI *PHASEOLUS VULGARIS* L.: STUDII COMPARATIVE ȘI PERSPECTIVE

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Abstract

FAO places Phaseolus vulgaris L. as the most important legume worldwide. Scarcity of water has a significant negative effect on the growth, development and hence crop production of Phaseolus vulgaris L. To assess the drought tolerance of Phaseolus vulgaris L., comparative morphological and physiological studies were carried out by subjecting three variants (two landraces with different geographical origin and one with increased drought resistance) to 8 and 12 days of water stress. The results revealed that the response to water lack is dependent on the intensity of the stimulus, the type of variant analyzed.

Rezumat

FAO plasează specia Phaseolus vulgaris L. pe primul loc, ca fiind cea mai importantă leguminoasă la nivel mondial. Lipsa apei are un efect negativ semnificativ asupra creșterii, dezvoltării și implicit a producției agricole, în cazul speciei Phaseolus vulgaris L. Pentru testarea toleranței la secetă a speciei Phaseolus vulgaris L. au fost efectuate studii comparative la nivel morfologic și, fiziologic, prin expunerea a trei variante (2 populații locale cu origini geografice diferite și o varintă cu rezistență mărită la secetă), la 8 și 12 zile de stres hidric. Rezultatele au arătat că, răspunsul față de lipsa apei este dependent de intensitatea stimulului, de tipul variantei analizate.

Keywords: drought, Phaseolus vulgaris L., physiological studies, morphological studies

Cuvinte cheie: *Phaseolus vulgaris L., secetă, fiziologie, morfologie*

INTRODUCTION

Plant species are considered, in a special kind of way, the lungs of the Earth. Plants are a major source of energy for the existence and evolution of species. FAO states that *Phaseolus vulgaris* L. is the most important legume in the world. This importance derives from the nutritional intake provided to the consumer, e.g. the protein concentration varies between 23-33%, depending on the variety, and the energy intake is provided by the high carbohydrate content (over 50%) (Hayat et al., 2014). Bean seeds are considered a key food in disadvantaged regions of the world, contributing greatly to the food security of the area concerned. Studies based on genome research are essential for understanding plant physiological processes and the changes that occur at the morphological, cellular or biochemical level when plant organisms are exposed to environmental factors less favorable for their growth and development. The information obtained can be essential, in particular, for breeding and can respond to current needs in direct proportion to climate change and, of course, to global population expansion.

Climate change causes severe changes in temperature or long periods of drought, which extremely affects crops regardless of its type by sharply decreasing production yield. If we talk about the production of *Phaseolus vulgaris* L., it can be affected up to 70% by lack of water (Polania et al., 2016).

For this reason, research studies based on the identification of individuals with increased resistance to various abiotic stress factors, mainly water stress, are of great importance at the present

time, and the findings may be of major importance, in the creation of resistant varieties to the current pedo-climatic conditions.

Phaseolus vulgaris L. is an important crop worldwide, and biotic and abiotic stresses can decrease bean yields by up to <600 kg/ha^{-1} in poor countries (Porch et al., 2013). Accelerated population growth is in direct proportion to the increasing demand for food, and global warming is resulting in lower crop yields due to reduced rainfall and higher temperatures, which ultimately leads to food insecurity, especially in underdeveloped countries.

Drought is considered to be the greatest abiotic stressor that limits plant growth and development by altering metabolic and biological activities. Currently, about 45% of the world's agricultural lands, where about 38% of the human population is counted, are subject to frequent or continuous drought. Every stage of plant growth is dependent on water, from the germination process to the maturity stage of plants, the lack of water results in crop failure and the socio-economic consequences can be far-reaching (Hussain et al., 2012).

Research studies based on the identification of individuals with increased resistance to various abiotic stress factors, mainly water stress, are of great importance at present, and the findings may be of major significance in the development of varieties resistant to current soil and climatic conditions.

MATERIAL AND METHOD

The biological material used is represented by two landraces (SVGB-1988 - Andean origin, SVGB-2087 - Mesoamerican origin) of the species *Phaseolus vulgaris* L., currently kept in the collection of the Plant Genetic Resources Bank "Mihai Cristea" Suceava, as well as a variety with increased resistance to water stress (Lechința, representing the positive control, the variety is in the collection of the Research and Development Institute for Legumiculture Bacău). A total of 3 variants of *Phaseolus vulg*aris L. were assessed.

To assess whether plants exposed to drought are morphologically affected, a phenotypic characterization of *Phaseolus vulgaris* L. plants from control and water-stressed groups was performed. The studies aimed to determine total plant height, root system length, above-ground height, root system biomass weight, shoot biomass weight and total weight, respectively. The determinations were performed for 5 individuals from each experimental lots.

In this study, the amount of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids were determined for both control and water-stressed samples. The amount of chlorophyll a and carotenoids, may indicate *Phaseolus vulgaris* L. variants with increased drought resistance. The higher the amount of chlorophyll, the higher the resistance of the plants during drought periods. The chlorophyll pigment IS content was determined by spectrophotometric readings in the UV-VIS range at a certain wavelength, dictated by the type of solvent used (Nayek et al., 2014).

The relative growth rate (RGR) can give an indication of the degree of plant growth under different conditions (normal growth conditions and water stress conditions respectively). This parameter indicates the growth rate of the plant per unittime. The RGR value can be determined by using a mathematical formula, where fresh plant weight values are entered for different growing days: : $\boldsymbol{RGR=(FW_{t22} - FW_{t30/34})/FW_{t30/34} \times 17}$;; where: FW_{t22}, FW_{t30} and FW_{t34}, represent the weight of the fresh plant at 22, 30 and 34 days of growth, respectively (Lizana et al., 2006). From each experimental batch, 5 individuals were evaluated.

The relative water content (RWC) was determined using the method developed by Weatherley (Weatherley, 1950). The method consists of using trifoliate leaves from the plant and weighing them immediately. They are placed in a container of distilled water and kept under these conditions for 4 hours at room temperature and light. The leaves are then weighed and the turgor weight is determined. The leaf tissue is further dried by forced air recirculation at 60ºC for 72 hours. The dried samples are weighed and then the weighed values are converted into the formula below, which indicates the relative water content (%):$\boldsymbol{RWC= (FW-DW)/(TW-DW)X100}$ (Qayyum et al., 2021).

RESULTS AND DISCUSSION

Different phenotypic changes and adaptation mechanisms were reported in *Phaseolus vulgaris* L., depending on the intensity of the applied water stress and the variety analyzed. Thus, morphological analysis of SVGB-1988 plants (from the Andean geographical basin) showed the presence of flowers in both irrigated and non-irrigated plants for 8 and 12 days (Figure 1). This is a classic phenomenon of adaptation to lack of water, referred to as drought escape, the plant channels all its energy and nutrients to complete the cell cycle, allowing flower and later fruit development.



Figure 1. Phenotypic study of the variety SVGB-1988 (Andean): a) control lot, irrigated for 30 days; b) water-stressed lot, 8 days; c) control lot, irrigated for 34 days; d) water-stressed lot, 12 days// Studiu fenotipic al soiului SVGB-1988 (Andean): a) lot de control, irigat timp de 30 de zile; b) lot sub stres hidric, 8 zile; c) lot de control, irigat timp de 34 de zile; d) lot sub stres hidric, 12 zile

For the genotype SVGB-2087, a less accentuated development of the individuals in the water-stressed lot was recorded for 8 and 12 days, respectively, compared to the plants irrigated throughout the experiment. Plants in the control plots showed much faster development, which can also be observed by the appearance and development of a much higher number of trifoliate leaves compared to the water-stressed plants with one or at most two poorly developed trifoliate leaf tiers. Figure 2 also shows the appearance of senescence in the leaf tissue for individuals irrigated for 8 days.



Figure 2. Phenotypic study of the variety SVGB-2087 (Mezoamerica): a) control lot, irrigated for 30 days; b) water-stressed lot, 8 days; c) control lot, irrigated for 34 days; d) water-stressed lot, 12 days// Studiu fenotipic al soiului SVGB-2087 (Mezoamerica): a) lot de control, irigat timp de 30 de zile; b) lot sub stres hidric, 8 zile; c) lot de control, irigat timp de 34 de zile; d) lot sub stres hidric, 12 zile

Although the Lechința variety is known to have a higher drought resistance, a simple morphological analysis of the plants shows a poor development of the non-irrigated individuals compared to the control plots (Figure 3), where the flower is also developed and the number of trifoliate leaves is much higher.

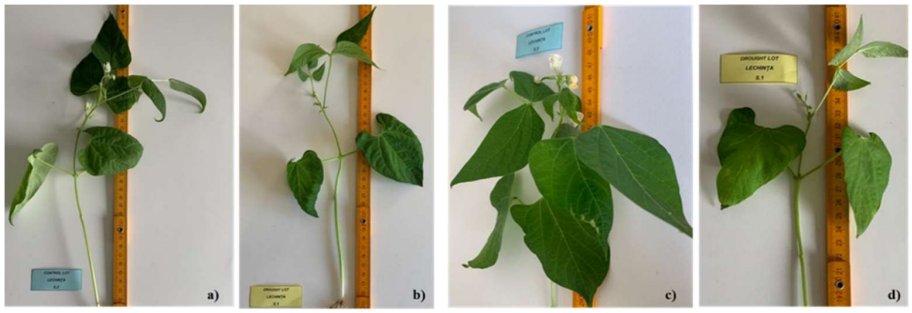


Figure 3. Phenotypic study of the variety Lechința: a) control lot, irrigated for 30 days; b) water-stressed lot, 8 days; c) control lot, irrigated for 34 days; d) water-stressed lot, 12 days// Studiu fenotipic al soiului Lechintima: a) lot de control, irigat timp de 30 de zile; b) lot sub stres hidric, 8 zile; c) lot de control, irigat timp de 34 de zile; d) lot sub stres hidric, 12 zile

After exposure of the *Phaseolus vulgaris* L. variants to 8 days (Figure 4) of water stress, the measurements showed significant and non-significant differences between the individuals belonging to the two experimental groups for all three varieties analyzed, according to the all morphological parameters evaluated.

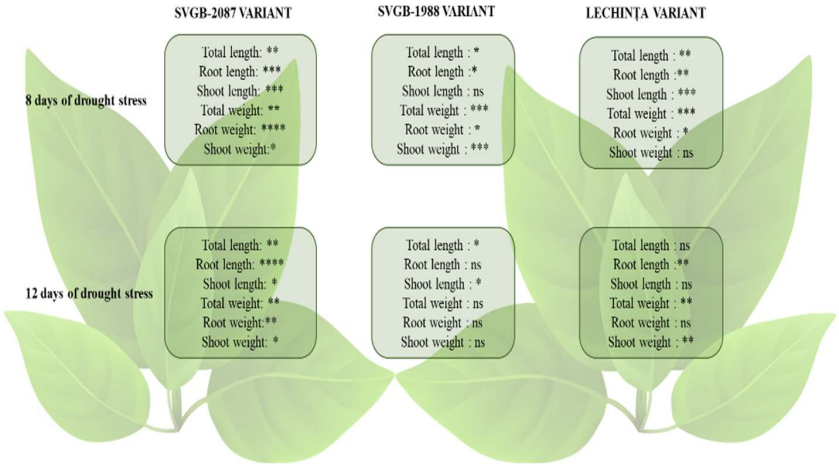


Figure 4. Summary of the differences achieved between irrigated and non-irrigated plots of the 3 varieties of *Phaseolus vulgaris* L., following the determination of morphological parameters and highlighting the statistical significance; with asterisks and "ns" indicating statistically significant differences: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$ and (****) $p \leq 0.0001$// *Rezumatul diferențelor obținute între parcelele irigate și neirigate din cele 3 soiuri de Phaseolus vulgaris L., în urma determinării parametrilor morfologici și evidențierea semnificației statistice; cu asteriscuri și "ns" indicând diferențe semnificative statistic: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$ and (****) $p \leq 0.0001$*

When plants do not have a sufficient water supply, they will undergo a variety of changes, not only morphologically, but also physiologically and biochemically. This category includes changes that occur during photosynthesis. Chlorophyll *a* is considered to be the pigment with the main role in photosynthesis, whereas chlorophyll *b* is the pigment that collects energy and passes it on for further processing, chlorophyll *a*. The 8 days of water stress led to a decrease in the concentration of chlorophyll *a* and *b*, but also for carotenoids, for water-stressed plants, compared to the control, for all the genotypes analyzed. However, the 12 days of drought led to a greater decrease in the concentration of assimilatory pigments in non-irrigated compared to irrigated samples. Analyzing from a biochemical point of view, the Lechința variety showed better drought resistance, while the Mesoamerica variety was the least resistant (Figure 5).

During periods of drought, plant organisms respond to these stressors by altering physiological and biochemical processes. By understanding the multiple mechanisms involved in plant resistance to water stress, it is possible to respond to the current challenge posed by global warming to identify specific plant species that can grow, develop and maintain increased yields under less favorable environmental conditions. By determining parameters such as relative water content (RWC) (Figure 6) and relative plant growth rate, it is possible to identify varieties of *Phaseolus vulgaris* L. with increased resistance to water stress factors. In theory, the value of these indicators is lower for drought-exposed individuals, which tolerate these environmental conditions more easily.

Relative leaf water content is an indicator of water content and reflects the balance between leaf tissue water supply and transpiration rate (Soltys-Kalina et al., 2016).

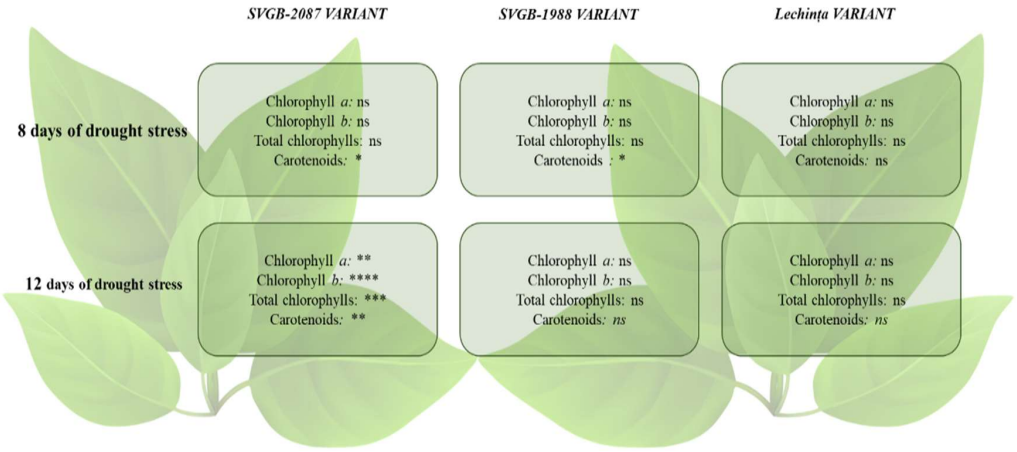


Figure 5. Summary of the differences achieved between irrigated and non-irrigated plots of the 3 varieties of *Phaseolus vulgaris* L., following the determination of some biochemical parameters and highlighting the statistical significance; with asterisks and "ns" indicating statistically significant differences: (*) $p \leq 0.05$, (**) $p \leq 0.01$, (***) $p \leq 0.001$ and (****) $p \leq 0.0001$// Rezumatul diferențelor obținute între parcelele irigate și neirigate din cele 3 soiuri de Phaseolus vulgaris L., în urma determinării unor parametri biochimici și evidențierea semnificației statistice; cu asteriscuri și "ns" indicând diferențe semnificative statistic: (*) $p \leq 0.05$, (***) $p \leq 0.001$ and (****) $p \leq 0.0001$

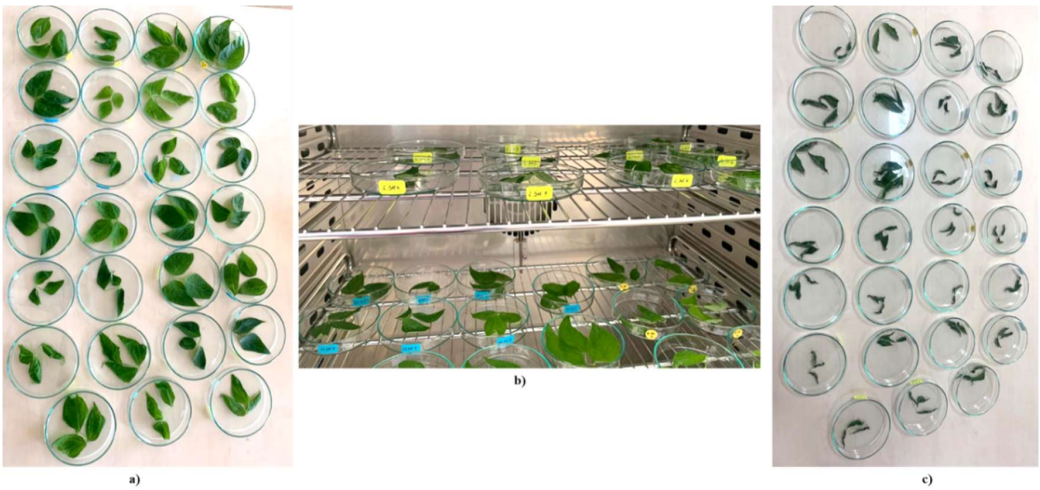


Figure 6. Emphasize the steps involved in the determination of the physiological index - relative water content: a) weighing trifoliate leaves immediately after sampling; b) placing the biological material in an oven at 60°C for 72 hours; c) preparing the dried plant material for final weighing// Evidențierea etapele implicate în determinarea indicelui fiziologic - conținutul relativ de apă: a) cântărirea frunzelor trifoliate imediat după prelevare; b) plasarea materialului biologic într-un cuptor la 60°C timp de 72 de ore; c) pregătirea materialului vegetal uscat pentru cântărirea finală

After 8 days of water stress, the RWC parameter for SVGB-1988 showed large differences between control and water-stressed individuals. For the SVGB-1988 variety, the same significant differences were observed between the individuals of the two groups after 12 days of water stress. The Lechința variety shows the smallest differences between individuals of the two plots (irrigated/non-irrigated), both at 8 days of water stress and at 12 days of water stress. Insignificant differences were observed between individuals of the two experimental plots (irrigated/non-irrigated) of SVGB-2087 after 8 days of water stress. In the case of the same genotype, SVGB-2087, after 12 days of water stress, the differences between the individuals of the two experimental groups were significant.

After 8 days of water stress, the relative plant growth rate varied according to the genotype analyzed. SVGB-1988 showed the greatest difference between individuals of the two experimental groups, both at 8 days of drought and at 12 days. In contrast, insignificant differences were reported

between individuals of the experimental plots (irrigated/non-irrigated) and for SVGB-2087 and Lechința varieties at 8 and 12 days of water stress.

CONCLUSIONS

1. Water stress is one of the most important factors limiting productivity in the agricultural sector and its effects are devastating. According to the Food and Agriculture Organization (FAO), by 2021, more than 828 million people were affected by hunger due to drought, with economic losses in the tens of billions of dollars worldwide.
2. Plants need water at all stages of growth and development. Lack of water causes changes and thus responses are generated at morphological, cellular, physiological, biochemical and molecular levels. However, the most important issue is the duration and intensity of water stress.
3. The phenotypic measurements indicated that Lechința and SVGB-1988 (originating from the Andean geographical basin) are the most drought resistant varieties, while SVGB-2087 shows a much lower adaptability to low water environment.
4. Quantification of assimilatory pigments, such as chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids again revealed that Lechința and SVGB-1988 are the most drought resistant varieties compared to SVGB-2087, a genotype with Mesoamerican origins.
5. The determination of physiological parameters such as RGR and RWC revealed the same aspects, the SVGB-2087 variety is the most poorly resistant to water shortage.

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COMPARATIVE MORPHOLOGICAL ANALYSIS OF TRIVALE AND BEZOSTAIA WHEAT VARIETIES WITHIN CONVENTIONAL AND ECOLOGICAL CROP SYSTEMS

ANALIZA MORFOLOGICĂ COMPARATIVĂ A SOIURILOR DE GRÂU TRIVALE ȘI BEZOSTAIA ÎN CADRUL SISTEMELOR DE CULTURĂ CONVENTIONALĂ ȘI ECOLOGICĂ

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Abstract

At the current stage in wheat, the aim is to improve the genetic endowment with as many supports as possible expressed through quantitative and qualitative valences. On the one hand, the aim is to increase the level of grain biomass, and on the other hand, it is also required to obtain sustainability performances for the cultivation environment. Recently, however, this sustainability is also oriented towards promoting the conventional system along with the ecological (green) one. From this point of view an analysis, even morphological aspects, some useful sustainability characters could be identified, with the promotion in fact for both cultivation systems. For the analysis of this material, two wheat varieties with a somewhat longer history in cultivation were used. These are: the Trivale variety, which has been cultivated for over 20 years, and the Bezostaia variety with a much longer history (the 1960s). Both here and elsewhere, it is considered that cereals of the type presented here could increasingly gain ground, given their variable genetic heritage, with greater resilience, high tolerance to biotic and abiotic stress and growth capacity.

Comparing the two varieties cultivated in the two systems: Conventional-Conv. and Ecological-Eco. it was found that the straw length was reduced by about 9 cm in the Trivale variety and by only 2 cm in Bezostaia. The thickness of the straw at the base was thickened in both varieties by the Eco type culture. The ears of the two varieties were shortened in ecological system by 1 cm. The weight of the ears was reduced in both varieties, by 0.8 g in Trivale and 0.4 g in Bezostaia, both by the ecological (Eco) culture. The number of spikelets in the ear was lower by 2 in both varieties in eco system. The membranes of the spikelets - the glumes and the blades were in the same order, shorter by 1 mm. The number of grains in the ear was 12 higher in the Trivale variety compared to Bezostaia, cultivated Conv., and by the Eco system. this number was reduced by 8 grains in Trivale and by 10 grains in Bezostaia in eco system. The weight of the grains in the two varieties was at the level of 0.6 g/Trivale Eco and 0.4 g/Bezostaia-Eco.

Grain length decreased slightly in both Conv. varieties, and grain thickness was greater in Bezostaia Eco. Crude protein remained above 12% in Trivale Conv., as in Bezostaia Conv. The Bezostaia-Eco. variety had a CP of about 11%. Good wet gluten for baking (minimum 22%) was obtained only in Trivale Conv. The Zeleny sedimentation index oscillated in both varieties between the percentages qualified as good-not so good only in the Trivale variety: 9.7 mm to 22.0 mm, while in the Bezostaia variety the percentages were not so good-good in the Conv. and Eco. systems: 21.0 mm to 37.6 mm. The correlations obtained between the main characters emphasized their support at significant positive levels in the Eco. crop system in both varieties. These results could prove to be supportive for the promotion of both current cropping systems and in the case of white luvic soils in the south of the territory.

Rezumat

În etapa actuală la grâu se dorește îmbunătățirea zestrei genetice cu cât mai multe susțineri exprimate prin valențe cantitative și calitative. Pe de o parte este vizată sporirea nivelului biomasei boabelor, iar pe de altă parte se cere și obținerea de performanțe de sustenabilitate pentru mediul de cultură. De curând însă această sustenabilitate este orientată și spre a promova sistemul convențional cu cel ecologic (green). Dintr-o astfel de analiză, chiar și prin aspectele morfologice, s-ar putea identifica unele caractere utile de sustenabilitate, cu promovarea de fapt pentru ambele sisteme de cultură. Pentru analiza din acest material s-au folosit două soiuri de grâu cu vechime ceva mai mare în cultură. Acestea sunt: soiul Trivale care se cultivă de peste 20 de ani și Bezostaia cu vechime mult mai mare (anii '60). Atât la noi, cât și în alte părți se consideră că cerealele de tipul celor de față ar putea să-și facă tot mai mult loc, având în vedere "variable genetic heritage, with greater resilience", cu toleranță ridicată la stresul biotic și abiotic și la creștere.

Comparând cele două soiuri cultivate în cele două sisteme: Convențional-Conv. și Ecologic-Eco. s-a constatat că lungimea paiului s-a redus cu circa 9 cm la soiul Trivale și cu numai 2 cm la Bezostaia. Grosimea paiului la bază a cunoscut îngroșări la ambele soiuri prin cultura de tip Eco. Spicele celor două soiuri s-au scurtat la ambele sisteme Eco. cu 1 cm. Greutatea spicelor s-a redus le ambele soiuri, cu 0.8 g la Trivale și 0.4 g la Bezostaia, ambele prin cultura Eco. Numărul de spiculețe din spic a fost mai mic cu 2 la ambele soiuri Eco. Membranele spiculețelor- glumele și paleile au fost în aceeași ordine, mai scurte cu 1 mm. Numărul de boabe în spic a fost cu 12 mai mare la soiul Trivale față de Bezostaia, cultivate Conv., iar prin sistemul Eco. acest număr s-au redus echivalent cu 8 boabe la Trivale și cu 10 boabe la Bezostaia. Greutatea boabelor la cele două soiuri s-a situat la nivelul 0,6 g/Trivale Eco. și 0.4 g/Bezostaia-Eco.

Lungimea boabelor a scăzut ușor la ambele soiuri Conv., iar grosimea boabelor de grâu a fost mai mare la Bezostaia Eco. Proteina brută s-a menținut la peste 12% la Trivale Conv., la fel ca și la Bezostaia Conv. Soiul Bezostaia-Eco. a avut PB de circa 11%. Glutenul umed bun de panificație (minim 22%) s-a obținut numai la Trivale Conv. Indicele de sedimentare Zeleny a oscilat la ambele soiuri între procentele calificate sub formă de bun-nu așa bun numai la soiul Trivale: 9.7 mm la 22.0 mm, în timp ce la soiul Bezostaia precentele au fost nu așa bun-bun îm sistemele Conv. și Eco. :21.0 mm la 37,6 mm. Corelațiile obținute între principalele caractere au accentuat susținerea acestora la nivele semnificative pozitive în sistemul de cultură Eco. la ambele soiuri. Aceste rezultate s-ar putea dovedi de susținere pentru promovarea ambelor sisteme actuale de cultură și în cazul luvosolurilor albce sin sudul teritoriului

***Keywords**: Conv, cropping systems, Eco, morphology, wheat*
***Cuvinte- cheie**: Conv, Eco, grâu, morfologie, sisteme de cultură*

INTRODUCTION

Being characterized as an ancestral species (Caligari & Bradham, 2001), wheat [Triticum aestivum (L.) Thell ssp. vulgare (Will.) M.K.], (pro syn. Triticum hybernum L., T. macha Dekap. & Menab., T. sativum Lam., T. sphaerococcum Percival, T. vulgare Will., common wheat, bread wheat) has been and still is one of the most important crop plants.

Triticum was adopted from the word threshing (bruising) which means to thresh, and aestivum characterizes the maturation of the plant in summer conditions. Threshing wheat is also called spelt. This characteristic together with genes from the species Aegilops tauschii, give this bread wheat the cold resistance, so necessary in temperate climates. In terms of surface area in the world, wheat actually ranks first, being cultivated in a multitude of ecological conditions.

The purpose of wheat cultivation is the production of grains, usually used in bread making (L-Baeckstrom et al., 2004). Qualitatively, the content of wheat grains in nutrients is considered both diverse (Mason et al., 2007) and balanced (Tester & Langridge, 2010) having a particularly high importance in human nutrition (Austin, 1999; Olesen et al., 2011). Over time, the plant has experienced particularly valuable developments (Bray & West, 2005) and namely through various specific characters.

Wheat represents one of the most diverse cereals/plants from a genetic point of view (Brenchley et al., 2012). Wheat has both autumn types and spring forms, some of which are rambling (Bonjean & William, 2001). There are also varieties with coated grains, while others are naked, with the membranes separated.

The present varieties, Trivale and Bezostaia have the hexaploid genome 2n = 6x (6x7) = 42, with six sets of chromosomes of the form AuAuBBDD (Li et al., 2014). The variety of these two varieties is erythrospermum Körn., with white spike, glabrous glumes and red grain.

The inflorescence of the plant is a terminal, distichous spike, 4-18 cm long, with sessile spikelets, attached solitary on the zigzag rachis. The spikelet is 10-15 mm long, being compressed laterally with two glumes and several flowers. The glumes have a short, blunt tooth-like tip, but also a short awn, 3-5 cm long. Each flower has a palea and lemma (lower membrane/pale). Depending on the variety, the lemma extends in the form of an awn, or as an extension in the form of a hood. When the palea and lemma adhere to the grain, it becomes covered. The grain (caryopses) is ellipsoidal in shape, with a central channel on one side. The grain is generally 4-12 mm long and 1.5-4.0 mm thick. The thousand grain weight (MMB) is between the wide limits of 15-60 g. The plant generally forms stems with heights ranging between 50 (60) and 140 (150) cm (Black & Halmer, 2006).

The research carried out to observe the variation of some morphological characters (Annicchiarico et al., 2010) of the two wheat varieties: Trivale and Bezostaia in the two crop systems - Conv. and Eco. included: i) the stem by the total length of the straw and the thickness of the basal internode, ii) the length and weight of the spike, iii) the number of spikelets/ spike, iv) the length of the external glume, the length of the lower blade (lemma), v) the number of grains/spike, their weight, vi) the dimensions of the grains (length and thickness) and the mass of one thousand grains (MMB). The results were obtained under the soil conditions of the location (Hillocks, 2012).

MATERIAL AND METHOD

The variants were cultivated in the more special climatic conditions of the 2024 harvest with the Trivale and Bezostaia varieties (Marrige, 1985), in two already known cropping systems: the conventional system - Conv. and the ecological system - Eco (Murphy et al., 2007; Przystalski et al., 2008). The experiments were set up at the same time in the two systems, according to the block method, with the variants in an area of 25 m^2 each, in 4 repetitions/ replications.

The technology used was the one recommended by the location for both systems (Tilman et al., 2002; Tomos, 2010). Their characteristic consisted in the fact that the culture soil was constituted by the stagnant albic luvic soil with its characteristics (Mader et al., 2002; Toncea, 2007). At full maturity, 25 plants/stems were randomly chosen from each repetition (100 in total), cut and brought to the laboratory. The 100 stems were measured and determined: total length of the straw, thickness of the basal internode, length and weight of the spike, number of spikelets in the spike, length of the glume and the panicle, number of grains in an ear and their weight, thousand grain mass (MMB), as well as grain dimensions: length and thickness.

The morphological characters (Hole et al., 2005) obtained were statistically analyzed by the histogram method (or frequency polygons-PF%). In their expression, class intervals established according to the specific series of values obtained were used. The study carried out highlighted several aspects, namely: i) the mode values (with the highest frequencies), ii) the limits of the variability intervals of the studied characters and iii) the specificity of each character of the wheat ecotypes in the analyzed area. Correlations were established between the analyzed characters, with the help of which their trends could also be observed within the studied crop systems. The Excel program was used to express the values. The significance of the correlation coefficients was obtained by comparing them with the r_{max} values (Erna Weber, 1961) for the 5%, 1% and 0.1% levels of transgression probabilities.

In the statistical calculation of all obtained values, the analysis of variance (Anova test) was used on the variation series. Statistical parameters were calculated using the formulas: $\bar{a} = \Sigma x/n$, where $\bar{a}$ = the average of the determinations, and x = the determined values, S^2 (variance) $= 1/(n-1)\ [\Sigma x^2-(\Sigma x)^2/n]$, S (standard error) $= \sqrt{S^2}$), S % (variation coefficient) $= S/\bar{a}.100$.

RESULTS AND DISCUSSIONS

Observations on climatic elements in a year with drought accents (Olsen et al., 2011). Regarding the monthly temperature regime, it was generally found that they exceeded the multiannual average by $2\text{-}4^0$ C (table 1). In a single month - May, the average value was slightly below normal. For the entire wheat vegetation, the average temperature achieved was $+3.2^0$ C. Under these conditions of excess heat (Mason et al., 2007) wheat plants showed either an acceleration of vegetation evolution in some plant morphological phases, or a relative stagnation of the deposition of reserve substances in grains (Eira & Caldas, 2000).

The precipitation regime that fell on the wheat vegetation experienced either slightly higher amounts close to normal, or at levels that induced the drought phenomenon. For the initial period, the month of September experienced a reduced level of rain, namely 120 mm. Under these conditions, both the soil water reserve and the establishment of wheat crops were favored. Against this background (Jones et al., 2010), the following periods of drought for wheat were in the months of

October, December and February. The months of November, January, March and April had ensured amounts of water that generally created somewhat more favorable conditions for the growth and development of wheat plants. In order to characterize the wheat crop year in more detail, the hydro-climatic index was used. From the data presented, it is found that for multiannual values, wheat has ensured water factor from sowing to February. For the period of intensive growth and maturity, the values are at sub-optimal levels. In the analyzed crop year, favorable situations were observed only in the months of November, January and March. Drought clearly set in during May and June. However, it is considered that for this year wheat benefited from a relatively good supply through the water reserve accumulated before the sowing period and through the periodic rains in January, March and April.

Table 1. Climatic factors evolution from winter wheat vegetation// *Evoluția factorilor climatici în perioada de vegetație a grâului de toamnă*

	Temperatures t^{0}C		Precipitations. mm		Hidro-climatic indices, %	
	Multi	2023-2024	Multi	2023-2024	Multi	2023- 2024
Oct.	11.1	15.1	45	7	**125**	19
Nov.	5.5	7.2	51	**67**	**283**	**112**
Dec.	0.7	3.7	44	18	**157**	97
Jan.	-1.1	0.8	40	**70**	**134**	**277**
Feb.	0.7	7.3	38	13	**136**	46
Mar.	4.8	8.1	37	**42**	92	**104**
Apr.	11.0	14.6	54	**45**	75	63
May	16.3	15.8	80	15	83	16
Jun.	19.5	24.7	91	16	80	14
Mean Sum	7.61	10.81	480	293	129.4	83,1
±		+3.20	±	-190	±	-46.3

HI* % = P mm/ETP.100 (ETP, evapotranspiration potential)

Variability of wheat straw dimensions. The wheat stalk or straw has a configuration with several internodes (usually 5-7) with their lengths increasing towards the ear. The actual straw has lengths between 50(60) cm and 150 cm.

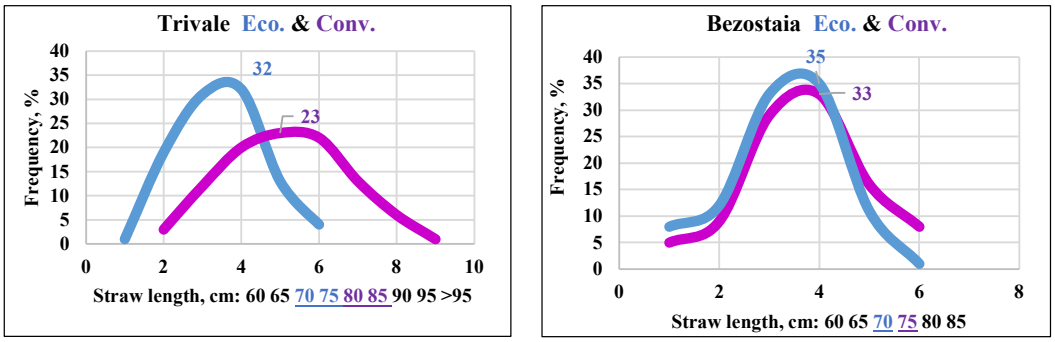


Fig. 1a- 1b. Frequencies of wheat straw length. // *Frecvențele lungimii paiului de grâu*

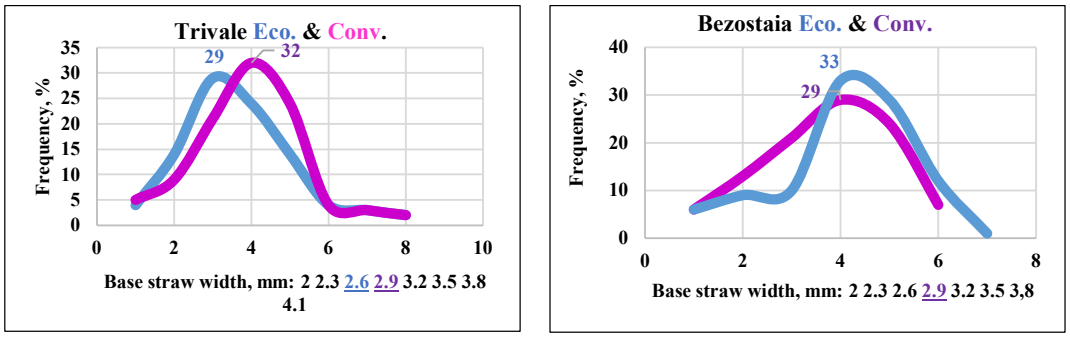


Fig. 2a- 2b. Frequencies of wheat straw diameter// *Frecvențele diametrului paiului de grâu*

At harvest maturity, the stems have a vertical position, with a relatively high task in both varieties. The measurements showed that the straw in the Trivale-Conv. variety was between 65 cm and over

95 cm. The straw of 80-85 cm (23%) had the highest frequency (figure 1a). In the case of Trivale-Eco. the straw had dimensions between 60 cm and 80 cm, with a maximum between 70-75 cm (32%). In the Bezostaia variety, the straws demonstrated a similarity between the systems, regarding length. These ranged from 60 to 85 cm, with the highest frequencies at 70-75 cm (33% and 35%, respectively) (figure 1b).

Regarding the thickness of the straw at the basal internode, specific situations were found depending on the crop system. Thus, in the Trivale variety, the straws had a diameter at the base between 2 mm and 4.1 mm (figure 2a). This size in the Eco. system was dominant at 2.6 mm, compared to Conv. in which 2.9 mm diameter met the highest percentages. The Bezostaia variety demonstrated somewhat smaller straw diameters, between 2 mm and 3.8 mm. The dominants were at the same thickness, namely 2.9 mm with 29% in Conv. and 33% in Eco. From the data in figure 2b, the straw thickness values were somewhat higher in the Eco system. In the images of the two varieties cultivated in the Conv. system, the specific appearance of the plants and ears in the post-flowering period is observed (figure 3a and 3b).



Fig. 3a- 3b. The two varieties of winter wheat, Trivale and Bezostaia, Conv. System // *Aspectul în cultură al celor două soiuri de grâu, Trivale și Bezistaia în sistemul Conv.*

Variability of wheat ears. The appearance and dimensions of the ear of these wheat varieties are characteristic, namely depending on the cropping system practiced. Thus, the length of the ear of the Trivale Conv. variety was between 7 and 11 cm. The length of 9 cm dominated (36%) (figure 4a). In the Eco system, the ears were shorter, between 5 and 10 cm, and the dominant ones were the ears with lengths of 7 cm (38%).

The variability of the length of the ears of the Bezostaia variety demonstrated the same limits: between 5 and 11 cm. In the Conv system, the ears with 8 cm length dominated (45%), while in the Eco system, the ear lengths with 7 cm length (57%) were highlighted (figure 4b).

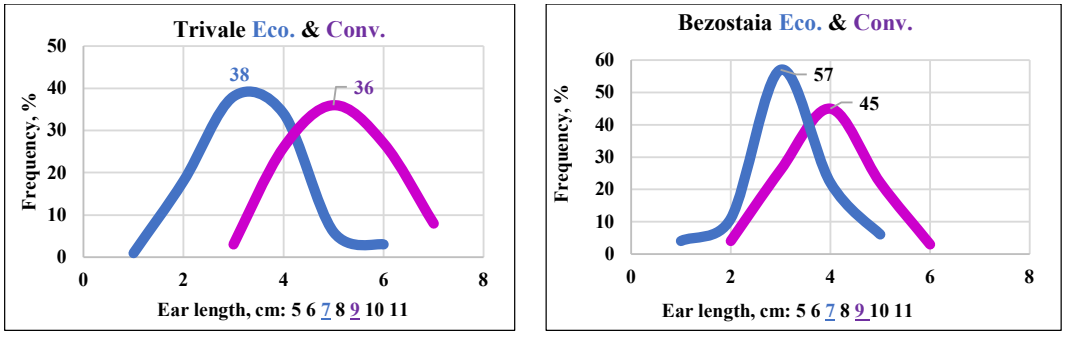


Fig. 4a-4b. Frequencies of wheat ear length. // *Frecvențele lungimii spicului de grâu*

Wheat ear weight showed a more obvious differentiation between the two winter wheat varieties. The Trivale variety formed ears with weights between 1.5 g and over 4 g. (Figure 5a). Spikes with 2.5 g biomass (30%) were dominant. In the Eco system, the Trivale variety formed spikes weighing between 1g and 3g, of which those with 1.5 g dominated. There is a separation of the heavier spikes

of the Trivale variety in the Conv. system towards weights of 3g and even 4g. The Bezostaia variety formed similar weights as a range of spike biomass (figure 5b). In the Conv. system, spikes with 2g weight (34%) dominated, while in the Eco system, the most obvious spikes (41%) were at 1.5 g. The graph also shows that the spikes in the Conv. system had an intention to move towards 2.5 g and even 3g.

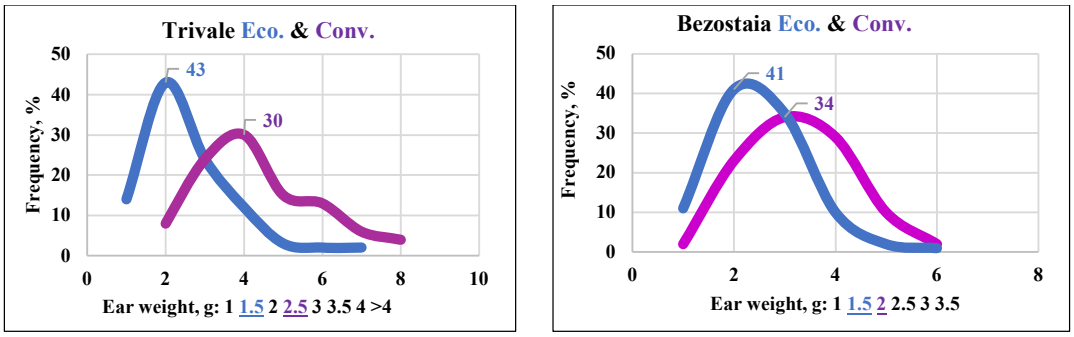


Fig. 5a-5b. Frequencies of wheat ear weight. // *Frecvențele greutății spicului de grâu*

Variability of the absolute weight of wheat grains. The mass of a thousand grains (MMB) showed slight differences in terms of variability (Trivale figure 6a and Bezostaia figure 6b). An analysis of the evolution of the absolute weight of the grains of the two wheat varieties shows the differentiation of the values towards higher thresholds through the Eco system. In the Trivale variety, the grains ranged between 25 g and over 50g (figure 7a). In the Conv. system, MMB was between 25g and 50g, with a maximum frequency at 35g (27-32%). In the Eco. system, the values were wider (as seen above), with a maximum at 40g (53%), as dominance.



Fig. 6a- 6b. The aspect of two wheat varieties from Eco. Systems // *Aspectul spicelor și al boabelor celor două soiuri de grâu cultivate în sistemul Eco.*

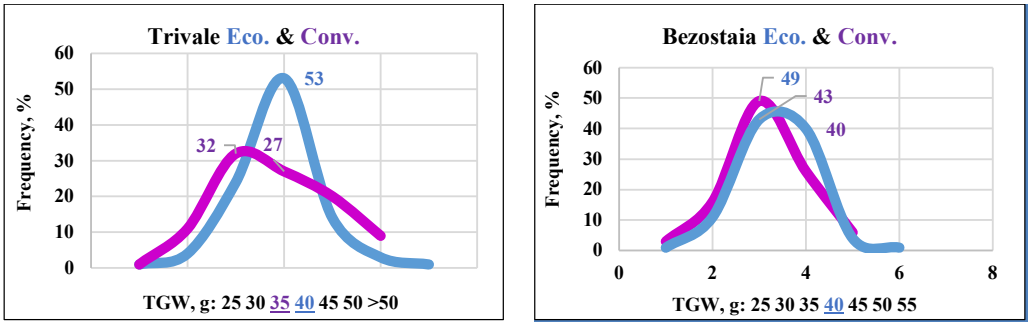


Fig. 7a-7b. Frequencies of TGW wheat grains // *Frecvențele masei a o mie de boabe.*

In the Bezostaia variety, the MMB limits were between 25g and 55g (figure 7b). And in this variety, the same thing was observed in a similar way: the grains obtained in the Eco system. tended to outpace those in the Conv system. The highest frequencies were obtained in both systems at 40g,

with frequencies higher than 49% in the Conv system. and also 40 g with higher frequencies at 40-43%.

Correlations between the main characters. The correlations obtained demonstrated positive aspects in their vast majority and with very obvious significance. This is the case of the two winter wheat varieties cultivated in the Eco system.

Thus, correlation values between 0.203 and 0.995 highlight the favorability of cultivating the Trivale variety in this new Eco system (table 2). By comparison with this, in the Conv. system a series of 6 negative correlations were obtained, in particular between straw length with: ear length (-0.065), grain weight (-0.062) and with the mass of a thousand grains (-0.153).

Table 2. Correlations between morphological characters of Trivale wheat from conventional and ecological systems// *Corelații între caracterele morfologice ale soiului de grâu Trivale cultivat în sistemele Conv. și Eco.*

Indices	Ear length, cm	Ear weight, g	No.grains/ ear	Grains weight, g	TGW*, g	Straw length, cm
	Trivale Conv.					
Ear weight, g	.709	1				
No. grains	.773	.933	1			
Grains weight, g	.703	.976	.934	1		
TGW, g	.394	.754	.555	.803	1	
Straw length, cm	-.065	-.048	.014	-.062	-.153	1
Straw diameter, mm	.322	.369	.387	-.079	.254	-.079
	Trivale Eco.					
Ear weight, g	.827	1				
No. grains	.843	.954	1			
Grains weight, g	.813	.995	.955	1		
TGW, g	.306	.525	.287	.541	1	
Straw length, cm	.551	.526	.547	.523	.203	1
Straw diameter, mm	.437	.490	.438	.483	.365	.256
LSD	(5%)- (0.195) (1%)- (0.254) (0.1%)- (0,32)					

TGW*, thousand grains weight

Table 3. Correlations between morphological characters of Bezostaia variety from Conv. and Eco. Sysstems // *Corelații între caracterele morfologice ale soiului Bezostaia cultivat în sistemele Conv. și Eco.*

Indicii	Length ear, cm	Ear weight, g	No.grains/ ear	Grains weight, g	TGW, g	Straw length, cm
	Bezostaia Conv.					
Ear weight, g	.719	1				
Nr. grains	.738	.953	1			
Grains weight, g	.693	.996	.950	1		
TGW, g	.407	.702	.488	.718	1	
Straw length, cm	**-.091**	.094	.063	.099	.194	1
Straw width, mm	.038	.383	.363	.384	.343	.079
	Bezostaia Eco.					
Ear weight, g	.823	1				
Nr. grains	.790	.961	1			
Grains weight, g	.804	.992	.950	1		
TGW, g	.020	.091	.488	.094	1	
Straw length, cm	.249	.298	.063	.312	.240	1
Straw width, mm	.412	.525	.363	.500	.220	.196
LSD	(.05)- (.195) (.01)- (.254) (.001)- (.320)					

Regarding the correlation values between 0.196 and 0.992, they also highlight the favorability of cultivating the Bezostaia variety in this new Eco system (table 3). By comparison, in the Bezostaia Conv. system, a single negative correlation was obtained, especially between straw length and spike length (-0.091).

From a comparative point of view, it is found that in the case of both wheat varieties, the stochastic links between the main morphological characters presented both positive and negative values through the Conv. system, while in the Eco. system these correlations were entirely favorable, positive and very well assured statistically (Wolfe et al., 2008).

Statistical analysis of the morphological characters variability. The results obtained in the morphological analysis of morphological characters in winter wheat showed specific aspects, in both

varieties and in both crop systems. Thus, in the Trivale variety the average straw length in the Conv. system was 9 cm higher than in the Eco. system, both with small variabilities (below 10%) (table 4).

Table 4.Statistical indices of some Trivale wheat plants morphology: straw, ear, base glume // Indicii statistici ai unor caractere morfologice la soiul de grâu Trivale: paiul, spicul, gluma inferioară

Indices	Straw length, cm	Ø straw, mm	Ear length, cm	Ear weight, g	No. spikelets	Glume length, mm
	Trivale Conv.					
Mean ā	**78.93**	**2.80**	**8.61**	**2.446**	**17.7**	**9.23**
Variance S^2	55.86	0.15	1.06	0.693	4.34	0.34
Standard deviation, S	7.474	0.39	1.03	0.832	2.08	0.59
Coeff.variability, CV,%	**9.47**	**13.9**	**12.0**	**34.03**	**11.8**	**6.36**
	Trivale Eco.					
Mean ā	**70.28**	**2.62**	**6.86**	**1.584**	**15.0**	**8.29**
Variance S^2	30.04	0.14	1.18	0.375	6.36	0.38
Standard deviation, S	5.481	0.37	1.09	0.613	2.52	0.62
Coeff.variability, CV,%	**7.80**	**14.3**	**15.8**	**38.71**	**16.9**	**7.43**

Table 5.Statistical indices of some Trivale wheat plants morphology: external palea, grains and TGW// *Indicii statistici ai unor caractere morfologice la soiul de grâu Trivale: palea externă, boabele şi MMB*

Indices	Palea length, mm	Grain no./ ear	Grain weight, g	Grain length, mm	Grain thick, mm	TGW, g
	Trivale Conv.					
Mean ā	**10.3**	**49.35**	**1.832**	**6.38**	**2.82**	**36.63**
Variance S^2	0.51	152.3	0.475	0.27	0.11	11.60
Standard deviation, S	0.71	12.34	0.689	0.52	0.33	3.405
Coeff.variability, CV,%	**6.91**	**25.01**	**37.61**	**8.12**	**11.9**	**9.296**
	Trivale Eco.					
Mean ā	**9.24**	**31.69**	**1.195**	**5.96**	**2.77**	**37.27**
Variance S^2	0.53	116.3	0.229	0.18	0.02	20.27
Standard deviation, S	0.73	10.79	0.478	0.42	0.13	4.502
Coeff.variability, CV,%	**7.89**	**34.04**	**40.05**	**7.07**	**4.83**	**12.08**

Table 6.Statistical indices of some Bezostaia wheat plants morphology: straw, ear, base glume / *Indicii statistici ai unor caractere morfologice la soiul de grâu Bezostaia: paiul, spicul, gluma inferioară*

Indices	Straw		Ears			
	Length, cm	Width, mm	Length, cm	Weight, g	No. spikelets	Glume, mm
	Bezostaia Conv.					
Mean ā	**71,6**	**2,75**	**7,50**	**1,95**	**18,1**	**8,51**
Variance S^2	22,5	0,05	0,66	0,18	3,83	0,24
Standard deviation, S	4,74	0,23	0,81	0,43	1,96	0,49
Coeff.variability, CV,%	**6,63**	**8,25**	**10,84**	**21,75**	**10,89**	**5,75**
	Bezostaia Eco					
Mean ā	**69,4**	**2,84**	**6,69**	**1,52**	**16,2**	**8,07**
Variance S^2	37,6	0,16	0,64	0,14	7,47	0,31
Standard deviation, S	6,13	0,40	0,80	0,39	2,73	0,56
Coeff.variability, CV,%	**8,83**	**14,07**	**11,92**	**24,21**	**16,84**	**6,90**

The straw thickness decreased in the Eco system by 0.2 mm, with average variability of the character (13.9-14.3%). In the same sense, the spike decreased in length by 1.7 cm, and both had average variability. The weight of the spike was lower by 0.8g in conditions of very high variability (34-38%). The number of spikelets was reduced by 2.7 at an average variability. The dimensions of the grain membranes: the glumes decreased in length by 1 mm, with low variability, and the blades were reduced very little, to 1 mm (table 5). The number of grains in an ear was reduced in the Eco system by 18 against a background of very high variability. The dimensions of the grains of the Trivale variety: the length and thickness had significant decreases, and the MMB was higher in the Eco system by 0.6 g at a low to average variability.

The characters of the Bezostaia variety demonstrated significant reductions in the morphological characters studied. Thus, the straw length decreased by only 2 cm under conditions of straw thickness greater by 0.1 mm in the Conv. system compared to the Eco. system and with similar, small and medium variabilities (table 6). The length of the spikelets decreased by 0.8 cm, and the weight by 0.4 g. The number of spikelets decreased by 2, the jokes being significantly equal. The palea decreased very little, while the number of grains per spike decreased by 10. In the Conv. system, the Bezostaia variety had an average of 37 grains, while in the Eco. system this number decreased to 28 (table 7).

The variability was greater in the Eco system. The weight of the grains per spike decreased by 0.4 g. The grain dimensions showed significant decreases in length and increases of 0.1 mm in thickness. MMB was 1.2 g higher in the Eco system.

Table 7.Statistical indices of some Trivale wheat plants morphology: external palea, grains and TGW// *Indicii statistici ai unor caractere morfologice la soiul de grâu Bezostaia: palea externă, boabele şi MMB*

Indices		Grains				
	Palea, mm	No. grains/ ear	Grains weight, g	Grain length, mm	Grain width, mm	TGW, g
		Bezostaia Conv.				
Mean ā	**10,18**	**37,26**	**1,48**	**6,27**	**2,62**	**38,38**
Variance S^2	0,47	23,0	0,01	0,12	0,03	5,36
Standard deviation, S	0,69	4,79	0,10	0,35	0,18	2,32
Coeff.variability, CV,%	**6,75**	**12,86**	**6,67**	**5,55**	**6,92**	**6,03**
		Bezostaia Eco				
Mean ā	**9,71**	**27,94**	**1,10**	**5,93**	**2,74**	**39,62**
Variance S^2	0,55	90,0	0,12	0,15	0,08	14,93
Standard deviation, S	0,74	9,49	0,35	0,39	0,28	3,86
Coeff.variability, CV,%	**7,61**	**33,95**	**31,94**	**6,61**	**10,04**	**9,75**

Aspects of the quality of wheat grains grown in the Conv. and Eco systems. Under the conditions of this year, the expression of quality indices (Hannell et al., 2004; Mader et al., Doltra et al., 2011) was somewhat better. Thus, the crude protein exceeded 12% in all cropping systems, which placed both the Trivale variety and the Bezostaia variety in the first quality, with one exception. The PB content of the Bezostaia variety in the Eco. system placed the grains in the second quality (below 12%). Starch was appropriate in all four situations: varieties and cropping systems. Wet gluten had better quality only in the Conv. system in both varieties, and in the Eco. system in both varieties it was unsatisfactory. The fourth character, the Zeleny sedimentation index, had good values in the Conv. system in the Trivale variety and in the Eco system. at Bezostaia (table 8).

Table 8. Quality indices of Trivale and Bezostaia varieties from the two crop systems// Indicii de calitate obținuți la soiurile Trivale și Bezostaia în cele două sisteme de cultură

System	Grain humidity, %	Crude Protein, %	Starch, %	Wet Gluten, %	Green index (Zeleny, ml)
	Trivale variety				
Conventional	12.6	**12.5**	**69.1**	**24.0**	39.7**
Ecological	15.0	**12.9**	**71.2**	18.8	22.0*
	Bezostaia variety				
Conventional	15.1	**13.6**	**71.6**	18.0	21.0*
Ecological	15.4	10.9	68.4	20.9	37.6**
Observations		Cal.I >12.0%	69.2-72.8 %, good conținut	Minim 22 %, bakery good	Not so good* Good**

It is possible that, given the specific climatic background with periods of drought, existing in the wheat crop, accumulations of nutrients in the grains have occurred, at somewhat better levels, even at higher categories.

CONCLUSIONS

1. The results of this analysis were obtained in a better climatic year: the temperatures during the wheat growing season were somewhat higher, and the rainfall received by the plants of the two wheat varieties was more inconstant. The hydro climatic index better expressed the fact that in three months the wheat benefited from normal rainfall, in two it rained with over 50% of the necessary amount, and in 4 months there were periods of drought. Due to the existing climatic inconstancy, specific morphological reactions were observed in the experiments carried out.
2. Among the morphological characters obtained, there were generally negative differences for the Eco. cultivation system conditions, in the case of both varieties used: Trivale and Bezostaia. There were also some exceptions. Thus, the thickness of the straw at the base of Bezostaia in the Eco system showed more obvious thickening tendencies compared to the Conv. system, and the

mass of a thousand grains expressed higher values in both varieties in the Eco system. From the point of view of improvement, these Eco. cultivation tendencies could also be used in the seed production system.

3. From the study of the correlations between the most important characters, very significant positive situations were found in both varieties in the Eco cultivation system. It is possible that through this cultivation system in the two varieties there was a greater harmonization between the growth and development factors of the plants.
4. The calculation of the studied statistical indices clearly highlighted the differences due to the two crop systems: Conv. and Eco. These calculations generally revealed negative differences in the case of the Eco system, for both varieties. The clearer differentiation between the two crop systems was observed in the case of the Trivale variety. In the case of the Bezostaia variety, the differences between the crop systems were relatively smaller, which leads to a practical advantage by which this very old variety is equally well suited to the Eco crop system. In addition, the thickness of the straw in the Bezostaia variety had larger dimensions, which highlights an increased resistance to plant fall.
5. The mass of a thousand grains demonstrated the achievement of higher values in the Eco system. compared to the Conv system. This actually demonstrated the favoring of this particularly important index in the possibilities of the increasingly broad promotion of the two varieties in the new system of sustainability of the culture environment.
6. The quality of the grains in the case of the two traditional wheat varieties was at relatively high levels, normal and good for baking. For quality, both the Trivale and Bezostaia varieties were also favored by the variability of the three vegetation factors: the warm thermal regime, the relatively sufficient precipitation on a background of water reserves in the soil, from the cold season, and the hydroclimatic index that better demonstrated the passage of the wheat plants this year, of periods with increased drought.
7. The present study demonstrated certain research performances of environmental sustainability by comparing morphological and quality characters of two old varieties, in two farming systems, Conv. and Eco. In addition, this study could also find some aspects of resilience and even tolerance to biotic and abiotic stress, demonstrated by both varieties.

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RESEARCHES ON THE EFFECTS OF CLIMATE CHANGE ON SUNFLOWER, MAIZE AND SORGHUM IN THE CONDITIONS OF THE SOUTH DOBROGEA PLATEAU

CERCETĂRI PRIVIND EFECTELE SCHIMBĂRILOR CLIMATICE LA FLOAREA-SOARELUI, PORUMB ȘI SORG ÎN CONDITIILE PODIȘULUI DOBROGEI DE SUD

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ABSTRACT

Climate change is a phenomenon affecting the plant growth and level, variability and quality of the obtained yields. Therefore, both researchers and farmers are interested to find the most suitable ways to adapt to the present climatic context. Starting from these ideas, the aim of this paper is to present the effects of climate change on sunflower, corn and sorghum in the conditions of the South Dobrogea Plateau, with an emphasis on the effects of hybrid and planting time. Research was performed under rainfed conditions, in the fields of S.C. SPORT AGRA S.RL. located in Amzacea Village, Constanța County, in the South Dobrogea Plateau (South-East of Romania). There were studied 3 crops (sunflower, corn and sorghum) in two years (2022 and 2024, except corn which was cultivated only in 2024), for each crop being tested several hybrids planted at different dates.

Under rainfed conditions, the actual climatic context affects significantly the spring crops (sunflower, corn, sorghum) yielding capacity in South Dobrogea Plateau (South-East of Romania). Among the studied crops, corn is by far the most affected by the climatic changes from the last years. In a context of less favourable climatic conditions, characterised by high temperatures and drought in the vegetation period of the cultivated plants, it becomes essential to choice the most suitable variety for cultivation and to adapt the crop technology. Adaptation of crop technology could be made through several ways, among which the early planting, as soon as the minimum germination temperature for each crop is reached in the soil, is essential. Generally, delaying the planting time is associated with important yield losses to all spring crops.

REZUMAT

Schimbarea climatică este un fenomen care afectează creșterea plantelor și nivelul, variabilitatea și calitatea recoltelor obținute. Prin urmare, atât cercetătorii, cât și fermierii sunt interesați să găsească cele mai potrivite modalități de adaptare la contextul climatic actual. Pornind de la aceste idei, scopul acestei lucrări este de a prezenta efectele schimbărilor climatice la floarea-soarelui, porumb și sorg în condițiile Podișului Dobrogei de Sud, cu accent pe efectele hibridului și momentului semănatului. Cercetările au fost efectuate în condiții de neirigat, pe terenurile S.C. SPORT AGRA S.RL. situate în satul Amzacea, județul Constanța, în Podișul Dobrogei de Sud (sud-estul României). Au fost studiate 3 culturi (floarea-soarelui, porumb și sorg) în doi ani (2022 și 2024, cu excepția porumbului care a fost studiat numai în 2024), pentru fiecare cultură fiind testați mai mulți hibrizi semănați la date diferite.

În condiții de neirigat, contextul climatic actual afectează semnificativ capacitatea de producție a culturilor de primăvară (floarea-soarelui, porumb, sorg) în Podișul Dobrogei de Sud (sud-estul României). Dintre culturile studiate, porumbul este de departe cel mai afectat de schimbările climatice din ultimii ani. Într-un context climatic mai puțin favorabil, caracterizat prin temperaturi ridicate și secetă în perioada de vegetație a plantelor cultivate, devine esențială alegerea celui mai potrivit cultivar și adaptarea tehnologiei de cultivare. Adaptarea tehnologiei de cultivare se poate face prin mai multe metode, printre care semănatul timpuriu, imediat ce se atinge temperatura minimă de germinare în sol corespunzător plantei cultivate, este esențială. În general, întârzierea perioadei de semănat este asociată cu pierderi importante de producție la toate culturile de primăvară.

Key words: *climate change, sunflower, corn, sorghum, hybrids, planting time.*
Cuvinte cheie: *schimbări climatice, floarea-soarelui, porumb, sorg, hibrizi, momentul semănatului.*

INTRODUCERE

Climate change is a global phenomenon that poses significant challenges to various sectors including agriculture, rising temperatures, altered precipitation patterns and more frequent extreme weather events transforming the agricultural landscape and posing risks to global food security (Vijai et al., 2023). Climate changes characterized by higher temperatures, extreme climatic hazards and low water for agriculture determine the extension of the areas affected by drought, the agriculture being the most affected by the climate variability and the extreme meteorological phenomenon (Prodan et al., 2021). Climatic variability is a major cause of inability of agricultural crops to achieve potential yield (Agele, 2003). Therefore, understanding crop responses to climate change is crucial for ensuring food security (Hu et al., 2024).

Climate is an important source for the variability of the length of the growth period of the crops (Bran and Ion, 2020), and finally of the variability concerning the obtained yield. The unprecedented rise in temperature has led to an increase in the incidence of heat waves, droughts, floods, and irregular patterns of precipitation; these changes have a dramatic impact on prevailing agricultural cropping systems, productivity, and food security of people regionally and globally (Alotaibi, 2023). Temperature affects the duration of all plant phenological stages (Alberio et al., 2015). Droughts occur naturally, but climate change has generally accelerated the hydrological processes to make them set in quicker and become more intense, with many consequences (Mukherjee et al., 2018).

In Romania, Dobrogea region in South-East Romania is the most drought area, the Constanta County from this region having the largest area cultivated with sunflower crop (Manole et al., 2023). Thus, in Contanța County, sunflower is cultivated on 115-120 thousand ha, while the corn decreased from 55 - 65 thousand ha in 2022 to 27 thousand ha in 2024, and sorghum increased from 155 ha in 2022 to 200 ha in 2024.

Sunflower is a temperate zone crop which can perform well under various climatic and soil conditions (Qadir et al., 2007). This is a versatile crop, which can grow on a wide range of soils in many latitudes and are tolerant of dry conditions (Myers, 2017). If drought resistant cultivars are developed, sunflower can be grown successfully in areas where water is a limiting factor (Farzad et al., 2013). Although sunflower is a moderately drought tolerant crop, severe drought causes reduction in the seed and oil production; therefore, to ensure sustainable sunflower achene and oil yields, it is very important to understand the relationship among the physiological, biochemical, genetic and agronomic basis of drought for its sustainable management (Hussain et al., 2018).

Corn's ecological flexibility makes it "the plant of choice" for grain and feed in climates raging from temperate to tropical as long as there is no frost and mean temperatures are mostly above 10^{o}C (Haraga and Ion, 2022). The great variety of genotypes and the high ecological plasticity made it possible to cultivate corn in different conditions in terms of soil, climate, and relief (Kusmec et al., 2018). Identifying corn hybrids with genetically improved characteristics and high level of adaptability in order to have low yielding losses is indeed relevant (Schitea and Motca, 2013).

Sorghum is one of the World's major cereal crops, it being valued for its grain, stalks and leaves; among different forms, grain sorghum is the most commonly cultivated agronomic type of sorghum worldwide (Dahlberg, 2011). It is particularly adapted to drought prone areas: hot, semi-arid tropical environments with 400-600 mm rainfall-areas that are too dry for other cereals (Dicko et al., 2006). Sorghum is tolerant to water stress at different stages of growth, but the water deficiency may exert its effect on yield and yield components (Abdel-Motagally, 2010).

Variety selection is one of the most important decisions a farmer makes each year for each planting area (Koonsanit et al., 2011). Planting time is important to improve the adaptation of cultivars to environmental conditions and to achieve high seed yields (Butkevičienė et al., 2021). Starting from these ideas, the aim of this paper is to present the effects of climate change on sunflower, corn and sorghum in the conditions of the South Dobrogea Plateau (in South-East Romania), with an emphasis on the effects of hybrid and planting time.

MATHERIAL AND METHOD

Research was performed under rainfed conditions, in the fields of S.C. SPORT AGRA S.RL. located in Amzacea Village, Constanța County, in the South Dobrogea Plateau (South-East of Romania).

There were studied 3 crops (sunflower, corn, and grain sorghum) in two years (2022 and 2024, except corn which was cultivated only in 2024), for each crop being tested several hybrids planted at different dates.

The soil where the research was performed is a cambic chernozem with a deeper profile than other chernozems and a blackish-brown layer of 40-50 cm thickness. The soil is characterised by the following characteristics: medium texture; nitrogen index of 3,34-4; phosphorus content of 72 mg/kg; potassium content of 140 mg/kg; humus content of 3.11%; neutral pH (7.2 value).

Constanta County has temperate continental climate, being characterised by an average annually air temperature of 10.8ºC and an average annually rainfall of 434 mm (Table 1). In the last 10-20 years, the weather were characterised by frequent droughts and high temperatures in the summer period.

Table 1. Average climatic conditions on 69 years in Dobrogea area (S.C.D.A. Valu lui Traian) / Condițiile climatice ca valori medii pe 69 de ani în zona Dobrogea (S.C.D.A. Valu lui Traian)

Month	Rainfall (mm)	Temperature (ºC)
January	58.9	-0.6
February	24.0	0.7
March	25.4	4.0
April	32.1	9.6
May	39.0	15.3
June	47.8	19.5
July	33.8	21.7
August	33.0	21.2
September	29.9	17.0
October	34.2	11.8
November	41.2	6.5
December	34.7	2.1
Annual sum/average	**434**	**10.8**

The water reserve from September to December 2021, for the year 2022, was of 296.5 mm, and the reserve in the same period, but for the year 2024, was of 168.6 mm (Tables 1 and 2). In the period March-July, the cumulated rainfall was of 158 mm in 2022 and of 267.5 mm.

The year 2022 was characterised by drought in May (with only 14 mm rainfall) and by severe drought in July and August (with only 10 mm rainfall in two months) (Table 2). In total, in the year 2022, the rainfall amount was of 336.5 mm.

The year 2024 was characterised by rainy periods in April (118 mm rainfall) and August (186 mm rainfall), but with drought in June and July (Table 3). In total, in the year 2024, the rainfall amount was of 751.5 mm.

For all the three study crops (sunflower, corn, sorghum), the preceding crop was winter wheat. After harvesting the wheat, the soil was loosened to a depth of 23-25 cm without turning the furrow. After that soil tillage, the soil was kept free of weeds by applying an herbicide based on glyphosate. Before winter, there were performed a soil work with a combinatory. In spring, the soil was worked before planting with a combinatory.

The weed control was performed by using herbicides according to crop, the diseases were control at sunflower crop, and the pest were control by using insecticides at sorghum crop (Table 4). For all the three crops, after 10-12 days from the herbicide application in the vegetation period (Table 4), there were performed the first mechanical weeding, and with the second mechanical weeding there was applied the ammonium nitrate fertilizer (34% N) in a rate of 200 kg/ha for sunflower and sorghum and 370 kg/ha for corn.

Table 2. Climatic conditions in the period September 2021 – December 2022 in Amzacea / Condițiile climatice în perioada septembrie 2021 – decembrie 2022 la Amzacea

Months in 2021					Months in 2022												
09	10	11	12	**Amount**	01	02	03	04	05	06	07	08	09	10	11	12	**Amount**
40	115	49	92.5	**296.5**	19	40	42	46,5	14	45,5	10	3	69,5	0	26	21	**336.5**

Table 3. Climatic conditions in the period September 2023 – December 2024 in Amzacea / Condițiile climatice în perioada septembrie 2021 – decembrie 2022 la Amzacea

Months in 2023					Months in 2024												
09	10	11	12	**Amount**	01	02	03	04	05	06	07	08	09	10	11	12	**Amount**
0	2.4	119.2	47	**168.6**	29	2.2	39.5	118	68	13.5	28.5	186	63	57	56	90.4	**751.1**

Table 4. Synthetically data on crop technology of the three analysed crops in 2022 and 2024 / Date sintetice cu privire la tehnologia de cultură la cele 3 culturile analizate în 2022 și 2024

Crop	2022	2024

Sunflower	- Herbicides applied in pre-emergence:
	  - Efica 960 EC (S-Metolachlor 960 g/l) in rate of 1.5 l/ha;
	  - Racer 25 EC (Fluorocloridon 250 g/l) in rate of 2 l/ha.
	- Herbicides applied in vegetation period:
	  - Trimmer 50 WG (Tribenuron-methyl 500 g/kg) in rate of 0.03 kg/ha, applied only at the resistant hybrids.
	- Fungicides applied in vegetation period:
	  - Mirage 45 EC (Prochloraz 450 g/l) in rate of 1 l/ha applied in the growth stage of 6-8 leaves;
	  - Pictor (Dimoxystrobin 200 g/l + Boscalid 200g/l) in rate of 0.5 l/ha applied in the growth stage of flower bud appears.
	- Planting density: 64,250 seeds/ha.
	- Harvesting date: 23 of August.
		- Herbicides applied in pre-emergence:
		  - Efica 960 EC (S-Metolachlor 960 g/l) in rate of 1.5 l/ha;
		  - Racer 25 EC (Fluorocloridon 250 g/l) in rate of 2 l/ha.
		- Herbicides applied in vegetation period:
		  - Pulsar Plus 25 EC (Imazamox 25 g/l) in rate of 2 l/ha, applied only at the resistant hybrids;
		  - Trimmer 50 WG (Tribenuron- methyl 500 g/kg) in rate of 0.03 kg/ha, applied only at the resistant hybrids.
		- Fungicides applied in vegetation period:
		  - Pictor (Dimoxystrobin 200 g/l + Boscalid 200g/l) in rate of 0.5 l/ha applied in the growth stage of flower bud appears.
		- Planting density: 64,500 seeds/ha.
		- Harvesting date: 14 of August.

Corn	-	- Herbicides applied in pre-emergence:
		  - Efica 960 EC (S-Metolachlor 960 g/l) in rate of 1.5 l/ha.
		- Herbicides applied in vegetation period:
		  - Pyxides (Dicamba 312.5 g/kg + Mesotrione 150 g/kg + Nicosulfuron 100 g/kg) in rate of 0.6 kg/ha applied in the growth stage of 4-6 leaves.
		- Planting density: 72,000 seeds/ha.
		- Harvesting date: 14 of August.

Sorghum	- Herbicides applied in pre-emergence:
	  - Efica 960 EC (S-Metolachlor 960 g/l) in rate of 1-1.5 l/ha.
	- Herbicides applied in vegetation period:
	  - Dicopur D (600 g/l acid 2.4 D from Dimethylamine salt) in rate of 1 l/ha applied in the growth stage of 3-4 leaves.
	- Insecticides applied in vegetation period for controlling *Schizaphis graminum*:
	  - Lamdex Extra (Lambda- cyhalothrin 25 g/kg) in rate of 0.30 kg/ha.
	- Planting density: 262,000 seeds/ha.
	- Harvesting date: 23 of August.
		- Herbicides applied in pre-emergence:
		  - Efica 960 EC (S-Metolachlor 960 g/l) in rate of 1-1.5 l/ha.
		- Herbicides applied in vegetation period:
		  - Basagran SL (Bentazona 480 g/l) in rate of 2 l/ha + Dicopur Top 464 SL (344 g/l acid 2.4 D from Dimethylamine salt + 120 g/l Dicamba) in rate of 1 l/ha applied in the growth stage of 3-4 leaves.
		- Insecticides applied in vegetation period for controlling *Schizaphis graminum*:
		  - Lamdex Extra (Lambda-cyhalothrin 25 g/kg) in rate of 0.30 kg/ha.
		- Planting density: 280,000 seeds/ha.
		- Harvesting date: 20 of August.

The seeds for all the three crops were treated with Cruiser 350 FS (Thiamethoxam 350 g/l) in a rate of 10 l/t for sunflower, 9 t/t for corn and 8 l/t for sorghum, for controlling especially the pests *Tanymecus dilaticolis* and *Agriotis* spp. At sunflower, the seed were also treated with the fungicide Apron XL 350 ES (Metalaxyl-M 350 g/l) in a rate of 3 l/t. The planting depth was of 5-6 cm for sunflower, 6-7 cm for corn and 4 cm for sorghum.

Together with planting, there were applied the complex fertilizer Granoro (N-10:P-20:K-0 + 4CaO + $20SO_3$ + 0.03Zn + 0.01B + 7.5C-Org 2N-Org) in a rate of 250 kg/ha in the year 2022, and the complex fertilizer Universal Up (N-9:P-30:K-0 + 6CaO + $11SO^3$) in a rate of 200 kg/ha in the year 2024.

There were tested several hybrids for each crop planted at different dates (Table 5), each hybrid being cultivated on a plot of 560 m^2.

Table 5. Synthetically data on planting date and tested hybrids of the three analysed crops in 2022 and 2024 / Date sintetice cu privire la data de semănat și hibrizii studiați la cele 3 culturi analizate în 2022 și 2024

Crop	2022		2024	
	Planting date	Tested hybrids	Planting date	Tested hybrids
Sunflower	March 26	- 6 hybrids with resistance at Tribenuron-Methyl: P64LE25; P64LE99; P64LE162; Suomi; Sureli; Aromatic - 13 hybrids with resistance at Imazamox: P64LP170; P64LP180; Odessa; Onestar; Michigan; Genesis; Janis; Anthemis; Acordis; Aluris; Dracaris; InSun 200; InSun 222	April 8	- 4 hybrids with resistance at Tribenuron-Methyl: Ceylon; P64LE99; P64LE162; P64LE280 - 7 hybrids with resistance at Imazamox: P64LP170; Genesis; Belfis; Coloris; InSun 222; Dragaris; Michigan
	April 8	- 2 sunflower hybrids with resistance at Imazamox: Genesis; In Sun 200		
Corn	-	-	April 8	- 7 hybrids: Maxxatac; Lipex; MyLady; LID3306; P8812; P8834; Chorintos
	-	-	April 20	- 1 hybrid: Maxxatac
Sorghum	March 26	- 3 hybrids: Anggy; Huggo; Icebergg	April 8	- 4 hybrids: Anggy; Legend; Feohn; Willy
	April 8	- 1 hybrid: Anggy	May 7	- 4 hybrids: Anggy; Legend; Feohn; Willy

RESULTS AND DISCUTIONS

In the two years of research, the climatic factors, especially air temperature and soil temperature, influenced the planting time and the growth phases, especially in the case of sunflower and corn crops, and less so in the case of grain sorghum.

In the year 2022, with a normal spring in terms of air temperature, the planting time was March 26, both in the case of sunflower and sorghum. In the year 2024, the late spring frosts that occurred in the last decade of March and the first decade of April made the planting time to be on April 08 for all the three studied crops, respectively sunflower, corn, and sorghum.

In the year 2022, the sunflower, as well as the sorghum developed well. At sunflower, the flowering process occurred in the period June 23 – 29, according to hybrid and planting time (Table 6). The plant height was in average of 188 cm for the resistant hybrids at Tribenurom-Methyl and of 179.5 cm for the resistant hybrids at Imazamox. Seed moisture content differed according to the hybrid, respectively with the precocity of the hybrid, and planting time. Hectolitre Mass was in average good, with values over 40 kg/100 l and a variation between 36.5 and 43.5 kg/100 l.

The grain yield registered good and very good values, being in average of 4358.7 kg/ha for the resistant hybrids at Tribenurom-Methyl and of 4430.4 kg/ha for the resistant hybrids at Imazamox (Table 6). For the resistant hybrids at Tribenurom-Methyl, the highest grain yield of 4608 kg/ha was registered at the Surely hybrid, while for the resistant hybrids at Imazamox the highest grain yield of 5260 kg/ha was registered at the InSun 222 hybrid.

Delaying the planting time from March 26th to April 08th delayed the flowering time, decreased the plant height, the Hectolitre Mass and the grain yield, and increased the seed moisture content at harvesting (Table 6). Delaying of planting time at Genesis hybrid decreased the grain yield by 63 kg/ha (1.4% yield decrease), while the delaying of planting time at InSun 200 hybrid decreased the grain yield by 852 kg/ha (19% yield decrease).

Table 6. Results obtained at sunflower in the year 2022 / Rezultate obținute la floarea-soarelui în anul 2022

Nr. Crt.	Hybrid	Planting date	Emergence date	Plants / m^2	Flowering date	Plant height (cm)	Seed moisture content (%)	Hectolitre Mass (kg/100 l)	Yield at 9% moisture (kg/ha)
Tribenurom-Methyl resistent hybrids									
1	P64LE25	26.03.2022	13.04.2022	6.5	28.06.2022	210	11.5	38.5	4525
2	P64LE99	26.03.2022	15.04.2022	6.5	28.06.2022	182	15.5	39.6	4590
3	P64LE162	26.03.2022	15.04.2022	6.0	28.06.2022	215	13.6	40.8	4258

Nr. Crt.	Hybrid	Planting date	Emergence date	Plants / m²	Flowering date	Plant height (cm)	Seed moisture content (%)	Hectolitre Mass (kg/100 l)	Yield at 9% moisture (kg/ha)
4	Suomi	26.03.2022	15.04.2022	6.5	21.06.2022	165	10.2	43	4322
5	Sureli	26.03.2022	13.04.2022	6.0	28.06.2022	180	12.9	41	4608
6	Aromatic	26.03.2022	15.04.2022	5.0	28.06.2022	176	12.1	40.4	3849
Average		**-**	**-**	***6.1***	**-**	***188.0***	***12.6***	***40.5***	***4358.7***
CV (%)		**-**	**-**	***8.77***	**-**	***9.68***	***13.21***	***3.40***	***6.03***
Imazamox resistent hybrids									
7	P64LP170	26.03.2022	13.04.2022	7.0	28.06.2022	171	12.1	36.5	4757
8	P64LP180	26.03.2022	13.04.2022	6.5	23.06.2022	192	12.7	38.5	4298
9	Odessa	26.03.2022	13.04.2022	6.0	23.06.2022	170	12.8	39.8	4766
10	Onestar	26.03.2022	13.04.2022	6.5	23.06.2022	174	10.8	43.3	4252
11	Michigan	26.03.2022	13.04.2022	6.0	28.06.2022	178	12.8	41	4336
12	Genesis	26.03.2022	13.04.2022	6.0	21.06.2022	168	8.6	42.6	4616
13	**Genesis**	**08.04.2022**	**27.04.2022**	**6.0**	**28.06.2022**	**165**	**9**	**41.4**	**4553**
14	Janis	26.03.2022	13.04.2022	6.0	23.06.2022	160	8.2	43.1	4364
15	Anthemis	26.03.2022	15.04.2022	6.0	28.06.2022	182	9.4	39.5	4401
16	Acordis	26.03.2022	13.04.2022	6.5	28.06.2022	180	12.7	37.8	4255
17	Aluris	26.03.2022	13.04.2022	6.5	29.06.2022	198	11	39	4112
18	Dracaris	26.03.2022	15.04.2022	6.0	23.06.2022	195	8.1	43.5	4368
19	InSun 200	26.03.2022	13.04.2022	6.0	29.06.2022	185	9.5	39	4485
20	**InSun 200**	**08.04.2022**	**28.04.2022**	**5.0**	**02.07.2022**	**183**	**12.1**	**37.1**	**3633**
21	InSun 222	26.03.2022	15.04.2022	6.5	28.06.2022	192	12.4	39.3	5260
Average		**-**	**-**	***6.2***	**-**	***179.5***	***10.8***	***40.1***	***4430.4***
CV (%)		**-**	**-**	***7.05***	**-**	***6.21***	***16.37***	***5.50***	***7.81***

For grain sorghum in 2022, the seed moisture content was below 15% but in the case of the Anggy hybrid planted later on April 8th, the registered seed moisture content was 16.8% (Table 7). The Hectolitre Mass was in average of 78.7 kg/100 l, with a variation from 77.7 to 79.7 kg/100 l, according to hybrid and planting time. The grain yield was in average of 6922.7 kg/ha, with a variation from 6418 kg/ha for the hybrid Icebergg and 7710 kg/ha for the hybrid Anggy planted on March 26th.

As in the case of sunflower, also for the sorghum the delaying the planting time from March 26th to April 08th increased the seed moisture content and decreased the Hectolitre Mass and the grain yield where registered (Table 7). The delaying of planting time at Anggy hybrid decreased the grain yield by 647 kg/ha (8.4% yield decrease).

Among the determined parameters, the highest coefficient of variation was registered for the seed moisture content, while the most stable one, with the smallest values of the coefficient of variation, was the Hectolitre Mass, both for sunflower and sorghum. The yield coefficient of variation for the sunflower hybrids resistant at Tribenurom-Methyl was of 6.03%, for the sunflower hybrids resistant at Imazamox it was of 7.81%, and for the sorghum hybrids was of 7.48%.

Table 7. Results obtained at sorghum in the year 2022/ Rezultate obținute la sorg în anul 2022

Nr. Crt.	Hybrid	Planting date	Emergence date	Plants / m²	Plant height (cm)	Seed moisture content (%)	Hectolitre Mass (kg/100 l)	Yield at 14% moisture (kg/ha)
1	Anggy	26.03.2022	30.04.2022	21	-	13.1	79.3	7710
2	**Anggy**	**08.04.2022**	**04.05.2022**	**21**	**-**	**16.8**	**78.0**	**7063**
3	Huggo	26.03.2022	30.04.2022	21	-	13.5	79.7	6500

Nr. Crt.	Hybrid	Planting date	Emergence date	Plants / m^2	Plant height (cm)	Seed moisture content (%)	Hectolitre Mass (kg/100 l)	Yield at 14% moisture (kg/ha)
4	Icebergg	26.03.2022	30.04.2022	21	-	14.8	77.7	6418
Average		**-**	**-**	***21***	**-**	***14.55***	***78.7***	***6922.7***
CV (%)		**-**	**-**	***0***	**-**	***9.92***	***1.07***	***7.48***

In the year 2024, the sunflower hybrids, as well as the corn hybrids and sorghum hybrids developed not so well. The high daily temperatures of June and July negatively influenced the pollination processes, the formation and filling of grains in sunflower and corn hybrids. The registered temperatures at the Amzacea weather station were: June 9th - 29.7ºC; June 11th - 31.3ºC; July 9th- 34ºC; July 12th - 35.3ºC; July 16th - 34.6ºC; July 18th - 34.7ºC; July 19th - 34ºC; July 28th- 33ºC.

At sunflower, the flowering process occurred in 2024 in the period of June 21-29, according to hybrid (Table 8). The plant height was in average of 170.7 cm for the hybrids resistant at Tribenurom-Methyl and of 173.5 cm for the resistant hybrids at Imazamox. Seed moisture content differed according to the hybrid, respectively with the precocity of the hybrid, this being smaller than that registered in 2022, with the smallest value reaching 6.4%. Hectolitre Mass was not as good as in 2022, with values less than 40 kg/100 l and a variation between 33.4 and 38.4 kg/100 l.

The sunflower hybrids yield registered values below 2000 kg/ha with two exceptions, respectively hybrid Ceylon with 2211 kg/ha and hybrid P64LE162 with 2148 kg/ha. The grain yield was in average of 2082.2 kg/ha for the resistant hybrids at Tribenurom-Methyl and of 1601.0 kg/ha for the resistant hybrids at Imazamox (Table 8).

At corn, the flowering process occurred in 2024 in the period of June 23 – July 5, according to hybrid and planting time (Table 9). The seed moisture content varied between 18.1% in the case of Maxxatac hybrid planted on April 8th and 35.3% in the case of the same hybrid but planted later on April 20th. The Hectolitre Mass registered the extreme values for the same hybrid Maxxatac, respectively 58.4 kg/100 l when the planting was on April 20th and 61.8 kg/ha when the planting was on April 8th. The yield varied between 966 kg/ha in the case of hybrid Chorintos and 3524 kg/ha in the case of Maxxatac hybrid planted on April 8th. Delaying the planting from April 08th to April 20th in the case of Maxxatac hybrid decreased the yield with 1565 kg/ha, representing 44.4% (Table 9).

As in 2022, in the case of sunflower and sorghum, delaying the plating time for corn in 2024 increased the seed moisture content and decreased the Hectolitre Mass and the yield.

If sunflower in 2024 registered the coefficient of variation with the smallest value for Hectolitre Mass (3.47%), a small valued for seed moisture content (6.88%) and a high value for grain yield (10.85%) (Table 8), the corn registered also the smallest value for Hectolitre Mass (2.58%), but a much higher value for seed moisture content (20.08%) and even a much higher values for yield (34.10%) (Table 9). This means a much more variation between corn hybrids for seed moisture content and grain yield than in the case of the sunflower hybrids.

Table 8. Results obtained at sunflower in the year 2024 / Rezultate obținute la floarea-soarelui în anul 2024

Nr. Crt.	Hybrid	Planting date	Emergence date	Plants / m^2	Flowering date	Plant height (cm)	Seed moisture content (%)	Hectolitre Mass (kg/100 l)	Yield at 9% moisture (kg/ha)
Tribenurom metil resistent hybrids									
1	Ceylon	8.04.2024	17.04.2024	6	23.06.2024	178	12.1	38.4	2211
2	P64LE99	8.04.2024	17.04.2024	6	27.06.2024	162	13.2	34	1979
3	P64LE162	8.04.2024	17.04.2024	6	26.06.2024	174	10.6	36.2	2148
4	P64LE280	8.04.2024	17.04.2024	6	24.06.2024	169	8.6	33.4	1991
Average		**-**	**-**	***6***	**-**	***170.7***	***11.1***	***35.5***	***2082.2***
CV (%)		**-**	**-**	***0***	**-**	***3.50***	***15.51***	***5.56***	***4.80***
Imazamox resistent hybrids									

Nr. Crt.	Hybrid	Planting date	Emergence date	Plants / m²	Flowering date	Plant height (cm)	Seed moisture content (%)	Hectolitre Mass (kg/100 l)	Yield at 9% moisture (kg/ha)
5	P64LP170	8.04.2024	17.04.2024	7	29.06.2024	188	7.9	35.9	1705
6	Genesis	8.04.2024	17.04.2024	7	23.06.2024	162	6.4	37.8	1628
7	Belfis	8.04.2024	17.04.2024	7	26.06.2024	165	7.5	37.9	1712
8	Coloris	8.04.2024	17.04.2024	8	29.06.2024	173	7.1	37.1	1314
9	InSun 222	8.04.2024	17.04.2024	7	26.06.2024	172	7.0	36.6	1618
10	Dracaris	8.04.2024	17.04.2024	6	26.06.2024	185	6.9	34.4	1317
11	Michigan	8.04.2024	17.04.2024	6	26.06.2024	155	7.7	34.5	1809
12	P64LP170	8.04.2024	17.04.2024	7	29.06.2024	188	7.9	35.9	1705
Average		***-***	***-***	***6.9***	***-***	***173.5***	***7.3***	***36.3***	***1601.0***
CV (%)		***-***	***-***	***7.05***	***-***	***6.76***	***6.88***	***3.47***	***10.85***

Table 9. Results obtained at corn in the year 2024 / Rezultate obținute la porumb în anul 2024

Nr. Crt.	Hybrid	Planting date	Emergence date	Plants / m²	Flowering date	Seed moisture content (%)	Hectolitre Mass (kg/100 l)	Yield at 14% moisture (kg/ha)
1	Maxxatac	8.04.2024	17.04.2024	7.0	23.06.2024	18.1	61.8	3524
2	Maxxatac	20.04.2024	5.05.2024	7.0	5.07.2024	35.3	58.4	1959
3	Lipex	8.04.2024	17.04.2024	7.0	24.06.2024	19.4	61.2	1978
4	MyLady	8.04.2024	17.04.2024	6.8	27.06.2024	25.6	60.7	2017
5	LID3306	8.04.2024	17.04.2024	7.0	26.06.2024	22.7	60.5	1640
6	P8812	8.04.2024	17.04.2024	7.0	26.06.2024	23.1	61.7	1785
7	P8834	8.04.2024	17.04.2024	7.0	27.06.2024	25.8	58.8	1837
8	Chorintos	8.04.2024	17.04.2024	7.0	01.07.2024	25.4	63.6	966
Average		***-***	***-***	***6.97***	***-***	***24.4***	***60.8***	***1963.2***
CV (%)		***-***	***-***	***0.95***	***-***	***20.08***	***2.58***	***34.10***

At sorghum in 2024, the plant height was in average of 120 cm, with a variation from 110 cm to 140 cm, according to hybrid and planting date (Table 10). Seed moisture content varied between 11.9 and 16.1%, and Hectolitre Mass varied between 70.2 and 76.9 kg/100 l, according to hybrid and planting date.

The average yield was of 3335.2 kg/ha, with limits of variation from 2768 kg/ha (Feohn hybrid planted on May 7th) to 3998 kg/ha (Legend hybrid planted on April 8th). In average for the four studied sorghum hybrids planted on April 8th 2024, the yield was of 3576.7 kg/ha, while when they were planted on May 7th 2024, the average yield was of 3093.7 kg/ha, which mean a decrease of 483 kg/ha, representing 13.5% (Table 10).

Also for sorghum in the climatic conditions of 2024, delaying the planting time increased the seed moisture content and decreased the plant height and the yield (Table 10). The coefficient of variation registered the smallest value for Hectolitre Mass (3.05%) and the highest value for yield (10.70%) (Table 10).

Table 10. Results obtained at sorghum in the year 2024/ Rezultate obținute la sorg în anul 2024

Nr. Crt.	Hybrid	Planting date	Emergence date	Plants / m²	Plant height (cm)	Seed moisture content (%)	Hectolitre Mass (kg/100 l)	Yield at 14% moisture (kg/ha)
1	Anggy	8.04.2024	17.04.2024	27	130	11.9	73.6	3281
2	**Anggy**	**7.05.2024**	**19.05.2024**	**26**	**115**	**16.1**	**74.3**	**2972**
3	Legend	8.04.2024	17.04.2024	27	115	14.5	70.2	3998
4	**Legend**	**7.05.2024**	**19.05.2024**	**27**	**110**	**14.6**	**71.3**	**3195**
5	Feohn	8.04.2024	17.04.2024	25	140	13.9	74	3396
6	**Feohn**	**7.05.2024**	**19.05.2024**	**26**	**120**	**14**	**76.9**	**2768**

Nr. Crt.	Hybrid	Planting date	Emergence date	Plants / m^2	Plant height (cm)	Seed moisture content (%)	Hectolitre Mass (kg/100 l)	Yield at 14% moisture (kg/ha)
7	Willy	8.04.2024	17.04.2024	26	120	12.3	77.1	3632
8	**Willy**	**7.05.2024**	**19.05.2024**	**27**	**110**	**12.6**	**74.9**	**3440**
Average		**-**	**-**	***26.4***	***120***	***13.7***	***74.0***	***3335.2***
CV (%)		**-**	**-**	***2.64***	***8.07***	***9.54***	***3.05***	***10.70***

Regarding the weed control, it has to be pointed out that the autumn application of the herbicide active substance glyphosate led to a good control of some weed species from the monocotyledonous group, such as *Sorghum halepense*, *Echinocloa* spp., *Setaria* spp. etc., as well as of some broadleaf weed species. In recent years, a whole series of weeds such as *Veronica* sp., *Chenopodium album*, *Convolvulus arvensis*, and *Apera spica-venti* cannot be completely controlled due to the lack of appropriate herbicide active substances.

In 2022, the weed control at the sorghum crop by applying in the vegetation period of the herbicide Dicopur D based on one active substance, respectively 2.4 D from Dimethylamine salt, was not enough for a good control of the weeds. In exchange, in 2024, the mixture of the three active substances, respectively Bentazona from Basagran SL herbicide, and 2.4 D from Dimethylamine salt + Dicamba from Dicopur Top 464 SL herbicide controlled well the range of weeds in sorghum crop. Also, in 2024, the use of the three active substances Dicamba + Mesotrione + Nicosulfuron from the Pyxides herbicide in post-emergence (4-6 leaves) realised a good weed control in corn crop.

Planting early the sunflower, corn, and sorghum crops has reduced the attack of corn leaf weevil (*Tanymecus dilaticollis* Gyll.), which is an important pest for these crops in Romania, especially in the areas from South and South-East. Also, planting early the sunflower crop reduced the attack of broomrape (*Orobanche cumana* Wallr.), which is very problematic in South-East of Romania.

CONCLUSIONS

1. Under rainfed conditions, the actual climatic context affects significantly the yielding capacity of the spring crops (sunflower, corn, sorghum) the spring crop (sunflower, corn, sorghum) yielding capacity in South Dobrogea Plateau (South-East of Romania). Among the studied crops, corn is by far the most affected by the climatic changes from the last years.
2. In a context of less favourable climatic conditions, characterised by high temperatures and drought in the vegetation period of the cultivated plants, it becomes essential to choice the most suitable variety for cultivation and to adapt the crop technology.
3. Adaptation of crop technology could be made through several ways, among which the early planting, as soon as the minimum germination temperature is reached in the soil, that being essential. Generally, delaying the planting time is associated with important yield losses to all spring crops.
4. Another important task to be reached in the crop technology is to have a well weed control, thus eliminating the weeds which are competitors to cultivated plants for growing resources.
5. For the studied area, early planting proved to be a good way to diminish the negative aspects related to the pest *Tanymecus dilaticollis* Gyll. and to the parasitic plant *Orobanche sp*.

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RESEARCH ON THE INFLUENCE OF SOME TECHNOLOGICAL SEQUENCES ON PRODUCTIVITY IN DRACOCEPHALUM MOLDAVICA UNDER ORGANIC FARMING

CERCETĂRI PRIVIND INFLUENŢA UNOR SECVENŢE TEHNOLOGICE ASUPRA PRODUCTIVITĂŢII LA SPECIA DRACOCEPHALUM MOLDOVICA ÎN CONDIŢII DE AGRICULTURĂ ECOLOGICĂ

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Rezumat.

În lucrarea de faţă se prezintă date privind influenţa epocii de semănat şi a spaţiului de nutriţie la specia Dracocephalum moldavica asupra producţiei de herba şi sămânţă, cultivată în sistem de agricultură ecologică în cadrul laboratorului de Plante medicinale şi aromatice de la S.C.D.A. Secuieni în perioada 2018-2020. În urma interacţiunii factorilor, distanţa între rânduri şi distanţa între plante pe rând s-a constatat că la varianta semănată la 50 cm între rânduri şi 25 cm între plante pe rând s-a evidenţiat cea mai ridicată producţie de sămânţă de 648 kg/ha. În ceea ce priveşte producţia de herba uscată, aceasta a oscilat între 9103 kg/ha la varianta semănată în decada a III-a a lunii martie şi 10236 kg/ha la varianta semănată în decada a II-a a lunii aprilie.

Abstract.

This paper presents data on the influence of sowing time and nutrition space at the Dracocephalum moldavica species on herb and seed production, cultivated in an organic farming system within the Medicinal and Aromatic Plants Laboratory at A.R.D.S. Secuieni during 2018-2020. Following the interaction of factors, row spacing and plant spacing, it was found that the variant sown at 50 cm between rows and 25 cm between plants per row showed the highest seed production of 648 kg/ha. As for dry herb production, it ranged between 9103 kg/ha in the variant sown in the third decade of March and 10236 kg/ha in the variant sown in the second decade of April.

Cuvinte cheie: *mătăciune, sămânță, herba*

Key words: *moldavian dragonhead, seed, herb*

INTRODUCTION

The moldavian dragonhead is an annual species native to the Himalayas, which is cultivated for its aerial part (herb) which contains a volatile oil similar to lemon balm oil, rich in citral a and b (up to 80%), geranyl acetate, linalool, nerol etc. Among the chemical elements contained are: gallo-tannins (5-10%), salicarin, ferric hydroxide, flavonoid substances, heterosides, orientin, anthocyanin pigments-diglycosides of malvidol and galactosides of cyanidol, choline, glucose, starch, choline, a phytoncide with antibiotic activity, antibiotic substances, pectins, carotenoids, mineral substances, traces of volatile oil. The butterbur is part of some medicinal teas used in diseases of the digestive system and the nervous system (Abedi et al.,2015; El-Baky et al., 2007).

The species *Dracocephalum moldavica* L. has antimicrobial and antibacterial properties, being widely used in the pharmaceutical, cosmetic, food and perfumery industries. In the form of tea it is used to alleviate headaches, abdominal pain, remedy in treating flu, nervous system pain, kidney pain, gastrointestinal and tooth pain. This plant can also be used as a poultice in rheumatic pain (Galambosi, 1989; Hassani, 2006; Omidbaigi, 2006).

The research was conducted between 2018-2020 at the Agricultural Research and Development Station Secuieni and aimed to establish the optimal sowing time and the optimal nutrition space for the *Dracocephalum moldavica* L. species in order to develop cultivation technology in an ecological system.

The research was carried out between 2018 and 2020, in the experimental field of the Agricultural Research and Development Station Secuieni, on a typical cambic phaeozem (chernozem) soil with a medium to neutral texture (pH/H2O – 7.26). The soil on which the experiment was located was characterized as being well supplied in active humus (2.33%), very well supplied in phosphorus (189 mg/kg), potassium (304 mg/kg), excessively supplied in Mg (253 mg/kg) and Mn (369 mg/kg), poorly supplied in nitrogen (9.4 mg/kg N – NO3) and Zn (1 mg/kg). The area where the unit is located has a temperate continental climate (D.f.b.x. Köppen), characterized by short springs, cool summers and harsh winters, with an average annual temperature of 10.1°C and an annual amount of precipitation of 537 mm (Trotuş et al., 2020).

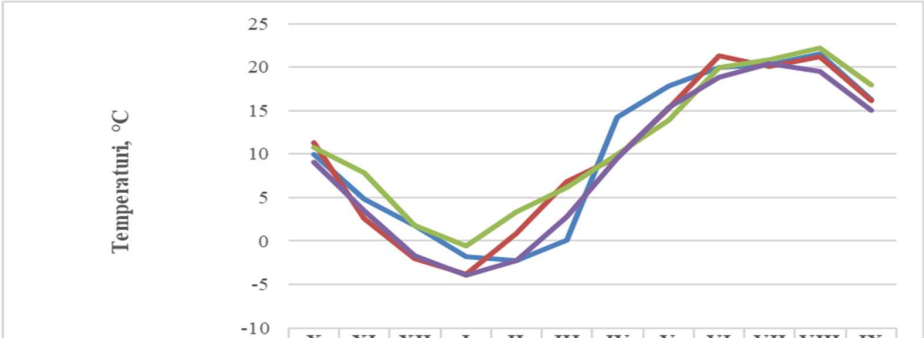
For the *Dracocephalum moldavica* species (Moldavian dragonhead), the establishment of technological links in the organic farming system was aimed at represented by the sowing period and the establishment of the optimal nutrition space.

Under the conditions at A.R.D.S. Secuieni, three sowing periods were experimented in a monofactorial experiment:
V1 – Epoch I - sowing in the first emergency (third decade of March - beginning of April - control);
V2 – Epoch II - sowing in the second emergency (second decade of April - third decade of April);
V3 – Epoch III - late sowing (last decade of April - beginning of May).

In order to establish the optimal nutrition space, a bifactorial experiment was set up according to the method of plots subdivided into 3 repetitions in which the following factors were experimented:

Factor A – distance between rows, with 3 graduations:
a1 – 25 cm,
a2 – 50 cm,
a3 – 70 cm.
Factor B – distance between plants per row, with 3 graduations:
b1 – continuous row (approx. 5 cm between plants/row),
b2 – 15 cm,
b3 – 25 cm.

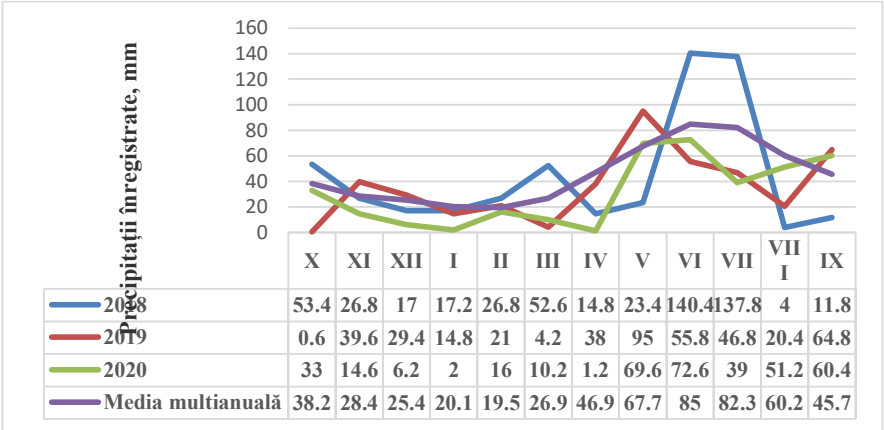
The 2017/2018 agricultural year was characterized by a late spring as a result of the negative average temperatures recorded in the first decade of March (-3.1ºC), as well as precipitation in the form of snow. The 2018/2019 agricultural year was characterized as warm in terms of temperatures and dry in terms of the annual amount of precipitation that was unevenly distributed during the plant vegetation period.



	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX
2017-2018	10	4,8	1,8	-1,8	-2,3	0,1	14,3	17,8	20	20,3	21,5	16,3
2018-2019	11,3	2,6	-2	-3,8	0,9	6,8	9,7	15,3	21,3	20,1	21,2	16,2
2019-2020	10,8	7,8	1,8	-0,6	3,4	6,2	10	13,9	20	20,9	22,2	18
Media multianuală	9,1	3,5	-1,7	-3,9	-2,2	2,8	9,5	15,4	18,8	20,4	19,5	15

Figure 1 Temperatures recorded at A.R.D.S. Secuieni in the period 2018-2020// Temperaturile înregistrate la S.C.D.A Secuieni în perioada 2018-2020

The 2019/2020 agricultural year was characterized by an early spring as a result of the high average temperatures recorded in March (6.2ºC), as well as the precipitation deficit of only 10.2 mm, with a deviation from the multiannual average of 26.2 mm. April was with 0.5 ºC warmer and 45.7 mm less in precipitation, while May was with 1.5 ºC colder than normal. Land preparation and sowing were difficult, with plant emergence being staggered and uneven.



	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX
2018	53.4	26.8	17	17.2	26.8	52.6	14.8	23.4	140.4	137.8	4	11.8
2019	0.6	39.6	29.4	14.8	21	4.2	38	95	55.8	46.8	20.4	64.8
2020	33	14.6	6.2	2	16	10.2	1.2	69.6	72.6	39	51.2	60.4
Media multianuală	38.2	28.4	25.4	20.1	19.5	26.9	46.9	67.7	85	82.3	60.2	45.7

Figure 2 Precipitation recorded at A.R.D.S. Secuieni in the period 2018-2020// Precipitațiile înregistrate la S.C.D.A. Secuieni în perioada 2018-2020

RESULTS AND DISCUSSION

Following the determinations made on the plants from the three sowing periods, it was found that the average height of the plants was between 60.50 cm at the 3rd sowing period and 86.12 cm at the 2nd sowing period. The average number of branches/plant ranged between 12.3 at the 3rd sowing period and 15.1 at the 2nd sowing period. Distinctly significant positive differences compared to the control (13.6) were recorded for the variant sown in the 2nd decade of April (1.5) (table 1).

Table 1. Biometric determinations of the species *Dracocephalum moldavica* L. in the period 2018-2020// Determinări biometrice la specia *Dracocephalum moldavica* L. în perioada 2018-2020

Variant	Average plant height (cm)	%	Diff. (cm)	Sign.
Epoch I	78.92	100	Ct.	
Epoch II	86.12	109.12	7.2	*
Epoch III	60.50	76.65	-18.42	ooo
LSD 5%= 5.2 cm 1%= 10.63 cm 0.1%= 17.41 cm				
Variant	Average number of branches/plant (no.)	%	Diff.	Sign.
Epoch I	13.6	100	Ct.	
Epoch II	15.1	111.02	1.5	**
Epoch III	12.3	90.44	-1.3	oo
DL5%= 0.52 1%= 1.15 0.1%=2.17				
Variant	Average plant weight (g)	%	Diff. (g)	Sign.
Epoch I	78.14	100	Ct.	
Epoch II	89.13	114.06	10.99	**
Epoch III	72.00	92.14	-6.14	o
LSD 5%= 4.86 g 1%= 8.04 g 0.1%=14.04 g				

Analyzing the influence of the sowing season on the average dry herb production, under the conditions at A.R.D.S. Secuieni, it is observed that it oscillated between 9103 kg/ha (epoch I) and 10236 kg/ha (epoch II) (table 2).

Table2. The influence of the sowing season on the production of dry herb at *Dracocephalum moldavica* species in the period 2018-2020 // Influenţa epocii de semănat asupra producţiei de herba uscată la specia *Dracocephalum moldavica* în perioada 2018-2020

Variant	Average dry herb yield (kg/ha)			
	Kg/ha	%	Diff. (kg/ha)	Sign.
Epoch I	9103	100	Ct.	
Epoch II	10236	112.44	1133	*
Epoch III	9538	104.77	435	
	LSD 5%= 894 (kg/ha) ; LSD 1%= 1436 (kg/ha) ; LSD 0,1%= 2347 (kg/ha)			

During the period under study, the average seed production varied between 750 kg/ha in the third epoch of sowing and 850 kg/ha in the variant sown in the second epoch. A statistically significant increase was obtained in the variant sown in the second decade of April (115 kg/ha) (table 3).

Table 3. The influence of the sowing season on the production of seed at *Dracocephalum moldavica species* in the period 2018-2020 // Influenţa epocii de semănat asupra producţiei de sămânță la specia *Dracocephalum moldavica* în perioada 2018-2020

Variant	Average seed yield (kg/ha)			
	Kg/ha	%	Diff. (kg/ha)	Sign.
Epoch I	778	100	Ct.	
Epoch II	850	109.25	115	*
Epoch III	750	96.40	-63	
	LSD5%= 75 (kg/ha); LSD 1%= 136 (kg/ha): LSD 0,1%= 205 (kg/ha)			

Table 4. The influence of the interaction between the distance between rows and the distance between plants in a row on the average production of dry herb at moldavian dragonhead in the period 2018-2020 // Influenţa interacţiunii dintre distanţa între rânduri şi distanţa între plante pe rând asupra producției medii de herba uscată la mătăciune în perioada 2018-2020

Distance between rows (A)	Distance between plants/rows(B)	Average dry herb yield (kg/ha)	%	Diff.	Sign.
a1-25 cm	b1-cca. 5 cm	9531	100	Ct.	
	b2-15 cm	9647	101,21	116	
	b3-25 cm	9822	103,05	291	
a2-50 cm	b1-cca. 5 cm	10360	108,69	829	**

Distance between rows (A)	Distance between plants/rows(B)	Average dry herb yield (kg/ha)	%	Diff.	Sign.
	b2-15 cm	10486	110,01	955	**
	b3-25 cm	10566	110,85	1035	**
a3-70 cm	b1-cca. 5 cm	10423	109,35	892	**
	b2-15 cm	10375	108,85	844	**
	b3-25 cm	10241	107,44	710	*
LSD 5%= 542 kg/ha LSD 1%= 821 kg/ha L 0.1%= 1318 kg/ha					

The interaction of the studied factors influenced the average dry herb production within limits ranging from 9531 kg/ha for the a1xb1 variant (25 cm between rows x 5 cm between plants/row) and 10566 kg/ha for the a2xb3 variant (50 cm between rows x 25 cm between plants/row) (table 4).

Regarding the average seed production at the tiller, positive, significant and distinctly significant differences were obtained for the variants sown at 50 cm and 70 cm between rows, the statistically assured production increases were between 137 kg/ha and 495 kg/ha (table 5).

Table 5. The influence of the interaction between the distance between rows and the distance between plants in a row on the average production of seed at moldavian dragonhead in the period 2018-2020 // Influența interacțiunii dintre distanța între rânduri și distanța între plante pe rând asupra producției medii de sămânță la mătăciune în perioada 2018-2020

Distance between rows (A)	**Distance between plants/rows(B)**	**Average seed yield (kg/ha)**	**%**	**Diff.**	**Sign.**
a1-25 cm	b1-cca. 5 cm	876	100	Ct.	
	b2-15 cm	833	95,09	-43	
	b3-25 cm	894	102,05	18	
a2-50 cm	b1-cca. 5 cm	1136	129,68	260	***
	b2-15 cm	1245	142,12	369	***
	b3-25 cm	1371	156,51	495	***
a3-70 cm	b1-cca. 5 cm	1236	141,10	360	***
	b2-15 cm	1115	127,28	239	***
	b3-25 cm	1013	115,64	137	*
LSD 5%= 77 kg/ha LSD 1%= 140 kg/ha LSD 0,1%= 210 kg/ha					

CONCLUSION

1. At the *Dracocephalum moldavica* L. species, the highest production of dry herb and seed was recorded in the variant sown in the second decade of April.
2. Analyzing the interaction of the studied factors on the average production of dry herb and seed, it was found that in the variants sown at distances of 50 cm and 70 cm between rows, the production differences were positive compared to the experimental control sown at 25 cm between rows.

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RESEARCH ON AGROTECHNICAL FEATURES OF CICHORIUM INTYBUS, PALATABILITY AS COWS' FEEDSTUFF AND THE EFFECTS ON MILK PRODUCTION

CERCETĂRI PRIVIND ASPECTELE AGROTEHNICE ALE CICORII FURAJERE, PALATABILITATEA ÎN FURAJAREA VACILOR ȘI EFECTELE ASUPRA PRODUCȚIEI DE LAPTE

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Abstract

The current study aimed to assess the Cichorium intybus agrotechnical and nutritional features, also the impact of it on milk production related to perennial gramineous and legumes. The agrotechnical evaluation based on the Braun-Blanchet scale was performed in the sequence of three vegetative cycles. Palatability was assessed based on analysis of variance. No significant differences ($p>0.05$) were recorded between alfalfa and chicory (90.14% vs. 87.56%). The lowest palatability was associated to gramineous (71.35%, $p\leq0.001$). The chicory shown an increased preferability degree in the sequence of daily feeding bouts, compared to alfalfa (82% vs 78%, $p\leq0,05$) also compared to gramineous or their mixtures (82% vs. 66%, $p\leq0.001$). The effects on milk production was assessed according to botanically structures. Comparable productions were recorded for alfalfa and chicory (17.5 kg vs. 17.2 kg, $p>0.05$). Positive effects were associated to chicory comparing to gramineous (17.5 kg vs. 14.6 kg, $p\leq0.001$) or its mixtures (17.5 kg vs. 15.5 kg, $p\leq0.01$). Chicory proved it efficiency in cows feeding comparing to legumes and gramineous, by an increased palatability and preferability degree. The positive effects on milk yield were comparatively evaluated according to the other botanically species. In this respect, based on the obtained results, the chicory proved to be rightfully considered as an alternative forage for dairy cows.

Rezumat

Studiul a urmărit aspectele agrotehnice, nutriționale și productive ale cicorii în raport cu gramineele și leguminoasele perene. Evaluarea agrotehnică în baza scalei Braun-Blanchet, s-a efectuat în succesiunea a trei cicluri vegetative. Palatabilitatea a fost evaluată pe baza analizei variației. Nu s-au înregistrat diferențe semnificative ($p>0.05$) între lucernă și cicoare (90.14% vs. 87.56%). Cea mai redusă palatabilitate a fost asociată gramineelor (71.35%, $p\leq0.001$). Cicoarea a prezentat un grad ridicat de preferabilitate în succesiunea tainurilor zilnice, comparativ cu lucerna (82% vs. 78%, $p\leq0,05$) dar și cu gramineele sau amestecuri ale acestora (82% vs. 66%, $p\leq0,001$). Efectele asupra dinamicii producției de lapte au fost evaluate în raport cu structura botanică. S-au înregistrat producții comparabile în cazul lucernei și a cicorii (17,5 kg vs. 17,2 kg, $p>0,05$). Efecte pozitive au fost asociate cicorii furajere comparativ cu gramineele (17,5 kg vs. 14,6 kg, $p\leq0,001$) sau amestecuri ale acestora (17,5 kg vs. 15,5 kg, $p\leq0,01$). Cicoarea și-a dovedit eficiența în cultură în relație cu leguminoasele și gramineele uzuale, fiind caracterizată și de un grad ridicat de palatabilitate și preferabilitate în furajarea vacilor de lapte. Efectele pozitive asupra dinamicii

producției lactate au fost evaluate comparativ între speciile botanice studiate, drept pentru care cicoarea furajeră se recomandă ca un furaj alternativ în dieta vacilor de lapte.

Keywords: *agro technics, chicory, cows, milk yield, nutrition*
Cuvinte cheie: *agrotehnică, cicoare, nutriție, producție lapte, vaci*

INTRODUCTION

Chicory was first mentioned as a forage plant early in 1915 in New Zealand, after which it remained understudied for a long time until 1978, when the chicory was defined as an excellent ruminant feed [Lancashire, 1978]. Drought-resistant, especially improved cultivars, among which the Puna variety stands out, have been licensed for commercialization in 1985. The research primarily focused on the agro-technics required for crops, their management and the chemical composition compared to other grasses, and to a lesser extent on the effects exerted on cow's production performances. Chicory is a perennial plant with an excellent harvest index [Lancashire, 1983; Hare *et al*., 1987], increased feed quality [Arnold, 1985; Hopkins *et al.*, 1995; Hoskin *et al.*, 1995; Nwafor *et al*., 2017;] and great results in animals fed with this fodder crop.

Chicory has long been an integral part of natural pastures in many parts of the world, but it only recently became a forage plant cultivated solely for this purpose. Numerous varieties have been created due to the implementation of genetic improvement programs for this species [David and Sears, 2007; Waugh *et al.*, 1998]. The average daily gain (ADG) of calves fed wiyh pure chicory crops in New Zealand was higher (900 g/day) [Han *et al.*, 2009] than the rate achieved in a conventional crop of perennial ryegrass (Lolium perenne L.), or white clover (Trifolium repens L.) and were similar to those obtained on some leguminous pastures such as alfalfa (Medicago sativa L.) [Ifeoma *et al.*, 2017].

Chicory for livestock forage has significantly expanded worldwide during the last decades [Sanderson *et al.*, 2003]. There is a lack of knowledge in the literature related to nutritional aspects of chicory, as well as the effects on the cow's feeding behaviour. An important aspect seems to be chicory's ability to mobilize soil resources and maintain higher levels of protein and dry matter in simple or complex mixtures. In terms of nutrition, chicory has a higher mineral content (with the exception of Ca) compared to other leguminous and gramineous plants. Reduced lignin content (15-20 g/kg DM) compared to alfalfa (70-75 g/kg DM) provides chicory a higher digestibility (70-85%) than alfalfa (50-70%). The high prevalence of lactones and chicory acid contributes to significant anthelmintic potential. However, the taste and smell associated with lactones could decrease palatability of chicory. According to Foster *et al.* (2006), a 0,58% lactone content is associated with Puna chicory variant compared to other floristic species with higher values ranging from 0.79% to 1,52 %, [Foster *et al*., 2006]. In light of these findings, Foster *et al.* (2002) highlights the anthelmintic activity of lactones in his research [Foster *et al*., 2002].

The use of chicory in cows' nutrition recorded positive results in terms of milk production. In this respect, an increased milk yield by 10-12% was observed compared to an alfalfa-based diet. Also, use of chicory in cows' diet lead to a different shape of the lactation curve, resulting in an early upward trend even from the beginning of lactation. Cows with an alfalfa diet have shown a constant milk yield. Compared to alfalfa, chicory has higher palatability (91-94% vs. 87-92) [Neciu, 2018]. The protein content of chicory is closely correlated with agro-technical and environmental factors, varying in relatively wide limits 10-18% according to Crush J.R. and Evans J.P.M. (1990) or 25% according to Wang Q. and Chiu J. (2010) [Crush and Evans, 1990; Wang and Ciu, 2010]. An important aspect is the quality of the available protein which proves to be superior to other plants, due to the 17 amino acids of which 9 are essential for animals and humans [Wang and Ciu, 2011]. The increased consumption of chicory in animal feed also comes from a lower lignin content (20 g / kg DM) compared to alfalfa (75 g / kg DM) with beneficial effects on digestibility (86% vs. 55%) [Terill *et al.*, 1992]. However, the effects on growth vary according to the morpho-productive type of cattle. In this respect, beef cattle recorded a higher weight gain (1-1.1 kg/day), compared to

Holstein cattle, which reach growth thresholds between 0.4-0.7 kg/day [Christina *et al.*, Laws and Genever, 2016]. Unrestricted administration of chicory has led to an increase in voluntary consumption from 1.7 to 3.6 kg / DM / hour. The structure of the chicory and especially its availability as fodder, can influence the feeding behaviour of the animals, with implications on the daily activity budget and implicitly on the productive performances. Unrestricted administration of chicory did not show an increase in the frequency of seizures, only in the quantity of forage associated with them, with the immediate effect of increasing the intake of dry matter and protein [Mc Coy *et al.*, 1997].

Plant diversity is progressively dwindling, due to the extended collapse of the processing industries. Each species plays a role in sustaining a productive ecosystem, supporting the increase of soil fertility and biomass production, according to several previous studies regarding plant biodiversity. The use of relatively new plants introduced into animal feed without being well known from a scientific point of view generates a certain reluctance from farmers. In general, chicory, viewed through the prism of different levels of knowledge (agro-technical, nutritional, chemical, morphological) still remains an unknown plant with tremendous potential, being a real alternative to the particularly conditions of soil, climate and agro-technics as well as the nutritional needs of cattle.

MATERIAL AND METHOD

The current study was performed at the Research and Development Station for Bovine Arad, Romania. This research was observational and non-invasive. Ethical considerations of this study were evaluated according to the European Union's Directive for animal experimentation (Directive 2010/63/EU) and the study was approved by the Scientific Council at the Research and Development Station for Bovine Arad.

Six botanical structures were included in the current study being allocated 4 ha/species. In this respect, in the study were included the following botanical structures:

1-Medicago sativa (100%)

2-Cichorium intybus (100%)

3-Medicago sativa+Cichorium intybus (50% / 50%)

4-Mixed gramineous (Dactylis glomerata 30%+Festuca arundinacea 30%+Loliun perenne 20%+Festuca pratensis 20%)

5-Dactylis glomerata 20%+Festuca arundinacea 20%+Loliun perenne 15%+Festuca pratensis 15%+ Medicago sativa 30%

6-Dactylis glomerata 20%+Festuca arundinacea 20%+Loliun perenne 15%+Festuca pratensis 15%+ Cichorium intybus 30%

The degree of emergence of the botanical structures was assessed by direct counting, using the geobotanical method based on the implementation of 1 m^2 grids. Five grids were placed for each hectare of crop. The direct counting allowed the evaluation of the botanical structure related to each phytocenosis and implicitly the expression in percentages and the reporting to the initial participation after sowing. The Braun –Blanchet scale (BB) was performed in each phytocenosis in order to assess the abundance and the dominance. The current evaluation involves the characterization of the botanical structures based on the 5 value points, as follows:

1 = numerous individuals, but with low soil coverage (1-10%);

2 = numerous individuals with soil coverage range between 11-25%;

3 = numerous individuals with soil coverage range between 26-50%;

4 = numerous individuals with an increase soil coverage range between, 51-75%;

5 = numerous individuals with highly soil coverage range between 76-100%.

The persistence of different botanical species in the culture was evaluated using the grid method, counting the individuals associated with different botanical species, reporting to their initial share. This study included 3 vegetative cycles. The palatability was evaluated based on the consummability but especially the preferability to the administered forage structures. The studies were conducted on randomized batches of 30 cows each. The first 10 days of feeding were considered as a period of adaptation to the new feeding structures. Daily feeding during the 3 repetitions of 7

testing days assessed the palatability, both in the control group (with alfalfa) and in the experimental groups. Daily feeding was divided out in 3 meals, included in the following time interval: meal 1 between 8.00 - 11.00, meal 2 between 11.00 - 16.00 and meal 3 between 16.00 - 19.00. The experimental feeding consisted in the daily administration of 70 kg/head/day of green fodder from the experimental crops as well as from the control crop (alfalfa). Every day, after each feeding, the uneaten feed residues were weighed and related to the initial amount administered. The obtained data were the basis of the palatability calculation system, for each dose of feed administered, for each feeding day and for the average of the experimental period.

The effects of botanical structures on milk yield were assessed using a factorial ANOVA protocol. Differences were tested using Tukey's test. The analysed data were expressed as least square means and standard errors of the mean. All the statistical inferences were carried out using the software package Statistica (StatSoft Inc., Tulsa, OK USA) [Hill and Lewicki, 2007].

RESULTS AND DISCUSSIONS

Emergence

Alfalfa registers an 88% of emergence, which is reduced to 76% in the succession of vegetation years. The total depreciation rate is 12% with an annual average of 4%. Chicory has an emergence rate of 87% and is already known as a late growing plant. During the 3 vegetative cycles, it proves to be a dominant plant, so that at the end of the period, a superior persistence compare to alfalfa (88%) was recorded, as well as a degree of appreciation of 1%. The mixt of the two species highlights varying percentages at emergence, with 48%/40% in favor of alfalfa. This ratio varies quickly, with alfalfa's presence declining at a total rate of 8% (2.7%/year) while chicory's presence increases moderately at a total rate of 10% (3.3%/year). Grasses enter the culture well (85%) but over time they reach their physiological limits quickly under the action of drought. The total depreciation rate was 15% with an annual average of 5%. The mixture of grasses and alfalfa enters the vegetation in a ratio of 60/27%, registering at the end of the cycles different losses between 8% for grasses and 6% for alfalfa, the average annual depreciation rates being 2.66% for grasses and 2% for alfalfa. The introduction of chicory in the mixture with grasses shows a good emergence of 62/26%, with chicory exerting severe dominance relationships and inducing grasses a decline rate of 13% (4.3%/year). Chicory dynamics register a slower pace defined by a total rate of 1% with an annual average of 0.33%.

Table 1 Comparative evaluation of the germination capacity of botanical structures// *Evaluarea comparativă a capacității de răsărire a structurilor botanice*

Botanical structure	Share at sowing (%)	Share at emergence (%)	Weeds (%)	Abundance (1-5)	Dominance (1-5)	Braun –Blanchet scale (1-5)
Medicago sativa	100	88	12	4	2	3
Chicorium intybus	100	87	13	4	3	3
Medicago sativa+Chicorium intybus	50/50	48/40	12	3	2	3
Gramineous	100	85	15	3	2	3
Gramineous +Medicago sativa	70/30	60/27	13	3	3	3
Gramineos+Chicorium intybus	70/30	62/26	12	3	4	4

Table 2 Comparative evaluation of persistence according to botanical structure// *Evaluarea comparativeă a persistenței în cultură a structurilor botanice*

Botanical structure	Share at sowing (%)	Share of species after 3 vegetative cycles (%)	Weeds (%)	Braun – Blanchet scale
Medicago sativa	100	76	12	**2!!!!!**
Chicorium intybus	100	88	9	**4!!!!!**
Medicago sativa+Chicorium intybus	50/50	40/50	8	3

Botanical structure	Share at sowing (%)	Share of species after 3 vegetative cycles (%)	Weeds (%)	Braun – Blanchet scale
Gramineous	100	70	9	2
Gramineous +Medicago sativa	70/30	52/21	8	2
Gramineos+Chicorium intybus	70/30	49/28	8	3

Palatability

The palatability of alfalfa is very high and is shown on an upward curve throughout the experimental period. The minimum value of 88.57% is associated with the first day of feeding, the upward trend being noted from that moment. The maximum value is quickly reached, even from the 3rd day, a sign that this type of forage is known and preferred by cattle. On average, the consumption of alfalfa is 89.14%. Chicory does not benefit from the previously acquired cognitive element, so in this sense, although registered on an upward tendency, it presents a lower initial palatability, with a minimum value of 77.14%, the maximum being reached only on the 8th day of trial. As cognitive relationships are established, chicory shows a significant increase in consumption, the average value reaching 94.26%. The mixture of alfalfa and chicory is preferred by cattle, the minimum palatability recorded in the first 2 days (73%) it increases and reaches the maximum threshold of 97.14% on the 8th day. The average consumption was 89.26%, a slightly increase compared to alfalfa, possibly due to the presence of chicory in the mixture. Grasses have the lowest palatability, with values range between 68.57-82.85% and with an average value of 71.35%. The dynamic over time is sinuous, a sign that cattle show a low preference for this type of fodder (only 66.23% at the terminal taint). The inclusion of alfalfa and chicory in the mixture of grasses improves the palatability. The presence of alfalfa increases the value to a maximum of 73% while chicory is preferred by animals, the degree of palatability being slightly higher (76.7%) due to the bitter taste conferred by the presence of tannins. At the same time, the presence of alfalfa or chicory in grass mixtures increases the preferability of the fodder structure, an increase consumptions at the terminal bout being registered compared to that associated with perennial grasses in simple mixtures.

Table 3 Comparative assessment of botanical structure palatability // *Evaluarea comparativă a palatabilității structurilor botanice*

Botanical structure	Palatability (%)	Preferability (%)		
		Bout I	Bout II	Bout III
Medicago sativa	89,14	100	87,83	**78,6**
Chicorium intybus	94,26	100	86,73	**82,97**
Medicago sativa + Chicorium intybus	89,26	96,25	85,01	80,53
Gramineous	71,35	79,16	66,66	66,23
Gramineous+Medicago sativa	73	81,5	70,83	**69,66**
Gramineous+Chicorium intybus	76,7	82,9	79,6	**76,7**

Milk production

A total number of 60 multiparous cows were randomly divided into 6 feeding groups of 10 heads/group, in order to estimate the effects of the studied botanical structures in the dairy cows feeding.

Feeding was carried out differently, each group of cows getting a particularly botanical structure. Feeding was carried out in 3 repetitions of 20 feeding days/plant. The efficiency of feeding with the new improved cultivars was studied according to the induced effects on milk yield as well as on its chemical composition.

In this respect, the analysis recorded a comparative production in the case of cows fed with alfalfa and chicory in pure culture, even if the gains obtained at the end of the study period was insignificant. The mixture of alfalfa and chicory maintains the productive level at comparable values to those associated with individual crops. Feeding with a mixture of grasses does not allow a high productive level, the differences generated compared to chicory being significant. The inclusion of

legumes in the mixture of grasses comes opportunely in the recovery of productive thresholds, although the performances obtained in the case of fodder with alfalfa or chicory could not be matched.

Table 4 The effects of botanical structures in milk yield // *Efectele structurilor botanice asupra producției de lapte*

Botanical structure	Daily milk yield (kg/day/period)	Dynamic of milk yield in the studied period (%)	Daily gain in milk (%)
Medicago sativa	17,2	+18,63	+1,33
Chicorium intybus	17,5	+19,1	+1,36
Medicago sativa + Chicorium intybus	16,6	+17,4	+1,24
Graminee	14,6	-13,92	-0,99
Graminee+Medicago sativa	15,7	-7,28	-0,52
Graminee+Chicorium intybus	16,7	-5,26	-0,37

Health status

Feeding with the new feed structures did not lead to abortions and did not disrupt reproductive activity in the experimental groups. Feeding with the new feed structures did not lead to the appearance of tympanums and did not facilitate the appearance of any other condition specific to this physiological and age category in the experimental groups.

CONCLUSIONS

1. Chicory has been shown to be a plant with an emergence capacity comparable to alfalfa and clearly superior to perennial grasses.
2. Related to the weeds, chicory proved a good dominance, largely inhibiting their excessive development.
3. Over time, chicory persistence in crops was superior to alfalfa and perennial grasses. Including it in cows diet proved a highly palatability, also high degree of preferability.
4. The highly nutritional value conferred by the increase protein content allowed to obtain an increased quantities of milk.
5. Last but not least, the administration of chicory as green forage eliminated the risks of specific nutritional disease in cattle.

ACKNOWLEDGMENT

This work was supported by a grant of the Romanian Ministry of Agriculture and Rural Development, throughout the project ADER 9.1.3. / 30.09.2019 project

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ESTIMATION OF CHLOROPHYLL CONCENTRATION IN POTATO LEAVES BASED ON SPAD MEASUREMENTS UNDER CONTROLLED CONDITIONS

ESTIMAREA CONCENTRAŢIEI DE CLOROFILĂ DIN FRUNZELE DE CARTOF BAZATĂ PE MĂSURĂTORI SPAD ÎN CONDIŢII CONTROLATE

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Abstract

The conventional potato breeding process has led toapproach this crop plant also under the implementation of modern biotechnology methods.Due to its capacity for in vitro regeneration, the potato has a rich history in tissue culture experiments.Micropropagation techniques are the safest, most elaborate and fastest way to provide planting material for a seed potato crop andalso for consumption.Laboratory monitoring, control in a protected space (insect-proof spaces), regarding the growth and development status of plantlets determines the assurance of phytosanitary quality according to the established standard.Biometric observations were made on the biological material taken into study,for three Romanian potato varieties - Asinaria, Darilena and Foresta, regarding the average height of the plant, the average number of shoots and leaves, and by using the SPAD device, measurements were made on the chlorophyll content of potato leaves at the time of transplanting the vitroplants and after the acclimatization period.

Key Words: *biotechnology, potato, protected space, chlorophyll index, SPAD*

Rezumat

Procesul de ameliorare convenţională a cartofului a condus la necesitatea abordării acestei plante de cultură şi prin prisma implementării metodelor moderne de biotehnologie. Datorită capacităţii sale de regenerare in vitro, cartoful are un istoric bogat în cadrul experimentelor bazate pe culturi de ţesuturi. Tehnicile de micropropagare constituie modul cel mai sigur, elaborat şi rapid pentru asigurarea materialului săditor necesar producerii cartofului pentru sămânţă şi, implicit producţiei destinate consumului. Monitorizarea în laborator, controlul în spaţiu protejat (solar), privind starea de creştere şi dezvoltare a plantulelor determină asigurarea calităţii fitosanitare conform standardului stabilit. Asupra materialului biologic luat în studiu, trei soiuri româneşti de cartof - Asinaria, Darilena şi Foresta, s-au făcut observaţii biometrice, referitoare la înălţimea medie a plantei, numărul mediu de lăstari şi frunze, iar cu ajutorul dispozitivului SPAD s-au făcut măsurători privind conţinutul de clorofilă al frunzelor de cartof, în momentul transplantării vitroplantelor şi după perioada de aclimatizare.

Cuvinte cheie: *biotehnologie, cartof, spaţiu protejat, indice de clorofilă, SPAD*

INTRODUCTION

Currently, the potato (*Solanum tuberosum* L.), due to its high adaptability to different pedoclimatic conditions, is cultivated in over 120 countries worldwide, with potato tubers being consumed daily by over one billion people (Faostat, 2023).Furthermore, this species is considered the fourth most cultivated crop plant in the world (Ceci et al., 2022), after maize (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.). Potatoes are cultivated throughout Romania, but

the largest areas are cultivated in the closed zone for the production of seed potatoes: Brasov, Covasna, Harghita, Bacau, Neamt, Suceava, Botosani counties.

Starting from the current state of research in the field of *in vitro* plant cultures, future development will be oriented, under the conditions of internal and international competition imposed by the market on seed potato producers, to ensure appropriate material (from all points of view: virological, physiological age, agronomic characteristicsetc.), which will also be accompanied by economic effects that create profit (Chiru, 2002).

Starting from the current state of research in the field of *in vitro* plant cultures, future development will be oriented, under the conditions of internal and international competition imposed by the market on seed potato producers, to ensure appropriate material (from all points of view: virological, physiological age, agronomic characteristics, etc.), which will also be accompanied by economic effects that create profit (Chiru, 2002).

Maximum production potential depends on an early development of source organs (leaf area) for optimal light interception and photosynthesis and sink organs (both initiation and extension of storage roots or tubers) in root crops (tubers) (Farooq, 2009).

By using the SPAD 502 device, the chlorophyll index was determined in potato seedlings under *in vitro* and *in vivo* conditions. This method, being an approach that presents advantages in experiments in which the leaf surface is small and the plants do not suffer mechanical and chemical accidents, and at the same time, extended determinations can be made on the same individual throughout development under different conditions and environmental factors.

The objective of this study was to establish a correlation between the SPAD 507 device readings of the potato varieties under study, at the time of transplantation from *in vitro* to *in vivo*, and at the same time performing biometrics regarding the height, number of shoots and number of leaves.

MATERIAL AND METHOD

The experience has as a starting point meristems of Romanian varieties Asinaria, Darilena and Foresta created at NIRDPSB Brasov. Meristems taken from potato sprouts are inoculated *in vitro* for obtaining a healthy pre-basic material. After a period of 6-9 months from meristems are developing plantles. To ensure phytosanitary accuracy is made ELISA test and infected clones are removed, continuing multiplication from healthy ones. The developed plantlets are cut to each internode level into minicuttings. The nutrient medium used was Murashige-Skoog including vitamins(supplemented with sucrose 2%, agar 0.85%, NAA 0.5 mg/l, and antimicrobial agent PPM 3 ml/l -Plant Preservation Mixture), the pH was reduced to 5.6-5.8, before sterilizing the culture medium in an autoclave. After preparing the culture medium, potato plantlets obtained from meristems were multiplied in order to obtain a stock of initial material. The culture recipients were incubated in the growth chamber, ensuring a photoperiod of 16 hours light/8 hours dark and a temperature of 19-21 ºC. For potato plantlets were made SPAD measurements. The potato plantlets were transplanted from *"in vitro"* conditions to *"ex vitro"*afterMay19, 2024.

They were planted in black plastic pots with 20 cm in diameter, filled with a substrate containing red peat, black peat and perlite (4:2:1), NPK 16:16:16 fertilizer was added to the composition. During the growing season treatments with foliar fertilizers were applied. After 2 months from the transfer of the plantlets to the protected space, determinations were made regarding growth and development parameters and also SPAD mesurements.

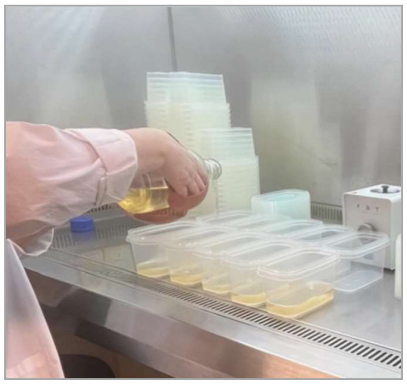


Fig. 1. Distribution of nutrient medium
Distribuirea mediului nutritiv



Fig. 2. Inoculation of mini-cuttings
Inocularea minibutasilor



Fig.3. Plantlets incubated in the growth chamber
Plantule incubate în camera de creştere



Fig.4. Acclimatized vitroplants
Vitroplante aclimatizate



Fig. 5 Potato plants in the protected space // *Plante de cartof în spaţiu protejat*

RESULTS AND DISCUSSION



Fig. 6 Biometrics// *Biometrizări*



Fig.7 SPAD device *//mAparatul SPAD*

Table 1. The influence of variety on the average plantlets height (cm) *in vitro*// *Influenţa soiului asupra înălţimii mediia plantulelor (cm) dezvoltate in vitro*

Variety	The plantlets height (cm)	Diff.	Sign.
Asinaria	8.90	0.8	ns
Darilena	12.03	3.88	*
Foresta (Ct.)	8.15	-	-
DL 5%=2.38 cm// DL 1%=5.50 cm// DL 0.1%=17.49 cm			

The statistical analysis performed for each varieties shows that plantlets height is bigger for Darilena variety, with a positive difference (3.88 cm) comparative with control variety (Foresta).

Table 2. The influence of variety over the number of leaves*in vitro*// *Influenţa soiului asupra numărului mediu de frunze alplantulelor dezvoltate in vitro*

Variety	Number of leaves	Diff.	Sign.
Asinaria	8.60	0.77	ns
Darilena	9.09	1.26	*
Foresta (Mt.)	7.83	-	-
DL 5%=1.17// DL 1%=2.70// DL 0.1%=8.58			

Analysis of the varieties used in the experiment shows that the best results were achieved for Darilena variety with a number of leaves by 9.09 and with a with positive difference of 1.26 leaves, comparative with control variety.

Table 3. The influence of variety on the average plants height*in vivo*// *Influenţa genotipului asupra înălţimii medii a plantelorin vivo*

Variety	The plantlets height	Diff.	Sign.
Asinaria	36.90	4,10	ns
Darilena	41.00	8,20	ns
Foresta (Mt)	32.80	-	-
DL 5%=9.93// DL 1%=13.67// DL 0.1%=18.82			

Table 4. The influence on variety on the average plants number of shoots*in vivo* // *Influenţa genotipului asupra numărului mediu de lăstari/planta in vivo*

Variety	Number of shoots	Diff.	Sign.
Asinaria	2.50	-0.80	ns
Darilena	2.10	-1.20	ns
Foresta (Mt)	3.30	-	
DL 5%=0.88// DL 1%=1.21// DL 0.1%=3.30			

Regarding the influence of genotype on the average height and number of shoots of plants acclimatized in the greenhouse, no significant differences were recorded compared to the control variety Foresta (Table 3 and 4).

The study of the influence of the variety on the number of leaves is highlighted with a very significant positive difference for the Darilena variety (45.20) (Table 5). The statistical interpretation of the results obtained highlighted the influence of the three potato genotypes on the growth and development rate, both *in vitro* and *in vivo*, where the Darilena variety stood out in particular.

Table 5. The influence of variety over the number of leaves/plant*in vivo*// *Influenţa genotipului asupra mediei numărului de frunze/plantă in vivo*

Variety	Number of leaves	Diff.	Sing.
Asinaria	42.00	-13.00	ns
Darilena	100.20	45.20	***
Foresta (Mt)	55.00	-	-
DL 5%=23.48// DL 1%=32.34 // DL 0.1%=44.52			

Following measurements of the chlorophyll concentration in the leaves of the seedlings of the varieties studied, at the time of preparation for transplantation, the average values recorded highlighted the Darilena variety (52.43), then the Asinaria variety (44.59) compared to the control variety Foresta (38.73).

Following the first measurements of chlorophyll concentration in leaves, carried out in a protected space, the recorded values are decreasing in all varieties, namely Darilena (51.06), Asinaria (38.64) and Foresta (34.44).

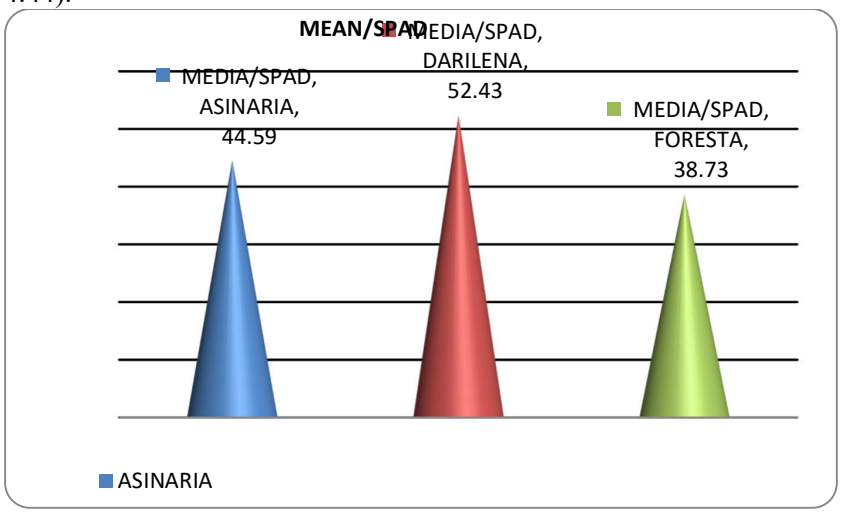


Fig . 8. SPAD values recorded *in vitro* plantlets (ready for transplanting) // *Valori SPAD înregistrate la plantulele din in vitro (pregătite pentru transplantare)*

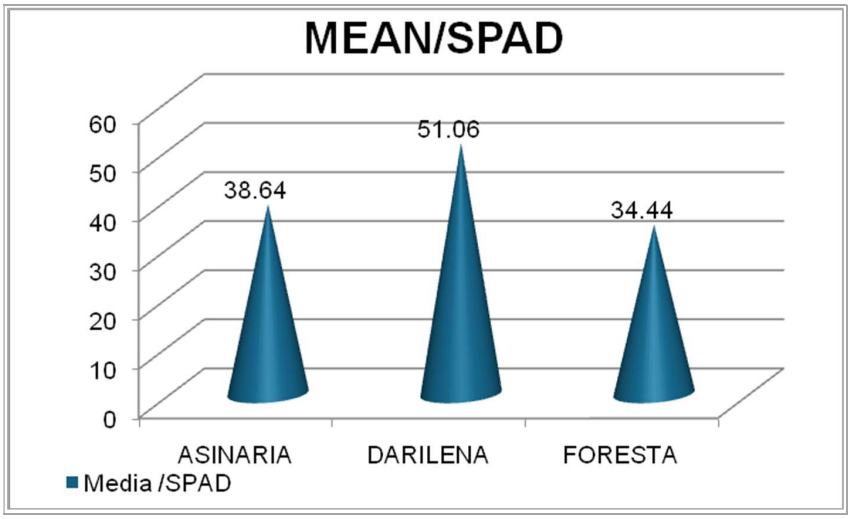


Fig. 9. Comparison of SPAD values of potato plants grown in greenhouse (after the acclimatization period) // *Comparaţia valorilor SPAD ale plantelor de cartofi cultivate în seră (după perioada de aclimatizare)*

The chlorophyll content of the leaves evaluated with the SPAD 502 equipment, at the time of the second measurement, in the solarium, similar to the first measurements, the readings taken again show a decrease in the average values for the varieties Darilena (42.8), Asinaria (37.26), only for the variety Foresta the average values show an increase (35.9).

The determination of the chlorophyll concentration at leaf level, through the measured values, provided us with a good assessment of the changes given by the acclimatization conditions and the health status of the plantlets in the protected space. Analyzing the results obtained, it is found that the Darilena variety has a better adaptation capacity, compared to the other varieties studied. Knowing the biological potential of potato varieties is an important aspect in breeding programs.

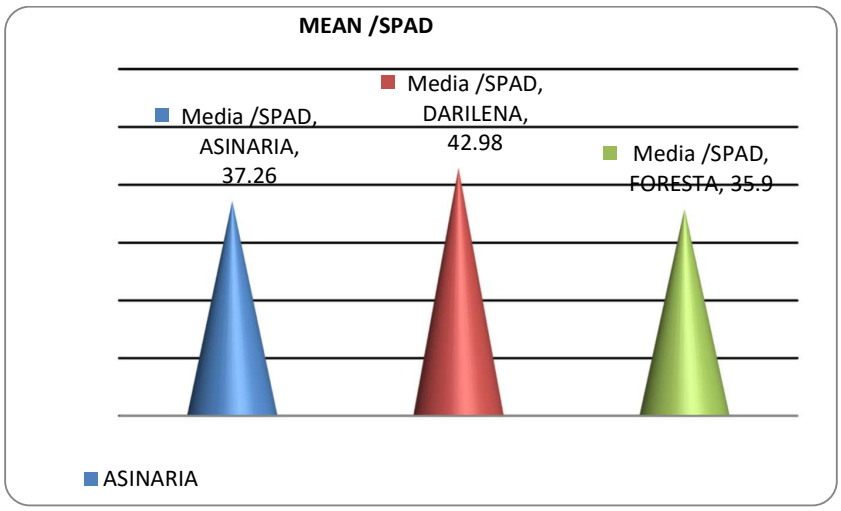


Fig. 10. Comparison of SPAD values of potato plants grown in protected space (after flowering) // *Comparaţia valorilor SPAD ale plantelor de cartofi cultivate în seră (după înflorire)*

CONCLUSIONS

1. Maximum acclimatization potential depends on early leaf development for optimal light interception;
2. Differences in chlorophyll index values between potato varieties act as a key factor in plant growth and development;
3. These experiments were performed to model the relationship between chlorophyll concentration measured both *in vitro* and *in vivo*.

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ANALYSIS OF FARMERS' PERSPECTIVE ON THE USE OF BIOTECHNOLOGIES AND IMPACT ON NATIONAL FOOD SECURITY

ANALIZA PERSPECTIVEI FERMIERILOR CU PRIVIRE LA UTILIZAREA BIOTEHNOLOGIILOR SI IMPACTUL ASUPRA SECURITĂȚII ALIMENTARE NAȚIONALE

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***Rezumat*:** *Într-un context agricol tot mai complex, marcat de schimbările climatice, scăderea fertilității solului, presiuni economice și incertitudini pe piețele de desfacere, biotehnologiile apar ca o opțiune strategică pentru creșterea sustenabilă a productivității și consolidarea securității alimentare la nivel național. Aceste tehnologii moderne, incluzând organisme modificate genetic, biofertilizanți, biopesticide și tehnici avansate de ameliorare genetică, pot contribui semnificativ la reducerea pierderilor din producție, optimizarea resurselor și adaptarea culturilor agricole la stresul abiotic. În acest context, înțelegerea modului în care fermierii percep și se raportează la biotehnologie devine esențială pentru succesul oricărei strategii de inovare în agricultură. Acceptarea socială a acestor tehnologii nu depinde exclusiv de eficiența lor agronomică, ci și de nivelul de încredere, informare și de percepția riscurilor asociate. Astfel, prezentul studiu propune o analiză detaliată a opiniilor fermierilor români cu privire la adoptarea biotehnologiilor agricole, investigând trei dimensiuni cheie: beneficiile percepute, obstacolele întâmpinate și gradul de cunoaștere a tehnologiilor disponibile. Prin această cercetare, se urmărește identificarea profilului fermierului deschis la inovație, precum și a factorilor care frânează tranziția către practici agricole moderne. Rezultatele obținute pot contribui la fundamentarea unor politici publice mai bine adaptate realităților din teren și la dezvoltarea unor programe eficiente de informare, formare și sprijin pentru adoptarea tehnologiilor biotehnologice în mediul rural.*

Abstract
*In an increasingly complex agricultural context, marked by climate change, declining soil fertility, economic pressures and uncertainties in markets, biotechnologies are emerging as a strategic option for sustainable productivity growth and strengthening food security at the national level. These modern technologies — including genetically modified organisms, biofertilizers, biopesticides and advanced genetic improvement techniques — can significantly contribute to reducing production losses, optimizing resources and adapting agricultural crops to abiotic stress. In this context, understanding how farmers perceive and relate to biotechnology becomes essential for the success of any innovation strategy in agriculture. The social acceptance of these technologies does not depend exclusively on their agronomic efficiency, but also on the level of trust, information and perception of the associated risks. Thus, this study proposes a detailed analysis of Romanian farmers' opinions on the adoption of agricultural biotechnologies, investigating three key dimensions: perceived benefits, obstacles encountered and the degree of knowledge of available technologies. Through this research, the aim is to identify the profile of the farmer open to innovation, as well as the factors that hinder the transition to modern agricultural practices. The results obtained can contribute to the foundation of public policies better adapted to the realities on the ground and to the development of effective information, training and support programs for the adoption of biotechnological technologies in rural areas.***

Cuvinte cheie: fermieri, biotehnologie, OMG, agricultură,
Keywords: farmers, biotechnologies, agriculture, GMO

INTRODUCTION

Biotechnologies play an increasingly essential role in transforming the modern economy, through their application in various industrial sectors and by capitalizing on the results of research and innovation. This strategic integration not only brings added value, but also directly contributes to economic growth (Pokataiev et al., 2023). The ecosystem formed by raw material suppliers, research actors and biotech investors is becoming a central pillar of the bioeconomy, stimulating sustainable development and global competitiveness (Lokko et al., 2018).

Biotechnology is a multidisciplinary field that uses biological processes, living organisms or their components to develop useful products and technologies in various sectors such as agriculture, medicine, industry and the environment (Munaweera et al., 2022). It combines knowledge from biology, genetics, chemistry, computer science and engineering to provide innovative solutions to some of humanity's most pressing challenges. Essentially, biotechnology involves controlled intervention in biological processes to obtain results of practical value, be it improving agricultural crops, producing medicines, treating waste or creating biodegradable materials (Harfouche et al., 2021; Gupta et al., 2017).

Contemporary agriculture is facing major challenges generated by climate change, global population growth and the need to ensure food security in an increasingly volatile economic context. In this regard, agricultural biotechnologies are promoted as innovative solutions that can contribute to increasing agricultural yields, reducing pesticide use and improving crop resistance to abiotic and biotic stress conditions (Das et al., 2023). For example, in the agricultural and agro-industrial sectors, biotechnology applications play a significant role. These applications range from increasing the productivity of primary production to processed finished products with increased added value, based on biotechnological processes (Hesham et al., 2021; Tyczewska et al., 2023).

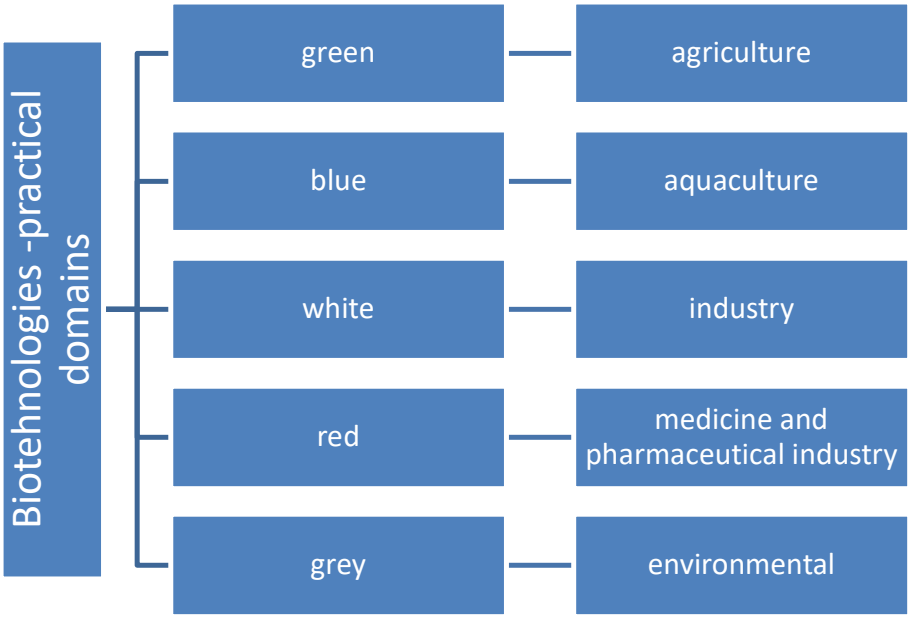


Figure 1. Practical domains of biotechnological applications // Domenii de aplicare ale biotehnologiei (Source: authors'own drawing)

Biotechnological applications (Figure 1) can be classified into the following areas, each representing a distinct domain of innovation with specific goals and impacts across various sectors: medical biotechnology (commonly referred to as red biotechnology), which focuses on pharmaceutical development and healthcare solutions; agricultural biotechnology (green biotechnology), dedicated to improving crop productivity, pest resistance, and environmental sustainability; industrial biotechnology (white biotechnology), which involves the use of enzymes and microorganisms in manufacturing processes to reduce environmental impact and increase efficiency; marine biotechnology (blue biotechnology), which explores oceanic and aquatic resources for novel compounds and genetic materials. and environmental biotechnology (grey biotechnology), which is aimed at bioremediation, waste management, and pollution reduction (Gupta et al., 2017) (Table 1) .

Table 1 Biotechnological applications// Aplicații biotehnologice

Classification	Description
green biotechnology	is biotechnology applied to agricultural processes, for example, obtaining transgenic plants resistant to adverse environmental conditions, adaptable to different soils, as well as plants resistant to diseases and pests.
blue biotechnology	includes marine and aquatic applications of biotechnology; this branch of biotechnology deals with the development of aquaculture, the care of marine creatures, the treatment of polluted or waste waters and the production of foods derived from the sea.
white biotechnology	is applied exclusively to the improvement of industrial processes; it uses yeast, molds, bacteria and enzymes with applications in industry. For example, engineering an organism to produce a useful chemical; the use of enzymes as industrial catalysts, either to produce valuable chemicals or to destroy dangerous and/or polluting chemicals;
red biotechnology	is applied to medical processes, e.g. engineering organisms to produce antibiotics; regenerative therapies and the application of genetic engineering to cure diseases.
grey biotechnology	Include environmental application of the biotechnology aimed to bioremediation, wast management pollution, reduction with other words, maintaining soil fertility and environmental health.

By using genetically modified organisms (GMOs), genome editing (such as CRISPR-Cas9) and biocontrol technologies, farmers can grow plants that are more resistant to disease, drought or salinity, thereby reducing dependence on pesticides and losses due to abiotic stress (FAO, 2021;Singh et al., 2025;Rasheed et al., 2021). At the same time, biotechnologies contribute to extending the shelf life of agricultural products through methods such as genetically modifying fruits to delay ripening or applying bioactive packaging, leading to more efficient resource management and reduced food waste (EuropaBio, 2023; OECD, 2022).

However, the adoption of agricultural biotechnologies remains a controversial topic, especially in Europe, where strict regulations, skeptical public attitudes and health and environmental concerns significantly limit the integration of these technologies into agricultural practices (Tunbull et al., 2021). In Romania, a country with considerable agricultural potential and a rural structure dominated by small farms, the debate on biotechnologies is marked by polarization and, possibly, lack of adequate information.

In particular, the potential impact of biotechnologies on farmers is twofold: on the one hand, these technologies could offer significant economic benefits, by increasing efficiency and reducing losses; on the other hand, they could increase dependence on global suppliers of seeds and inputs, creating the risk of concentration of economic power in agriculture. In this context, it is essential to understand the perspectives of farmers, the main actors of the agri-food system, on the opportunities and risks associated with biotechnologies.

Analyzing farmers' perspectives on the use of biotechnologies is essential for understanding the level of acceptance and challenges associated with their implementation in agriculture. In a context where biotechnologies are becoming increasingly relevant for food security and agricultural sustainability (Ranja et al., 2022), it is important to investigate not only the technological impact, but also the perception of the direct users, namely the farmers (Wozniak et al., 2022).

In this regard, this article aims to conduct a comprehensive analysis of farmers' opinions on the adoption of biotechnologies, focusing on three major areas: perceived benefits (such as increased productivity and reduced losses), barriers encountered (including lack of information, implementation costs or reluctance to change) and the level of knowledge of available technologies. The overall goal of the approach is to highlight the dynamics of perception among farmers, to identify obstacles that hinder the widespread adoption of biotechnologies and to propose directions for action for more effective agricultural policies and information programs. This approach contributes to a more nuanced understanding of the context in which technological innovation meets realities on the ground.

RESEARCH METHODOLOGY

To conduct the analysis of farmers' perceptions of the use of biotechnologies in agriculture, a quantitative approach was adopted, based on the application of a standardized questionnaire. The study was conducted between January and March 2025. The sample consisted of 103 active farmers.

The questionnaire included both closed questions (with Likert-type answers) and open questions, targeting the following dimensions:

- level of knowledge of biotechnologies;
- general attitude towards their use;
- perception of benefits (production, climate resilience, economic efficiency);
- barriers encountered in the adoption process;
- information sources

RESULTS AND DISSCUSSIONS

This study analyzed farmers' perception, level of knowledge and openness towards agricultural biotechnologies, based on a questionnaire applied to a sample of 87 respondents. General data on the profile of the participants indicate a predominance of male farmers, aged between 35 and 60. Most of them own medium-sized farms and have high school or post-high school education, working predominantly in vegetable agriculture.

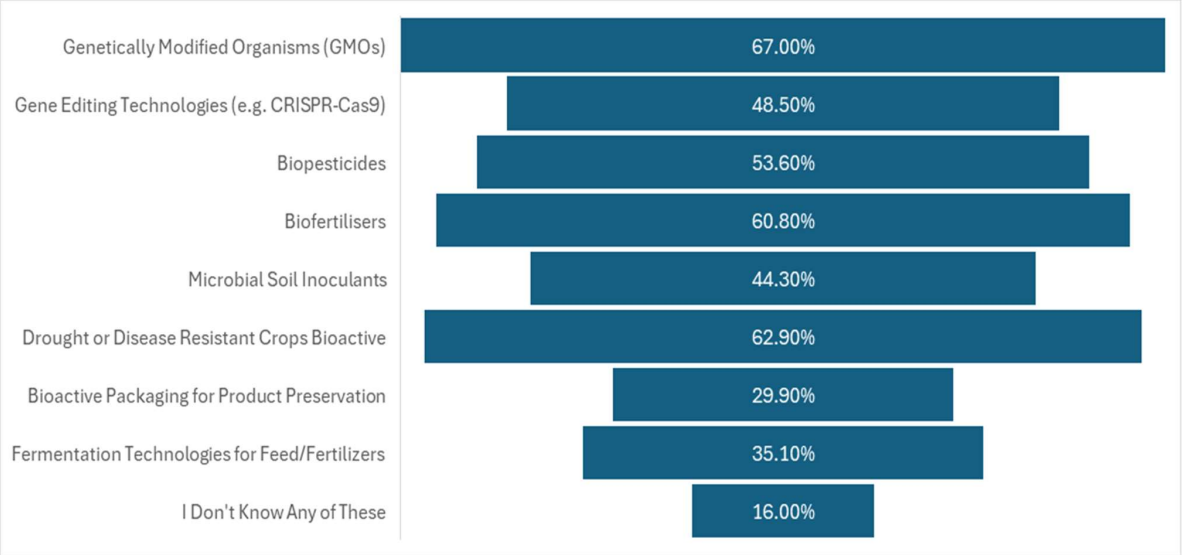


Figure 2. Awareness of Agricultural Biotechnologies Among Farmers // Biotehnologii agricole cunoscute de fermieri

The majority of respondents (64%) stated that they had heard of agricultural biotechnology. Next, to assess the level of detailed knowledge of farmers regarding agricultural biotechnology, one of the questions in the questionnaire asked to identify, from a list of options, technologies that can be considered biotechnology (Figure 2) . The list included concrete and relevant examples, such as genetically modified organisms (GMOs), gene editing technologies (e.g. CRISPR-Cas9), biopesticides, biofertilizers, microbial soil inoculants, drought or disease resistant crops, bioactive packaging and fermentation technologies applied in the production of feed and fertilizers. Each of these options represents valid and current categories of biotechnology used in agriculture, either for the genetic improvement of plants or for the optimization of resources and reduction of environmental impact. The analysis of the responses revealed that, although a significant number of farmers declare that they are familiar with the term “biotechnology”, many of them fail to correctly recognize all the concrete examples, especially in the case of small farms or those with less experience.

This discrepancy highlights the difference between declarative familiarity and applied knowledge, suggesting the need for better targeted information campaigns adapted to the profile of the Romanian farmer. Therefore, the most recognized examples of biotechnologies are genetically modified organisms (GMOs) and drought- or disease-resistant crops, a sign that more publicized terms are better associated with the idea of agricultural innovation.

However, more recent or specialized biotechnologies, such as CRISPR-Cas9, bioactive packaging or fermentation for feed, are significantly less known. This distribution suggests that although there is a general awareness of biotechnologies, the degree of detail and accuracy of knowledge remains low.

An interesting element is the fact that approximately 16% of respondents ticked "I don't know any of these", even in combination with other options, which indicates either conceptual confusion or lack of confidence in their own knowledge. Also, farmers with small farms (under 5 ha) and with little experience (under 10 years) are predominantly those who provide wrong or incomplete answers.

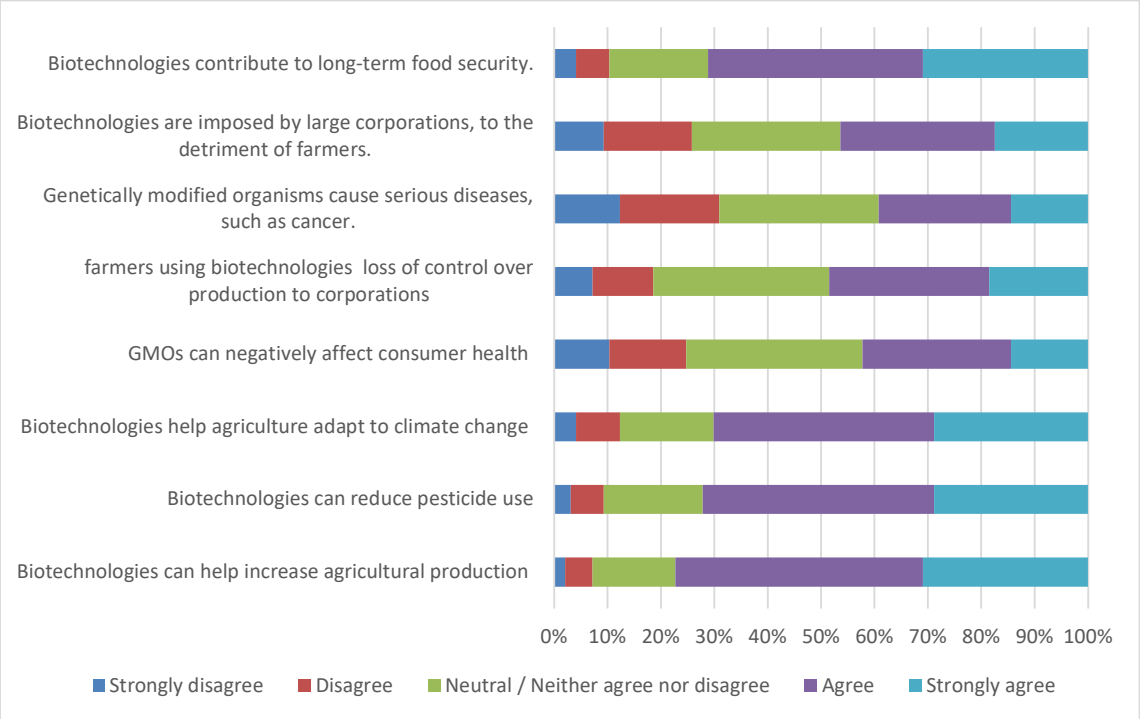


Figure 3. Distribution of responses regarding the perception of biotechnologies // Distribuția răspunsurilor privind percepția biotehnologiilor

The results analyzed indicate that Romanian farmers have a predominantly favorable perception of agricultural biotechnologies, but not without reservations. Regarding the benefits, 77% of respondents agree or strongly agree that biotechnologies can contribute to increasing agricultural production, while only 2% strongly disagree, reflecting a high level of confidence in their agronomic potential. Similarly, 72% of farmers believe that these technologies can reduce the use of pesticides, and 70% agree that biotechnologies help agriculture adapt to climate change. A consistent percentage, 71%, believes that biotechnologies contribute to long-term food security.

On the other hand, the analysis also highlights a number of persistent concerns. Approximately 42% of participants agree that genetically modified organisms can negatively affect consumer health, while 24% declare themselves in total or partial disagreement with this statement, suggesting a clear polarization of opinion.

Also, 48% believe that the use of biotechnologies can lead to the loss of control over production in favor of large corporations, a perception supported by the 47% who state that biotechnologies are imposed by industrial actors, to the detriment of farmers. Regarding one of the most problematic perceptions – that GMOs cause serious diseases, such as cancer – 39% of respondents agreed and 31% disagreed, which suggests the significant presence of misinformation.

Romanian farmers have a largely positive attitude towards the benefits of biotechnology, especially in terms of productivity, sustainability and food security. However, concerns persist

regarding perceived health risks and control over production, indicating that full acceptance is still conditional on accurate information and institutional transparency.

Among the main perceived benefits, farmers mentioned: increased yield per hectare (54%), reduced losses caused by pests and diseases (46%) and reduced costs of chemical inputs (38%) (Figure 4).

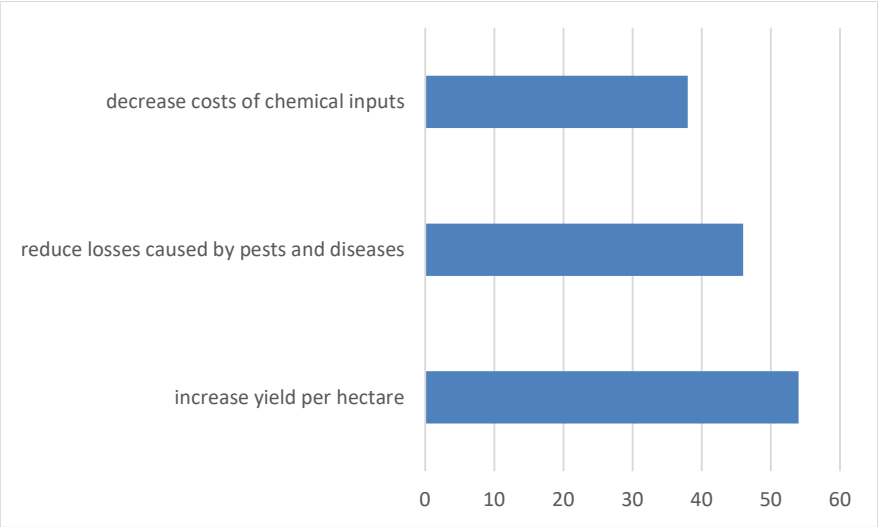


Figure 4. Farmers' perception on advantages that biotechnologies may bring// Percepția fermierilor asupra beneficiilor biotehnologiilor

Notably, farmers with medium and large holdings were more open to adopting biotechnologies, considering them a necessary tool for competitiveness in the agricultural market. These data align with the conclusions of other European research (EuropaBio, 2023), which shows a positive correlation between farm size and openness to innovation.

Regarding the obstacles to the adoption of these technologies, the most frequently mentioned barriers are the lack of clear information, high costs and difficulty in accessing the technology. Also, concerns about health or environmental impact continue to influence farmers' decisions. Despite these challenges, about half of the respondents said they were willing to test or adopt a biotechnology on their farm.

The highest interest is expressed for technologies such as biofertilizers, biopesticides and microbial inoculants, which are perceived as more environmentally friendly and less invasive solutions compared to GMOs.

In terms of information sources, the internet and social networks are the main channels through which farmers access information about agricultural technologies, followed by other farmers, consultants and participation in profile events. However, trust in these sources varies significantly. The most trusted sources are universities and research centers, followed by public institutions. Agricultural technology producers and online press are viewed with more reserve, signaling the need for transparent and scientifically validated communication.

CONCLUSIONS

1. The results of this survey suggest that Romanian farmers generally have a moderately positive attitude towards agricultural biotechnologies, indicating a real potential for integrating these technologies into current agricultural practices. Most respondents recognize the essential benefits offered by biotechnologies, such as increased yield per hectare, reduced losses caused by pests and diseases, and lower costs of chemical inputs. This openness is more pronounced among medium- and large-scale farmers, who are more oriented towards innovation and economic competitiveness.

2. The analysis of perceptions highlights a balance between openness to the benefits of biotechnology and the existence of significant concerns. Respondents tend to agree that biotechnology can contribute to increasing agricultural production, reducing pesticide use and adapting agriculture to climate change. At the same time, a relevant part of the participants expresses fears about the potential negative effects on consumer health, especially in relation to GMOs, as well as a perception that the use of biotechnology may lead to the loss of control over agricultural production by farmers in favor of corporations. However, the degree of acceptance of biotechnologies remains closely linked to several limiting factors. A key element is the level of information: farmers with limited access to credible sources of information or with a lower educational level are more likely to reject or not fully understand the real advantages of these technologies. Also, perceptions of risks — including fears about health effects or the influence of multinational corporations — continue to negatively influence the decision to adopt.
3. Implementation costs and the lack of effective technical and financial support programs are also major barriers reported by respondents. Even among interested farmers, the willingness to adopt biotechnologies is often conditioned by economic accessibility and trust in the legislative and institutional framework.
4. Therefore, for the full potential of biotechnologies to be exploited in Romanian agriculture, it is essential that public policies and science communication initiatives directly target these sensitive points. Investments in training, agricultural advisory services and subsidy programs can contribute to increasing trust and accelerating the transition to a more innovative, resilient and sustainable agricultural system.

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RESULTS OF THE MULTI-YEAR TESTING OF ROMANIAN WINTER WHEAT VARIETIES IN DIFFERENT AREAS OF THE COUNTRY

REZULTATE ALE TESTĂRII MULTIANUALE A SOIURILOR ROMÂNEȘTI DE GRÂU DE TOAMNĂ ÎN DIFERITE ZONE ALE ŢĂRII

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Abstract

Cultivars can play a key role in ensuring high wheat grain yields and reducing its variation under the increasing environmental variability. We analyzed data on grain yield and coefficients of variation from ninety-four yield trials with Romanian winter wheat cultivars, conducted in four regions of the country, under different crop management, for four years (2021-2024). Despite the large variation of environments, we identified cultivars with higher yields in most

conditions and with lower coefficients of variation, which could contribute to higher and more stable yields in farms. Results are also useful for establishing priorities breeding future cultivars.

Rezumat

Soiurile pot juca un rol decisiv în asigurarea unor producţii ridicate şi în reducerea variabilităţii acestora în condiţiile unei variabilităţi tot mai mari a condiţiilor de mediu. Am analizat datele privind producţia de boabe şi coeficienţii de variaţie a producţiilor din nouă zeci şi patru culturi comparative cu soiuri româneşti de grâu de toamnă, efectuate în patru regiuni ale ţării, cu diferite variante tehnologice, vreme de patru ani (2021-2024). Deşi variaţia condiţiilor de mediu a fost foarte mare, am identificat soiuri cu producţii ridicate în majoritatea condiţiilor şi cu coeficienţi de variaţie a recoltelor mai mici, care pot contribui la obţinerea de recolte mai mari şi mai stabile în ferme. Rezultatele sunt deasemenea utile pentru stabilirea priorităţilor pentru ameliorarea viitoarelor soiuri.

Key words: *wheat, yield, variation, ranks*
Cuvinte cheie: *grâu, producţie, variaţie, clasificare*

INTRODUCTION

Most farmers recognize cultivars as one of the most dynamic and economically efficient factors in wheat production, with a decisive influence on yield level and response to environmental conditions. Public breeding programs in Romania have continuously contributed to providing better cultivars to farms (Paraschivoiu et al., 1983; Botezan et al., 1994; Tabără et al., 2008; Bunta and Bucurean, 2010; Mustăţea and Săulescu, 2011; Moldovan et al., 2012; Marinciu et al., 2015; Saulescu et al., 2024.). As a result, from 1971 to present, Romanian wheat cultivars have been grown on about 40 to 69 % or agricultural wheat surface. To promote the best new cultivars, NARDI Fundulea has coordinated, since its foundation, a National uniform yield trial with winter wheat cultivar, at sites all over the country. The present paper analyzes average yields and yield variation recorded in these trials conducted during 2021-2024.

MATERIAL AND METHODS

We analyzed yield trials results obtained in Romanian winter wheat cultivars originated from the National Agricultural Research & Development Institute Fundulea (NARDI), and from the Agricultural Research & Development Stations Turda, Lovrin – Oradea, Șimnic and Caracal, along with the historical check Bezostaya 1. Yield trials were conducted countrywide to test winter wheat yielding ability and adaptation to various regions of Romania. The set of cultivars tested in the Southern and Western regions was different from the set tested in the Central and Eastern regions. Table 1 presents the cultivars tested in the four regions in alphabetical order.

We analyzed yield results grouped as follows:

1. Trials with crop management recommended for each environment, including the recommended Nitrogen fertilization, which varied from 82 to 143 kg. N/ha, conducted at sites in:

1.1. Southern region including:

- Agricultural Research & Development Station (ARDS) Valu lui Traian – Lat. 44º16’ N, Long. 28º51’E;
- National Agriculture Research and Development Institute (NARDI) Fundulea – Lat 44°30`N, Long 26°51`E. At this site two trials with different sowing dates were analyzed;
- ARDS Teleorman (Drăgăneşti-Vlaşca) – Lat. 44°07’N, Long. 25°45’E;
- ARDS Mărculeşti – Lat. 44°25′N, Long. 27°29′E ;
- ARDS Brăila – Lat. 45°16′N, Long. 27°57′E ;
- ARDS Piteşti – Lat 44º81’N, Long.24 º86’E;
- Craiova University - Agricultural Research Station Caracal – Lat. 44º11’N. Long. 24º37’E;

- ARDS Șimnic – Lat 44°20′N, Long. 23°49′E.

1.2. Western region including:
- ARDS Lovrin – Lat 45°57'N, Long. 20°46';
- ARDS Oradea – Lat. 47°02'N Long. 21°54'E;
- ARDS Livada – Lat. 47°52'N, Long. 23°08'E;

1.3. Eastern region including:
- ARDS Secuieni – Lat. 46ºN, Long. 26º 86'E ;
- [15] Research & Development Station for Soil Erosion Control „Mircea Moțoc" (RDSSEC "MM") Perieni – Lat. 46°18′N, Lat. 27°37′E.

1.4. Central region including:
- ARDS Turda – Lat. 46º58' N, Long. 23º78'E ;
- RDSCB Târgu-Mureș – Lat. 46°32'N, Long. 24°33'E.

Table 1. Cultivars tested in yield trials// *Soiurile testate în culturi comparative*

Southern and Western regions					**Central and Eastern regions**			
	Cultivar	**Breeder**	**Status**		**Nr**	**Cultivar**	**Breeder**	**Status**
1	A 4-10	ARDS Pitești	Breeding line		1	ANDRADA	ARDS Turda	Released 2012
2	AMURG	NARDI Fundulea	Breeding line		2	BEZOSTAYA 1	Russia	Historical check
3	BEZOSTAYA 1	Russia	Historical check		3	CEZARA	ARDS Turda	Released 2020
4	BIHARIA	ARDS Lovrin	Released 2021		4	CODRU	ARDS Turda	Released 2015
5	CAROM	ARDS Caracal	Relased 2024		5	FDL ABUND	NARDI Fundulea	Released 2022
6	DACIC	ARDS Lovrin	Released 2019		6	FDL COLUMNA	NARDI Fundulea	Pending release 2025
7	FDL ABUND	NARDI Fundulea	Released 2022		7	FDL CONSECVENT	NARDI Fundulea	Released 2024
8	FDL COLUMNA	NARDI Fundulea	Pending release 2025		8	FDL MIRANDA	NARDI Fundulea	Released 2011
9	FDL CONSECVENT	NARDI Fundulea	Released 2024		9	GLOSA	NARDI Fundulea	Released 2025
10	FDL MIRANDA	NARDI Fundulea	Released 2011		10	LUMINITA	ARDS Turda	Released 2023
11	GLOSA	NARDI Fundulea	Released 2005		11	OTILIA	NARDI Fundulea	Released 2013
12	IZVOR	NARDI Fundulea	Released 2007		12	PITAR	NARDI Fundulea	Released 2015
13	OTILIA	NARDI Fundulea	Released 2013		13	SEMNAL	NARDI Fundulea	Released 2017
14	PITAR	NARDI Fundulea	Released 2015		14	T. 42-17	ARDS Turda	Breeding line
15	SIMNIC1619	ARDS Simnic	Breeding line		15	T. 61-18	ARDS Turda	Breeding line
16	URSITA	NARDI Fundulea	Released 2021		16	T. 75-16	ARDS Turda	Breeding line
17	VOINIC	NARDI Fundulea	Released 2019		17	T109-12	ARDS Turda	Breeding line
					18	URSITA	NARDI Fundulea	Released 2021
					19	VOINIC	NARDI Fundulea	Released 2019

2. Trials with reduced Nitrogen fertilization (where N fertilizers were only applied in autumn at rates from 0 to 50 kg N s.a./ha), conducted at:
   - ARDS Valu lui Traian
   - NARDI Fundulea
   - ARDS Teleorman (Drăgăneşti-Vlaşca)
   - ARDS Pitești
   - Craiova University - Agricultural Research Station Caracal.

3. Trials conducted with organic agriculture management for two years at;

- ARDS Valu lui Traian
- NARDI Fundulea

The testing sites covered a large diversity of soils, from chernozems to luvisoils, with pH from 5.02 to 7.6, and humus content from 1.71% to 3.6%. Weather conditions during 2021-2024 at all these sites were diverse and reflected present climate changes, as illustrated by rainfall, which varied from 211.2 to 613.8 mm/year.

We analyzed results from the cultivars tested all testing years and calculated the average yield and the coefficient of variation (s% = standard deviation/grain yield*100), for each cultivar and group of trials.

RESULTS AND DISCUSSION

In thirty-six trials from the **Southern region**, average yields varied from about 5500 kg/ha in Bezostaya1 to more than 7300 kg/ha in FDL Columna, while coefficients of variation varied from 22.1% in FDL Consecvent to 30% in line A4-10 (Table 2). The large variation observed in A4-10 was due to the fact that the region included ARDS Pitesti, where the line is very adapted, but also sites where lodging affected its yields.

Table 2. Average grain yield and coefficients of variation for seventeen wheat cultivars tested in thirty-six trials in the Southern region // *Producţiile medii şi coeficienţii de variaţie pentru 17 soiuri de grâu testate în 36 culturi comparative în zona de Sud*

Cultivar	Average grain yield (kg/ha)	Percentage of Glosa (%)	Coefficient of variation (s%)
FDL COLUMNA	7329	109.6	22.9
URSITA	7255	108.5	23.6
FDL ABUND	7176	107.3	23.9
FDL CONSECVENT	7150	106.9	22.1
BIHARIA	7079	105.9	26.0
CAROM	6820	102.0	25.8
Simnic1619	6816	101.9	24.3
VOINIC	6797	101.7	23.2
GLOSA	6687	100.0	23.2
OTILIA	6644	99.4	23.1
AMURG	6591	98.6	23.6
FDL MIRANDA	6553	98.0	25.2
DACIC	6546	97.9	28.3
IZVOR	6522	97.5	24.7
PITAR	6479	96.9	22.6
A 4-10	6469	96.7	30.0
BEZOSTAYA 1	5538	82.8	27.8
AVERAGE	6732		24.7

Cultivars FDL Consecvent, FDL Columna, Ursita and FDL Abund had highest average yields and above average yield stability, while cultivar Biharia, which was also high yielding, showed higher yield variation.

In twenty trials with **reduced Nitrogen fertilization**, average yields varied from less than 4800 kg/ha in cultivar Bezostaya1, to more than 6600 kg/ha in cultivars FDL Consecvent, FDL Columna and Ursita (Table 3). Cultivar FDL Abund had larger yield variation in these trials. Coefficients of variation varied from about 18% in cultivars Carom and A4-10 to more than 26% in Bezostaya1. The highest yielding cultivars (FDL Consecvent, FDL Columna, Ursita and Biharia) also had better than average yield stability in these conditions.

Table 3. Average grain yield and coefficients of variation for seventeen wheat cultivars tested in twenty trials with reduced Nitrogen fertilization in the Southern region // (*Producţiile medii şi coeficienţii de variaţie pentru 17 soiuri de grâu testate în 20 culturi comparative cu fertilizare redusa cu azot în zona de Sud*

Cultivar	Average grain yield (kg/ha)	Percentage of Glosa (%)	coefficient of variation (s%)
FDL CONSECVENT	6717	111.7	20.50934

Cultivar	Average grain yield (kg/ha)	Percentage of Glosa (%)	coefficient of variation (s%)
FDL COLUMNA	6670	110.9	21.30424
URSITA	6614	110.0	19.69744
BIHARIA	6560	109.1	20.94902
FDL ABUND	6534	108.7	24.47419
A 4-10	6286	104.5	18.1825
VOINIC	6223	103.5	20.08725
CAROM	6131	102.0	17.69162
OTILIA	6097	101.4	19.19114
FDL MIRANDA	6088	101.2	23.99814
Simnic1619	6023	100.2	20.56955
GLOSA	6013	100.0	23.26138
IZVOR	5959	99.1	22.16443
DACIC	5953	99.0	23.97849
AMURG	5929	98.6	20.70563
PITAR	5855	97.4	23.51689
BEZOSTAYA 1	4735	78.7	26.71709
Average	6204.404	102.1	21.6

In twelve trials from the **Western region** average yields were higher, varying from 4928 kg/ha in cultivar Bezostaya1 to 8042 kg/ha in FDL Columna (Table 4).
Coefficients of variation varied from about 29 in cultivars Otilia, Biharia and FDL Consecvent to 37.7% in the breeding line Amurg. High yield variation was also observed in cultivars FDL Miranda and Carom.

It is worth mentioning that cultivars FDL Consecvent and Biharia proved to be at the same time the highest yielding and among the cultivars with smallest yield variation caused by environmental conditions in this region.

Table 4. Average grain yield and coefficients of variation for seventeen wheat cultivars tested in twelve trials in the Western region //
Producţiile medii şi coeficienţii de variaţie pentru 17 soiuri de grâu testate în 12 culturi comparative în zona de Vest

Cultivar	Average grain yield (kg/ha)	Percentage of Glosa (%)	coefficient of variation (s%)
FDL COLUMNA	8042	119.7	32.3
FDL CONSECVENT	7869	117.2	29.6
FDL ABUND	7785	115.9	31.9
BIHARIA	7783	115.9	28.9
URSITA	7367	109.7	34.6
OTILIA	7202	107.2	28.7
VOINIC	7173	106.8	31.5
A 4-10	6953	103.5	34.2
PITAR	6904	102.8	33.7
Simnic1619	6864	102.2	31.3
CAROM	6806	101.3	35.8
IZVOR	6767	100.7	30.7
DACIC	6718	100.0	31.9
GLOSA	6717	100.0	33.1
FDL MIRANDA	6619	98.5	36.0
AMURG	6555	97.6	37.7
BEZOSTAYA 1	4928	73.4	32.3
Average	7003		32.6

Averaged over eleven trials conducted in the **Eastern region**, yields varied from 4826 kg/ha in cultivar Bezostaya 1 to 6756 kg/ha in cultivar FDL Columna (Table 5). Coefficients of variation varied from around 31% in cultivars Pitar and Voinic, to 40% in cultivar Codru. In contrast with other regions, no cultivar combined best values of yield and s%. The highest yielding cultivars (FDL Columna and FDL Consecvent) had average yield variation. The best combination of yield and stability was present in cultivar Voinic, the third highest yielding, which was the second in yield stability. Two lines from the breeding program of ARDS Turda had better than average yield and coefficient of variation, while most cultivars from that breeding program had lower yields and larger yield variation, associated with their relative lateness and lodging susceptibility, which had a negative influence on yield in this region.

Table 5. Average grain yield and coefficients of variation for nineteen wheat cultivars tested in eleven trials in the Eastern region //
Producţiile medii şi coeficienţii de variaţie pentru 19 soiuri de grâu testate în 11 culturi comparative în zona de Est

Cultivar	Average grain yield (kg/ha)	Percentage of Glosa (%)	coefficient of variation (s%)
FDL COLUMNA	6756	116.2	34.8
FDL CONSECVENT	6638	114.2	35.3
VOINIC	6315	108.7	31.5
SEMNAL	6298	108.4	36.1
FDL ABUND	6255	107.6	34.8
URSITA	6148	105.8	37.5
T. 75-16	6122	105.3	34.6
T. 61-18	6062	104.3	34.4
OTILIA	5990	103.1	35.2
CODRU	5877	101.1	40.0
CEZARA	5863	100.9	38.1
GLOSA	5812	100.0	33.7
ANDRADA	5800	99.8	35.6
T109-12	5738	98.7	38.7
PITAR	5701	98.1	30.6
T. 42-17	5696	98.0	37.9
LUMINITA	5680	97.7	37.3
FDL MIRANDA	5678	97.7	33.5
BEZOSTAYA 1	4827	83.0	31.3
Average	5961	102.6	35.3

In the eleven trials conducted in the **Central region** (Transylvania) yields were highest of all regions, average yields varying from 6617 in cultivar Bezostaya1 to 8842 kg/ha in cultivar FDL Consecvent (Table 6). Coefficients of variation varied from 8.5% in cultivar FDL Consecvent to 19.5% in cultivar FDL Miranda.

More cultivars than in the other regions, such as FDL Consecvent, FDL Abund, Luminiţa, Semnal, Ursita and FDL Columna, combined high yields with low yield variation.
Cultivars Glosa and Pitar, showed good yield stability but lower yields in this region, related to their earliness, which does not allow full use of the available weather resources.

Table 6. Average grain yield and coefficients of variation for nineteen wheat cultivars tested in eleven trials in the Central region //
Producţiile medii şi coeficienţii de variaţie pentru 19 soiuri de grâu testate în 11 culturi comparative în zona de Centru

Cultivar	Average grain yield (kg/ha)	Percentage of Glosa (%)	coefficient of variation (s%)
FDL CONSECVENT	8842	112.8	8.5
T. 75-16	8739	111.4	15.2
T. 42-17	8724	111.3	12.9
FDL ABUND	8687	110.8	9.8

Cultivar	Average grain yield (kg/ha)	Percentage of Glosa (%)	coefficient of variation (s%)
LUMINITA	8658	110.4	10.2
SEMNAL	8633	110.1	9.4
URSITA	8580	109.4	10.9
FDL COLUMNA	8572	109.3	8.8
T109-12	8571	109.3	13.6
CEZARA	8530	108.8	16.5
CODRU	8507	108.5	13.9
OTILIA	8497	108.4	10.3
T. 61-18	8434	107.6	16.5
VOINIC	8390	107	9.1
ANDRADA	8184	104.4	16.4
PITAR	8148	103.9	9.4
FDL MIRANDA	7920	101	19.5
GLOSA	7842	100	10.9
BEZOSTAYA 1	6617	84.4	17.1
Average	8372	106.8	12.6

In the four trials conducted in **organic agriculture**, yields were lower and average yields varied from 2935 kg/ha in Bezostaya1 to 4374 kg/ha in FDL Consecvent (Table 7).
Yield variation was larger than with conventional crop management, coefficients of variation varying from 36.2% in cultivar Carom to 56.1% in cultivar Glosa.

Results obtained by cultivar FDL Consecvent were outstanding, as it was the highest yielding in organic agriculture and had lower than average variation of yields, it is also bunt resistant, an important trait in organic agriculture.

T**able 7. Average grain yield and coefficients of variation for eighteen wheat cultivars tested in four trials under organic agriculture system**.//
Producţiile medii şi coeficienţii de variaţie pentru 18 soiuri de grâu testate în 4 culturi comparative în sistem de agricultură ecologică

Cultivar	Average grain yield (kg/ha)	Percentage of Glosa (%)	coefficient of variation (s%)
FDL CONSECVENT	4374	130.1	43
FDL EMISAR	4258	126.7	50.7
URSITA	4185	124.5	46.2
FDL DARNIC	4088	121.6	53.3
FDL ABUND	4085	121.5	45.4
FDL MIRANDA	4046	120.3	55
OTILIA	3997	118.9	45.3
FDL EVIDENT	3877	115.3	46.7
FDL COLUMNA	3860	114.8	46
CAROM	3849	114.5	36.2
BIHARIA	3806	113.2	40.5
SIMNIC 1619	3770	112.1	46.4
DACIC	3729	110.9	51.9
PITAR	3604	107.2	38.3
A 4-10	3589	106.7	53.8
GLOSA	3362	100	56.1
VOINIC	3227	96	49.2
BEZOSTAYA 1	2935	87.3	47.2
Average	3813	113.4	47.3

To summarize the cultivar behavior, we analyzed their ranking in each group of trials. Rankings can facilitate conclusions about cultivar suitability to environments and can also provide information about their stability.

Table 8 presents the rankings of cultivars based on average yields in the analyzed trial groups.

Table 8. Cultivar ranking for yield in the analyzed groups of trials.// *Clasificarea soiurilor în grupele de culturi comparative analizate*

Cultivar	South		West	Average	Cultivar	East	Central	Average
	Full N	Reduced N						
FDL COLUMNA	1	2	1	1.3	FDL CONSECVENT	2	1	1.5
FDL CONSECVENT	4	1	2	2.3	FDL COLUMNA	1	8	4.5
URSITA	2	3	5	3.3	FDL ABUND	5	4	4.5
FDL ABUND	3	5	3	3.7	T 75-16	7	2	4.5
BIHARIA	5	4	4	4.3	SEMNAL	4	6	5.0
VOINIC	8	7	7	7.3	URSITA	6	7	6.5
OTILIA	10	9	6	8.3	VOINIC	3	14	8.5
CAROM	6	8	11	8.3	T 42-17	16	3	9.5
Simnic1619	7	11	10	9.3	OTILIA	9	12	10.5
A 4-10	16	6	8	10.0	CEZARA	11	10	10.5
GLOSA	9	12	14	11.7	CODRU	10	11	10.5
FDL MIRANDA	12	10	15	12.3	T 61-18	8	13	10.5
IZVOR	14	13	12	13.0	LUMINIŢA	17	5	11.0
DACIC	13	14	13	13.3	T 109-12	14	9	11.5
PITAR	15	16	9	13.3	ANDRADA	13	15	14.0
AMURG	11	15	16	14.0	GLOSA	12	18	15.0
BEZOSTAYA1	17	17	17	17.0	PITAR	15	16	15.5
					FDL MIRANDA	18	17	17.5
					BEZOSTAYA 1	19	19	19.0

If we compare the cultivar ranking in different conditions, it is obvious that despite the strong effect of environments, cultivars FDL Columna and FDL Consecvent ranked on the top for yield, while Bezostaya1 and Pitar had low rankings in most environments.

Cultivar rankings for yield in the four trials conducted in organic agriculture were highly variable and differed from those observed under recommended crop management. However, cultivars Ursita and FDL Consecvent had better rankings than other tested cultivars (Table 9).

Table 9. Cultivar ranking in organic agriculture.// *Clasificarea soiurilor în agricultura ecologică*

Cultivar	Fundulea 2024	Valu Traian 2024	Fundulea 2023	Valu Traian 2023	Average
URSITA	4	4	7	6	5.2
FDL CONSECVENT	1	14	1	7	5.7
FDL ABUND	16	2	5	5	7.0
OTILIA	7	8	8	9	8.0
CARO	8	18	3	4	8.2
MIRANDA	2	7	13	14	9.0
BIHARIA	14	12	11	3	10.0
A 4-10	10	11	20	2	10.7
SIMNIC 1619	5	16	12	11	11.0
FDL COLUMNA	11	10	4	19	11.0
PITAR	18	17	6	15	13.2
GLOSA	20	5	14	20	13.5
VOINIC	15	19	16	17	17.5
BEZOSTAYA1	19	20	19	16	18.7

CONCLUSIONS

Testing wheat cultivars in ninety-four yield trials conducted during four years in diverse conditions allowed identification of cultivars able to provide higher yields, with smaller variation induced by environmental conditions.

1. Data included in this paper can facilitate a better choice of cultivars for each of the analyzed regions and this could contribute to higher and more stable wheat production in farms.
2. Presented results can also be useful for establishing priorities in breeding future cultivars.

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MORPHOLOGICAL ASPECTS ON THE *IN VITRO* DEVELOPMENT OF THE PLANTLETS FROM THE LOCAL POTATO VARIETIES COLLECTION, PRESERVED IN *SLOW GROWTH* CONDITIONS AT SUCEAVA GENEBANK

ASPECTE MORFOLOGICE ALE DEZVOLTĂRII *IN VITRO* A PLANTULELOR DIN COLECȚIA LOCALĂ DE VARIETĂȚI DE CARTOF, CONSERVATE ÎN CONDIȚII DE *CREȘTERE LENTĂ* LA BANCA DE GENE SUCEAVA

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Abstract

Based on the phenotypic evaluation of the over 100 potato genotypes from the "in vitro" collection, the analysis of the modes of manifestation for the main morphological aspects of the plantlets (type of shoots, leaflets and roots, generation of microtubers etc.), a large number of reactions to slow growth conservation conditions were identified. Variability appeared mainly through the way of reaction of different genotypes, the morphological appearance of the plantlets for the same genotype, on different culture media, being relatively similar.

Rezumat

Pe baza evaluării fenotipice a celor peste 100 de genotipuri de cartof din colecția "in vitro", a analizei modurilor de manifestare a principalelor aspecte morfologice ale plantulelor (tipul lăstarilor, al frunzulițelor și rădăcinițelor, generarea de microtuberculi etc.), a fost identificat un număr mare de reacții la condițiile de conservare prin creștere lentă. Variabilitatea a apărut mai ales prin modul de reacție a diferitelor genotipuri, aspectul morfologic al plantulelor la același genotip, pe diferite medii de cultură, fiind relativ similar.

Keywords: *potato, Solanum tuberosum, "in vitro culture", osmotic stress, slow growth*
Cuvinte cheie: *cartof, Solanum tuberosum, cultură "in vitro", stres osmotic, creștere lentă*

INTRODUCTION

The collection of local potato populations (*Solanum tuberosum* L.), maintained in the experimental field, in the *in vitro* growth and conservation chambers of the Suceava Gene Bank, includes mainly old varieties, collected especially from mountainous areas of Romania, the potato being, along with peas and faba beans, among the few crops that reach maturity in these areas with short vegetation periods.

The development of micropropagation techniques through *in vitro* cultures responded to practical needs, including the rescue of species that are "recalcitrant" to seed conservation, or those with vegetative multiplication, such as the potato (CIP Training Manual; 2014).

In vitro culture is, in itself, a method of conservation and conservative propagation, through cloning. However, the method involves material costs, energy consumption, and labour and presents the risk of accidental contamination and, over time, possible genetic changes. Typically, a culture must be re-subcultured on fresh medium at least once every 2-3 months, which justifies the efforts directed towards finding methods to reduce growth, increase the intervals between two subcultures, which is the objective of *in vitro* conservation, thus reducing costs and eliminating the various deficiencies that can be caused by frequent manipulations of the preserved biological material.

The *in vitro* reactivity of potato is generally very good, but that of local genotypes can be quite different, in terms of growth rate, leaf size, plantlets vigour or how it reacts to the stressful conditions of *slow-growing* conservation. (Ibănescu M., Constantinovici D. and Strajeru S., 2007).

The main objective of the work, at this moment, is to preserve the inoculums in the collection by *slow growth*. In this context, the beneficial effects of combining 2 limiting factors of development were found. In order to generate plantlets, the uninodal microcuttings are periodically subcultured on media with higher osmotic pressure, by adding mannitol, sorbitol, sucrose, or in other substances with a retardant effect (daminozide, cycocel) are introduced, simultaneously with a decrease in the ambient temperature from 22^0C to 6 – 10^0C.

MATERIAL AND METHOD

All the experiments were carried out on the basis of the subcultivation of uninodal microcuttings, resulting from the plantlets of local potato varieties collection, previously regenerated *in vitro* (Constantinovici D., 2005).

The culture media were based on the MURASHIGE-SKOOG recipe (MS-1962) (Murashige T., Skoog F., 1962). The growth regulators used were kinetin (K), benzyladenine (BA) and μ naphthyl acetic acid (ANA) in low concentrations, so as not to stimulate the growth of the inoculums. To extend the time between two subcultures, the osmotic pressure was increased by adding 40 g of mannitol in **C_1** medium, and sorbitol to the second culture media, **C_2**.

The medium (20 ml) was dispensed into 170 ml vials of (5.5 cm diameter / 9.5 cm height). The vials were covered with aluminum foil and sterilized in an autoclave for 20 min at 121^0C. After placing the inocula on the medium, the vials were covered with double polyethylene film, fixed with rubber rings and were transferred to the growth chamber at a temperature of 20 – 22^0C, and photoperiod of 16/24 hours, with a light intensity of 2000 - 2500 lx.

After resuming the inoculum growth processes, the vials were transferred to the conservation room, under slightly restrictive conditions, with a temperature varying between 6 and 10^0C, photoperiod 10/24 hours, and the light intensity of 1000 lx, generated by LED tubes.

RESULTS AND DISCUSSION

The potato is a crop of great importance, which multiplies vegetatively and for which rapid micropropagation procedures have been developed. The entire methodology, starting from meristem inoculation and subculture of microcuttings is dependent on the pre-existence of axillary buds. Elongation of shoots could be obtained on a simple medium, while tuberization requires higher concentrations of sucrose, cytokinins and various growth regulators (CachiThe potato genotypes from the collection maintained under *slow growth* conditions, for periods exceeding two years, while preserving viability and vitality, show morphological differences, but the main effect is the reduction of the height of the plantlets (2-4 cm), by shortening the internodes as well as the size of the leaflets. These often become scaly (1.5-2 mm), many of them growing on microshoots developed on the surface of microtubers or some hypertrophied-looking formations. (photo 1 - 3).

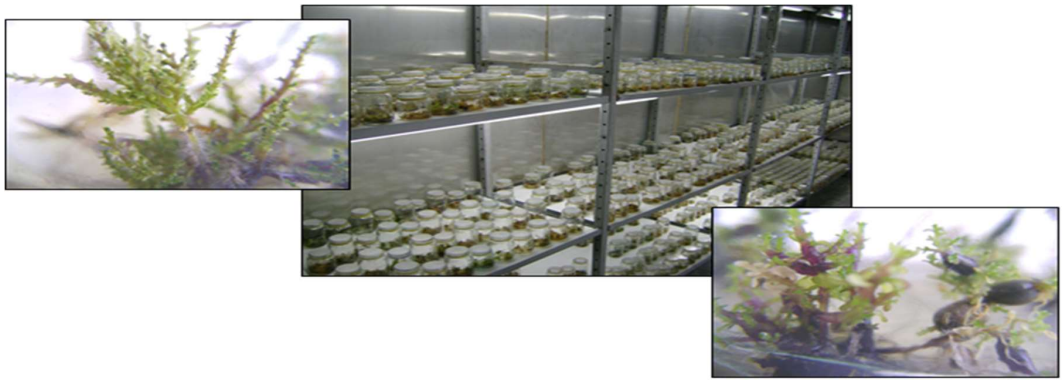


Photo 1 – 3. Plantlets from local potato populations (*Solanum tuberosum* L.) preserved *in vitro* by *slow growth* on mannitol or sorbitol media, with details on the appearance of the inocula after more than 24 months// Foto 1 - 3. Plantule din populaţii locale de cartof (*Solanum tuberosum* L.) conservate *in vitro* prin *creştere lentă* pe medii cu manitol sau sorbitol, cu detalii privind aspectul inoculilor după mai e luni

The double polyethylene foil ensures, in most cases, sterility and, above all, avoids dehydration of the culture media.

Variability appeared mainly through the way different genotypes reacted, the morphological appearance of the plantlets, of the same genotype, on the two culture media, being relatively similar (photo 4-6)



Photo 4 - 6. Potato plantlets of the SVGB-5126 (Ulma, Suceava County), preserved *in vitro* by *slow growth* on mannitol (left) and sorbitol (right) media, with details regarding the appearance of the inocula after more than 24 months.

Foto 4 - 6. Plantule de cartof ale populației SVGB-5126 (Ulma, Județul Suceava), conservate *in vitro* prin *creștere lentă* pe medii cu manitol (stânga) și sorbitol (dreapta), cu detalii privind aspectul inoculilor după mai mult de 24 de luni.

During the conservation period, the plantlets generate morphological structures that store water and ensure the viability of the buds located at the base of the leaflets (Tanasă A.C., Constantinovici D. and Străjeru S., 2023). The development of microtubers or aerial roots is also stimulated. The shoots of the genotypes in the collection have a wide variety of shapes, being simple or branched and growing both on the surface and inside the culture media. After the end of the conservation period, when performing the apex subcultivation operations, the last observations were made regarding the influence of the conservation media (**C_1** and **C_2**) on the evolution of potato plantlets during *slow growth*. (Photo 7 - 14).



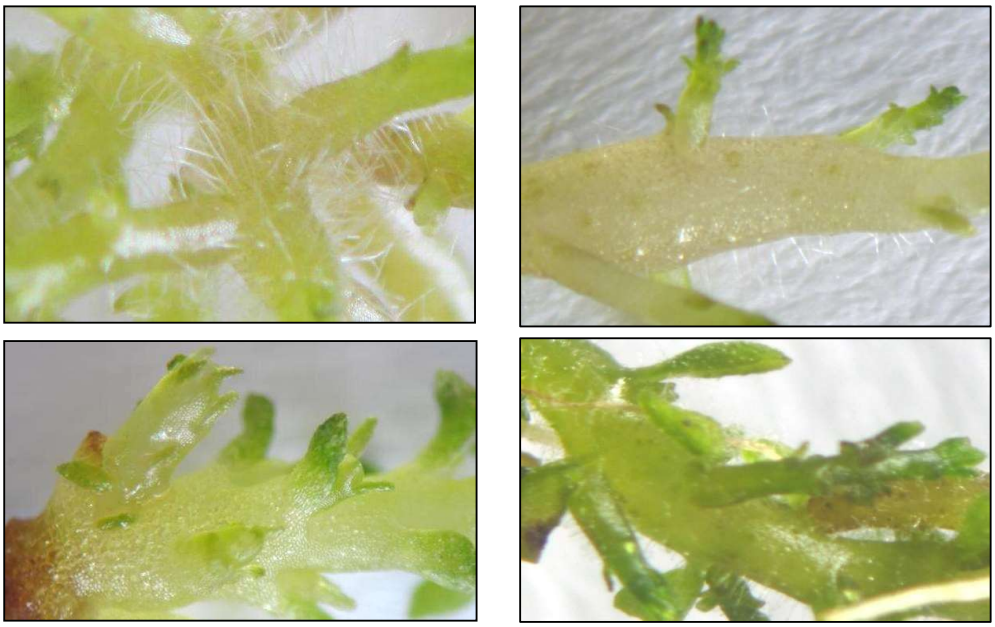


Photo 7 - 14. Shoots developed during the conservation period on culture medium with mannitol (left) and sorbitol (right), in the four potato genotypes: SVGB-12496 (Tupilati, Neamt County), SVGB-15102 (Sânpetru Almasului, Sălaj County), SVGB-15140 (Agarbiciu, Cluj County) and SVGB-15242 (Grosuri, Hunedoara County) //

Foto 7 - 14. Lăstari dezvoltați în perioada de conservare pe mediu de cultură cu manitol la genotipurile (stânga) și sorbitol (dreapta), la patru genotipuri de cartof: SVGB-12496 (Tupilati, județul Neamț), SVGB-15102 (Sânpetru Almasului, județul Sălaj), SVGB-15140 (Agarbiciu, județul Cluj) și SVGB-15242 (Grosuri, județul Hunedoara).

The decrease in the concentration of inorganic compounds in the MS culture media, together with the increase in osmotic pressure, led to the development of plants with reduced height and short internodes, but nevertheless allowed an acceptable evolution of the basal roots, sometimes also of the aerial ones, being favourable for the medium-term conservation of the collection of local genotypes, representing the main concern of the in vitro culture laboratory at the Suceava Gene Bank.

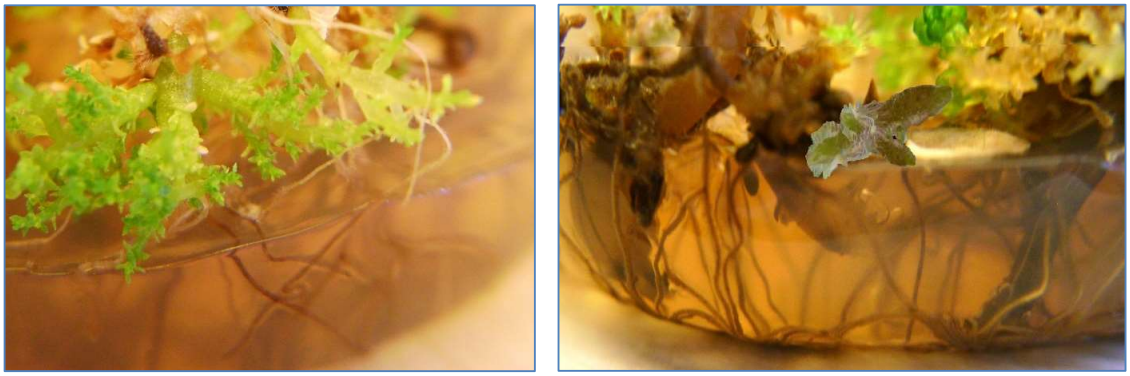


Photo 15 - 16. Basal roots developed during the conservation period on culture medium with sorbitol in the genotype SVGB-15244 (Grosuri, Hunedoara County) //

Foto 15 - 16. Rădăcini bazale dezvoltate în perioada de conservare pe mediu de cultură cu sorbitol la genotipul SVGB-15244 (Grosuri, județul Hunedoara).

The possibility of maintaining all varieties at temperatures between 6 – 10^0C is an element of great importance in extending the time periods between two subcultures and postponing the appearance of senescence phenomena, which have proven to be closely related to the genotype but also to the culture medium.

The apices of potato plantlets grown on the two culture media containing mannitol and sorbitol as inhibitory agents were taken and subcultured on fresh medium. After the regeneration of

normally shaped plants, a second subculture followed for the multiplication of the genotypes, starting from young, actively growing tissues.

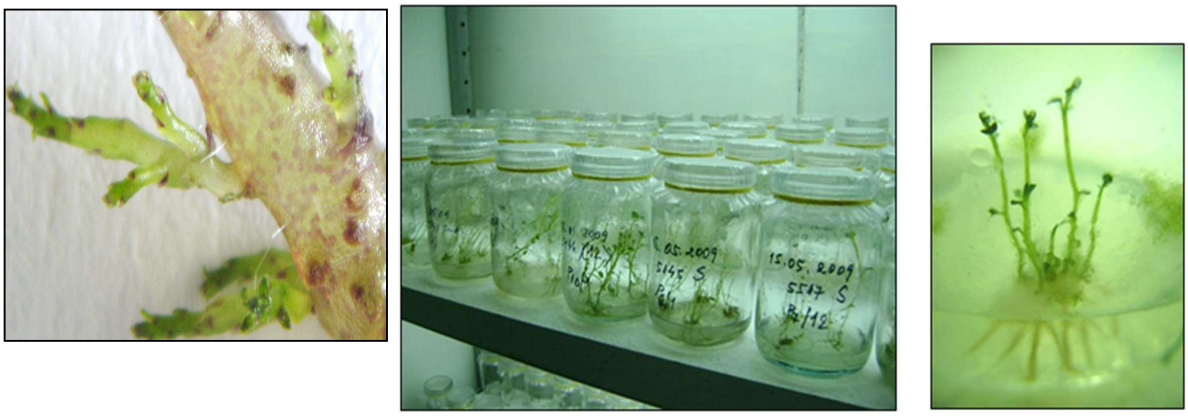


Photo 17 - 18. Plantlets from local potato genotypes, regenerated after "*in vitro*" conservation for over two years through *slow growth.*//
Foto 17 - 18. Plantule din genotipuri locale de cartof, regenerate după o conservare *in vitro* timp de peste doi ani prin *creştere lentă*.

New *in vitro* preservation technologies are always being researched, but the *slow growth* method is accessible in most cases. *In vitro* conservation does not intend to replace the conventional *in situ* and *ex situ* methods of preserving plant genetic resources, being only a complementary methodology.

CONCLUSIONS

6. The composition of culture media influences the *in vitro* development of potato inocula, in all phases helping to extend conservation periods through *slow growth*, and to regenerate plantlets after conservation.
7. The preservation media induced a slowdown in the growth of the inoculums, the preservation of their viability and vitality, the manifestation of some differences in the type of growth of the genotypes, but the main effect is that of reducing the height of the plantlets (2-4 cm), by shortening the internodes, modifying their morphological appearance, as well as the dimensions of the leaflets.
8. The existence of the possibility to reduce the temperature in the preservation room to 6-10^{0}C, is one of the main beneficial factor for extending the periods of time between two subcultures and postponing the appearance of senescence phenomena.
9. *In vitro* preservation reduces the risk of material loss due to environmental conditions from the experimental field, as it is carried out in laboratory, without the influence of seasons. After eliminating viruses or other infections, cultures can be kept free of pathogens and can be available to users.
10. The *in vitro* conservation method is one of the most effective for a large number of potato genotypes, for a developing collection.

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ACTA AGRICOLA ROMANICA

Volume 7, Year 7, No. 7
August 2025

Horticulture, Forestry and Agricultural Economics

BUCHAREST

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The magazine appears annually, in the second semester of the year
ISSN – L 2784 – 0948

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):

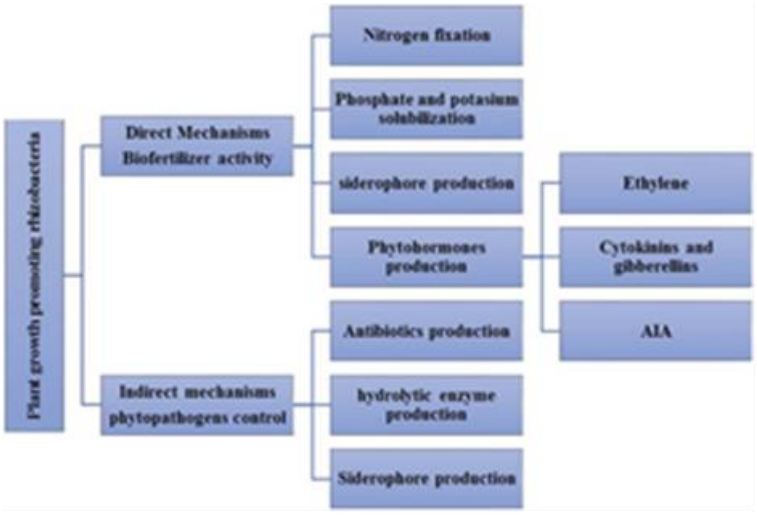


Figure 1. PGPR mechanisms of plants / Mecanismele de acțiune ale plantelor

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.

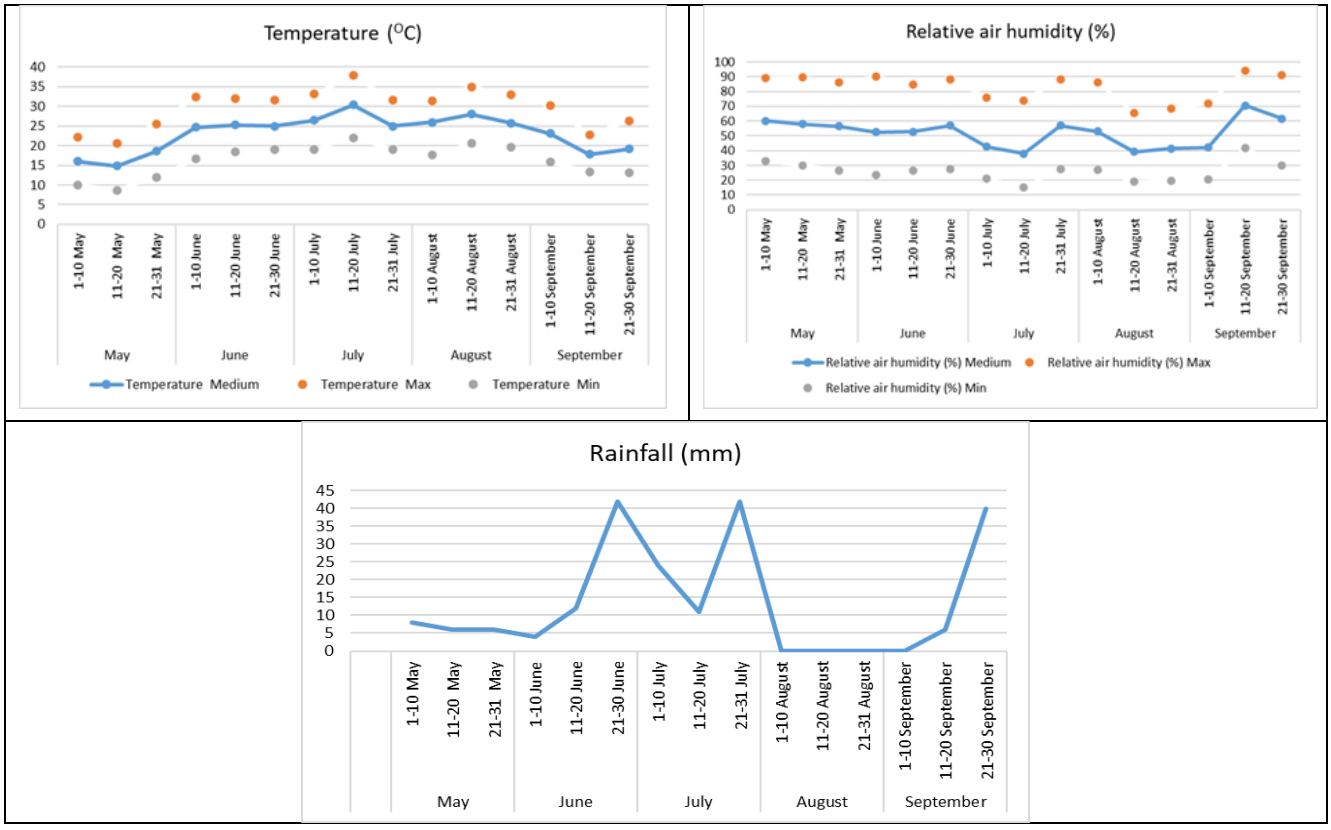


Figure 2. Atmospheric temperature, relative air humidity and precipitation amount for the period May - September 2024, in Vidra, Ilfov county / *Temperatura atmosferică, umiditatea relativă a aerului și cantitatea de precipitații în perioada mai - septembrie 2024, în Vidra, județul Ilfov*

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.

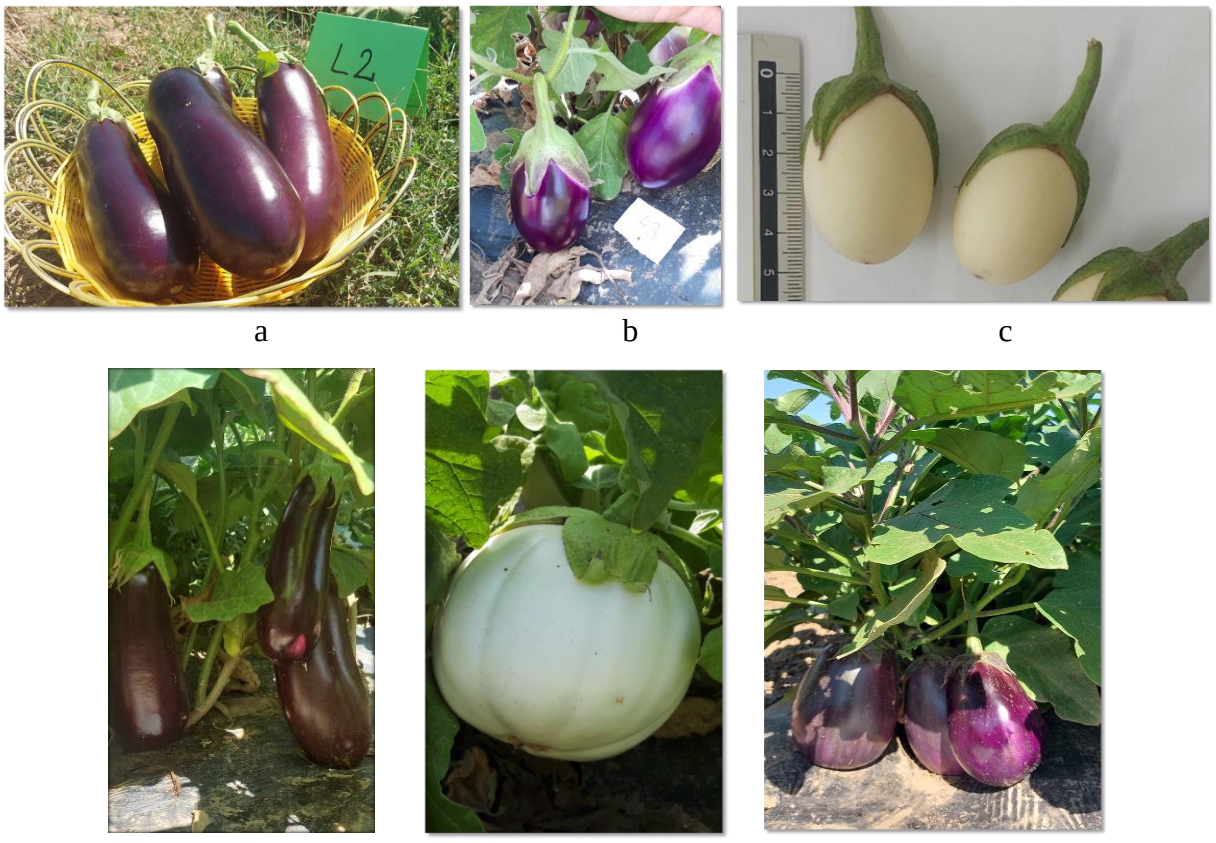
The eggplant harvest was carried out in stages, at the technological maturity of each genotype. The first harvest was carried out at the technological maturity date, followed by two more harvests. No fruit was harvested from any diseased plants, and fruit affected by blossom end rot were removed and 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.

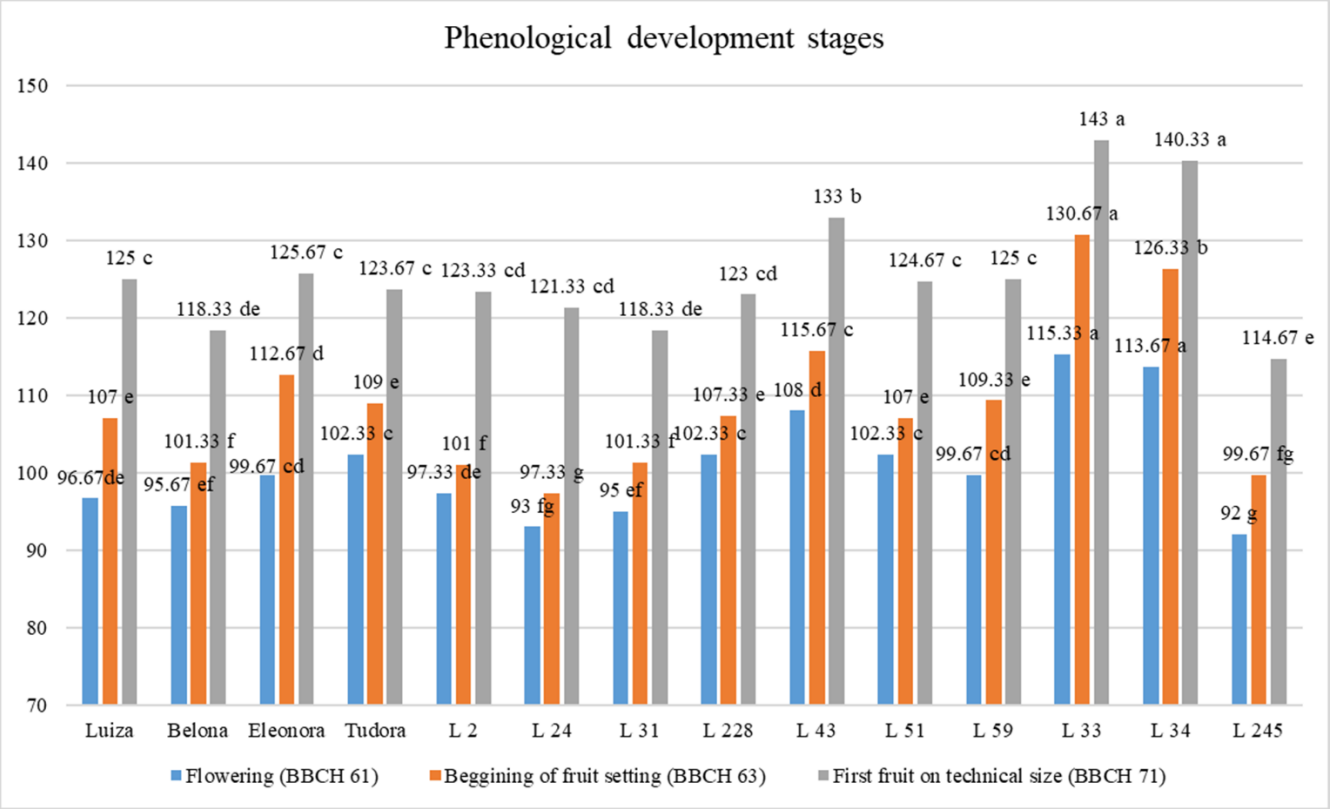


Figure 5. Phenological development stages at the studied eggplant cultivars and genotypes (days) */ Stadii de dezvoltare fenologică la soiurile și genotipurile de vinete studiate (zile)*

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

The present scientific experiment was carried out within Project 1159 – “Obtaining homozygous lines for the creation of F1 varieties and hybrids for the vegetable species tomatoes, pepper and eggplant cultivated in the field and

protected areas; Evaluation and conservation of genetic resources", financed from the state budget through The Academy of Agricultural and Forestry Sciences.

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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).

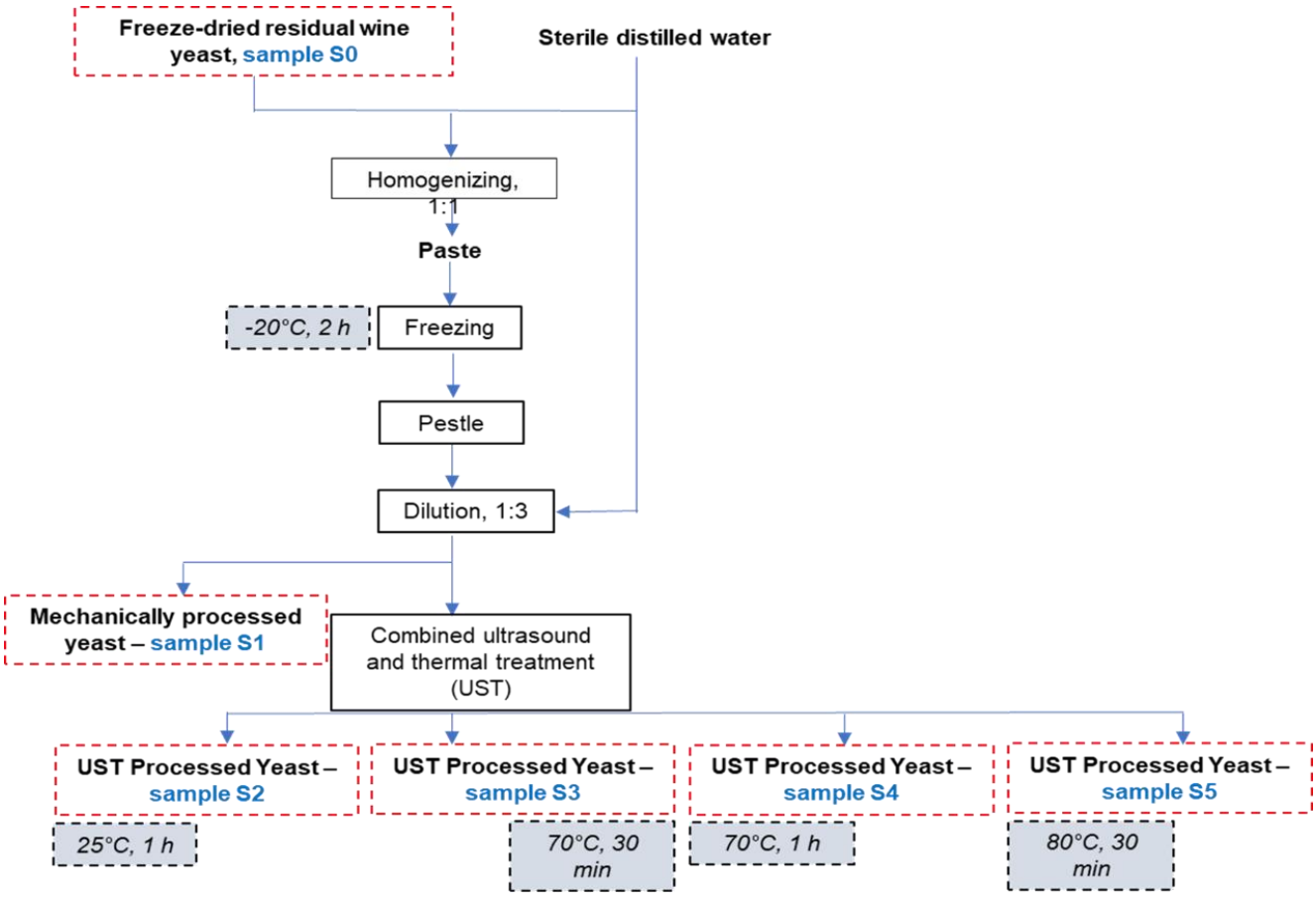


Figure 1. Red wine *Băbească neagră* lees biotication by release of postbiotics and obtaining parabiotics (Cotârleț *et al*, 2025) / Bioticarea drojdiei reziduale de vin (*Băbească neagră*) prin eliberarea postbioticelor și obținerea paraprobioticelor (Cotârleț și colab., 2025)

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.

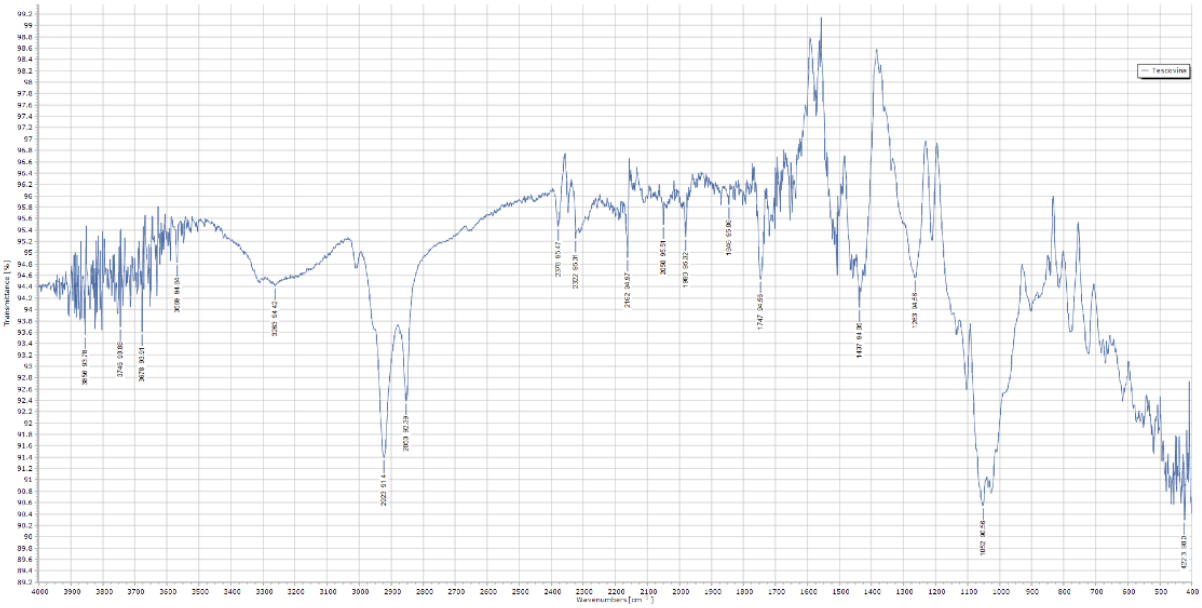


Figure 2. FTIR spectrum of dried *Băbească neagră* pomace / Spectrul FTIR al tescovinei (*Băbească neagră)* uscată

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.

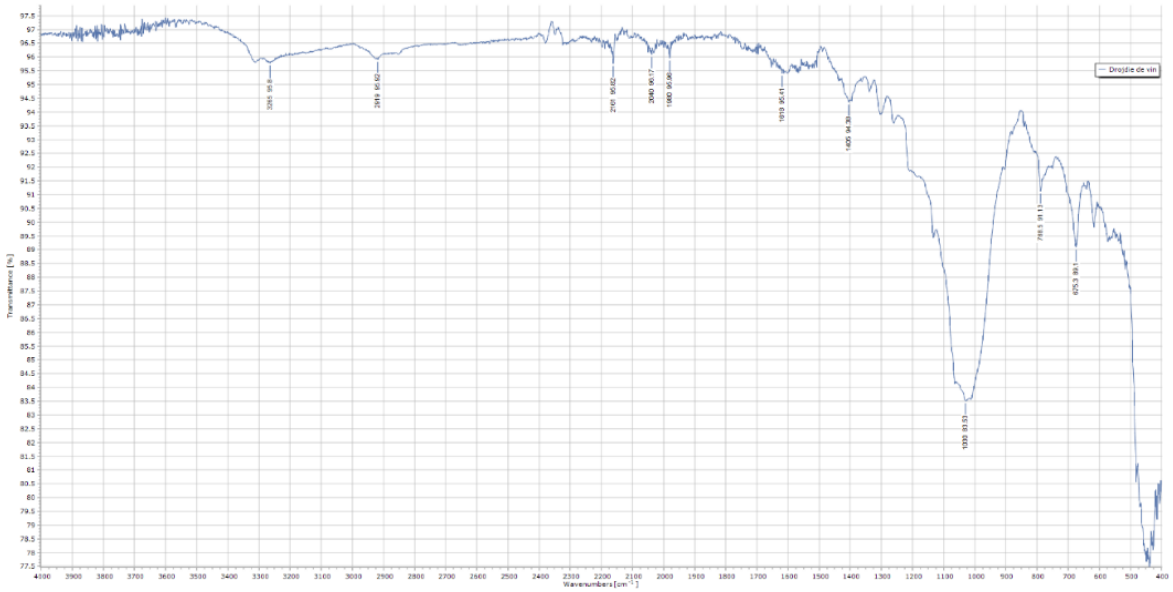


Figure 3. FTIR spectrum of freeze-dried lees (*Băbească neagră* wine) / Spectrul FTIR al drojdiei reziduală de vin (*Băbească neagră*) liofilizată

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.

ACKNOWLEDGMENTS

This research agrees with the objectives of the project PNRR/2022/9/MCDI/I5, Financing contract 760005/30.12.2022, Project ID 2, " Establishment and operationalization of a Competence Center for Soil Health and Food Safety (CeSoH)", Component Project 4 " Innovative and emerging solutions for smart valorisation of residual resources impacting health and safety of soil-food axis (InnES)".

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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.

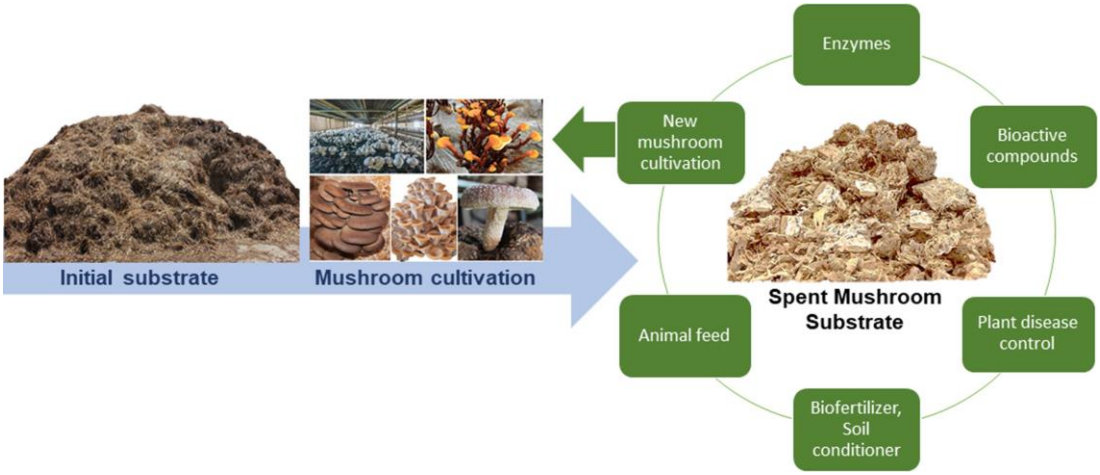


Fig. 2. Use of SMS in a circular economy / Utilizarea SMS în economia circulară
(Source/Sursa: Martin, C., Georgios I. Zervakis C, Shaojun X., Georgios Koutrotsiosc, and Knut Olav, 2023)

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.

ACKNOWLEDGEMENTS

Special thanks are extended to all the researchers whose work has significantly contributed to the expansion of knowledge regarding the applications of spent mushroom substrate in agriculture. Their pioneering efforts have laid the foundation for future innovation and sustainable development in fruit-growing systems.

This synthesis was made possible through collective efforts and both national and international institutional expertise. We gratefully acknowledge the financial and logistical support provided by SCDP Băneasa and ICDP Pitești-Mărăcineni through the Thematic Plan for the Implementation of the ASAS Strategy on Research–Development–Innovation in Pomology for the period 2021–2027. This work is part of Theme II: Pomological technologies with a favorable impact on the environment and rural development, Subtheme 2.1: Technological sequences for land preparation for establishing orchards, fruit tree cropping systems, and soil maintenance systems through mechanized operations.

We also extend our sincere thanks to the national network of fruit research stations for their ongoing collaboration and support.

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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).

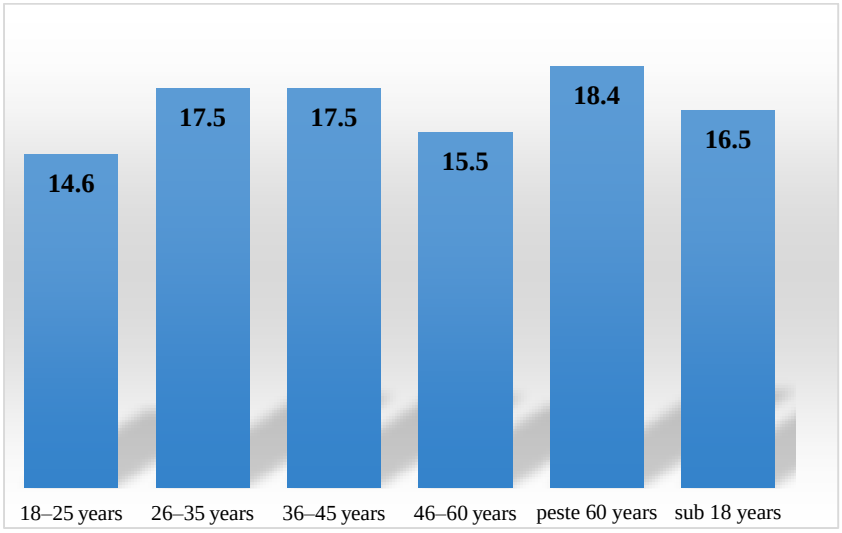


Figure 1. Distribution of respondents by age category / Distribuția respondenților pe categorii de vârstă
Source: processing based on questionnaire data / Sursa: procesarea datelor din chestionare

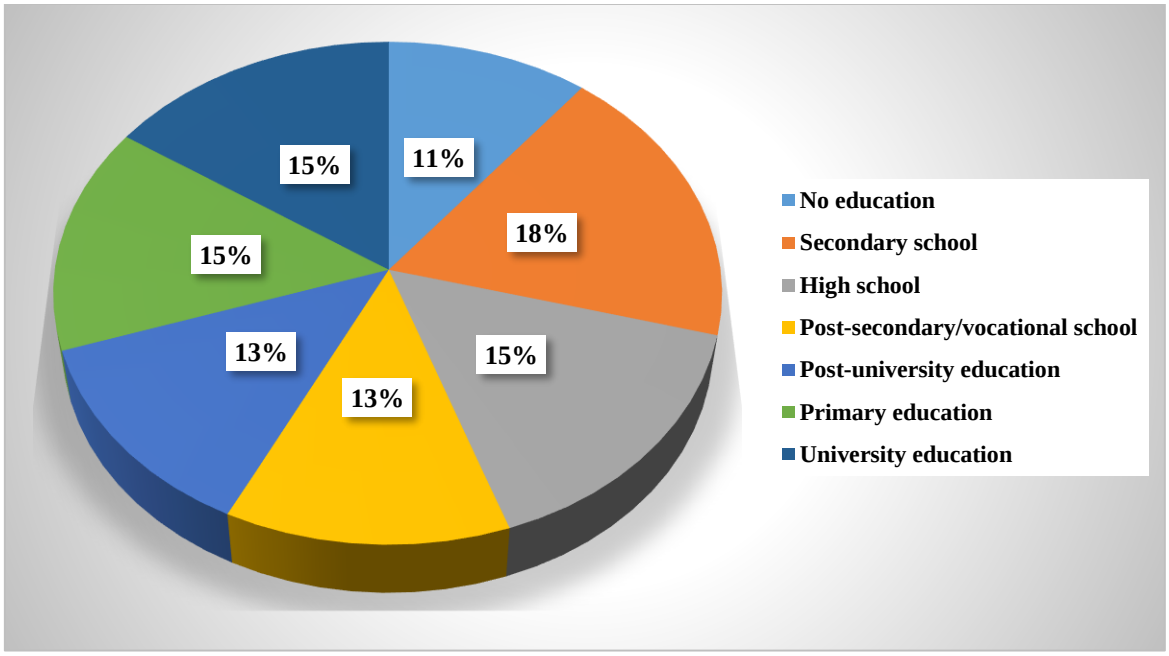


Figure 2. Distribution of respondents by level of education / Distribuția respondenților pe nivelul de studii
Source: processing based on questionnaire data / Sursa: procesarea datelor din chestionare

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).

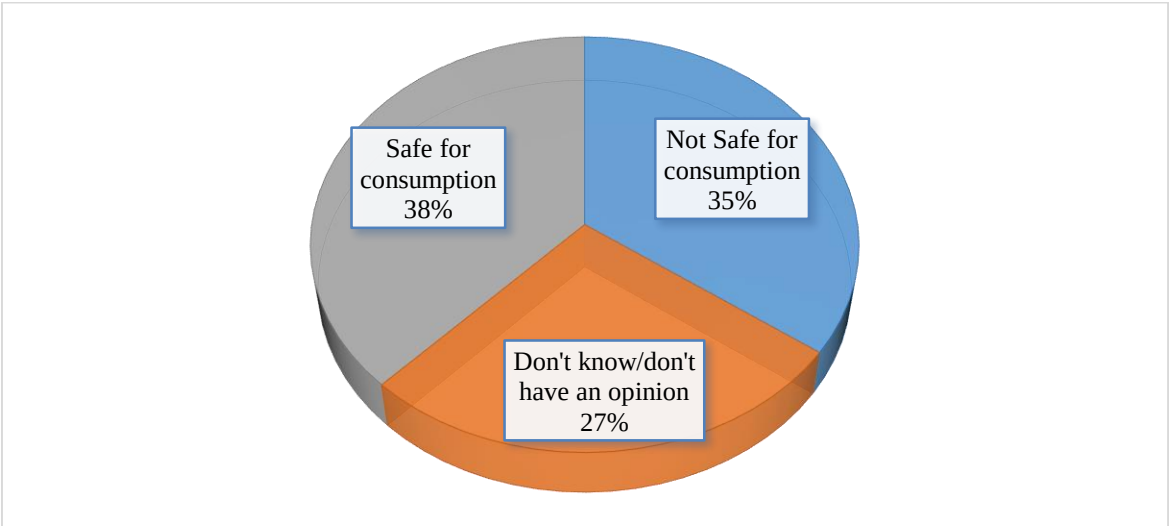


Figure 3. Respondents' perception of the safety of GMOs / Percepția respondenților privind siguranța OMG-urilor
Source: processing based on questionnaire data / Sursa: procesarea datelor din chestionare

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.

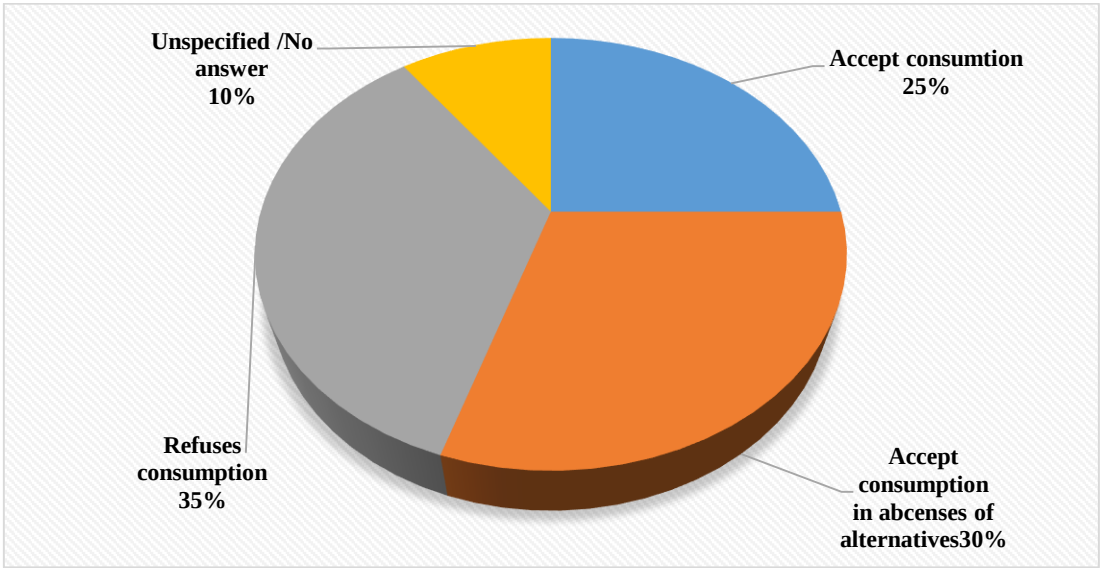


Figure 4. Respondents' perception of GMO consumption / Percepția respondenților față de consumul de OMG-uri
Source: processing based on questionnaire data/Sursa: procesarea datelor din chestionare

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).

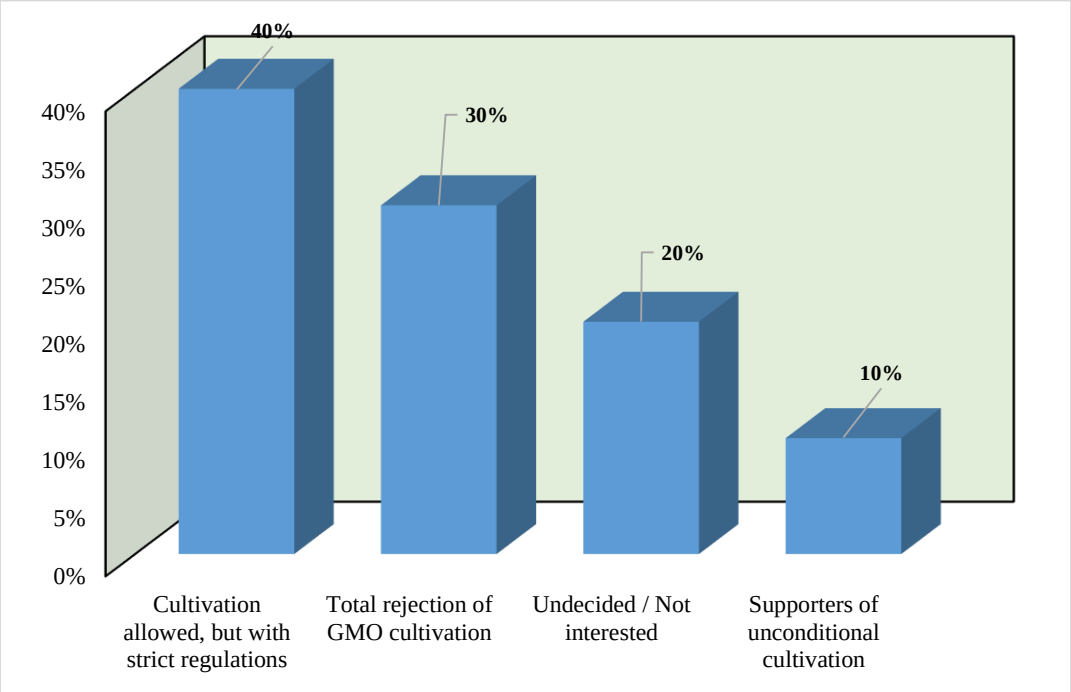


Figure 5. Respondents' perception of GMO cultivation / Percepția respondenților față de cultivarea OMG-urilor
Source: processing based on questionnaire data / Sursa: procesarea datelor din chestionare

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

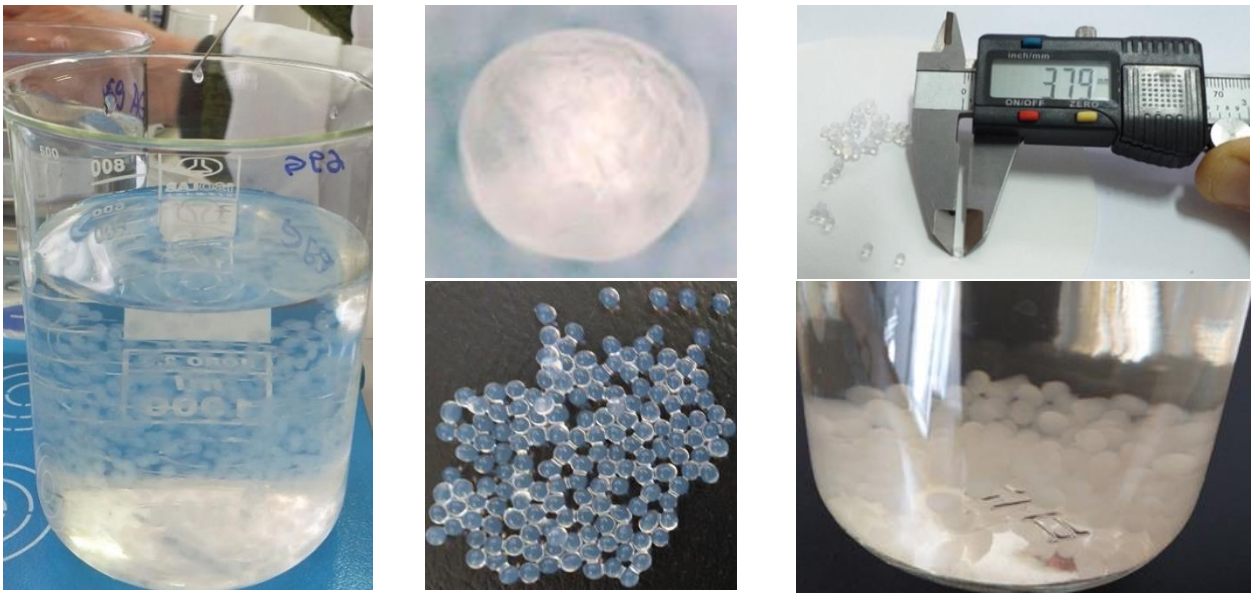
distance travelled by the solvent (Bele and Khale, 2011). The residual L-malic acid was determined using an enzymatic kit (BioSystems, Spain), involving the activity of L-malate dehydrogenase and glutamate-oxaloacetate transaminase (GOT) at 37 °C and reading the optical density at λ 340 nm. To highlight 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).

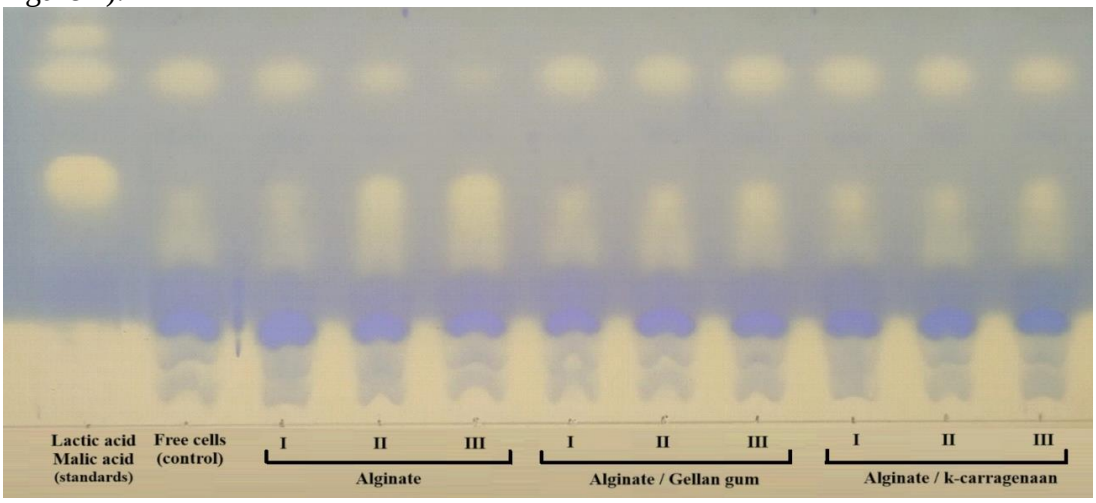


Figure 2. Monitoring the MLF process in synthetic wines by thin layer chromatography in three successive fermentative cycles with immobilized LAB cells / *Monitorizarea fermentației malolactice în vin sintetic prin cromatografie în strat subțire, în trei cicluri fermentative succesive realizate cu celule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

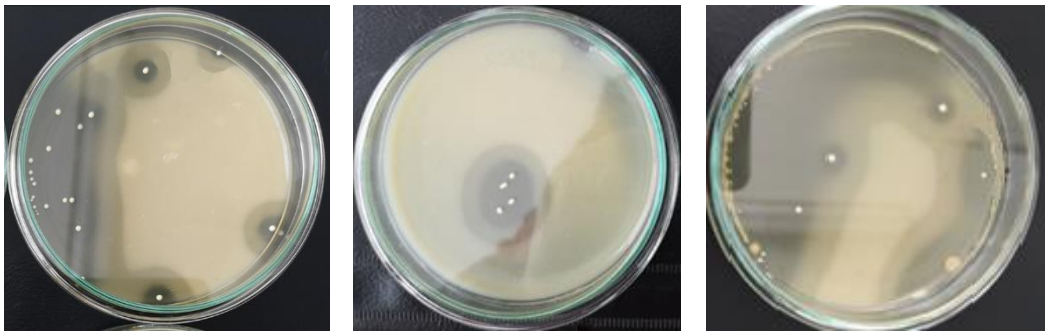
Determination of synthetic wine turbidity by measuring transmittance at 550 nm was used as a qualitative indicator of the mechanical stability and integrity of the beads, as previously proposed by Iurciuc *et al*. (2017). It is possible that at the end of the MLF, some polymer fragments or LAB cells 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.

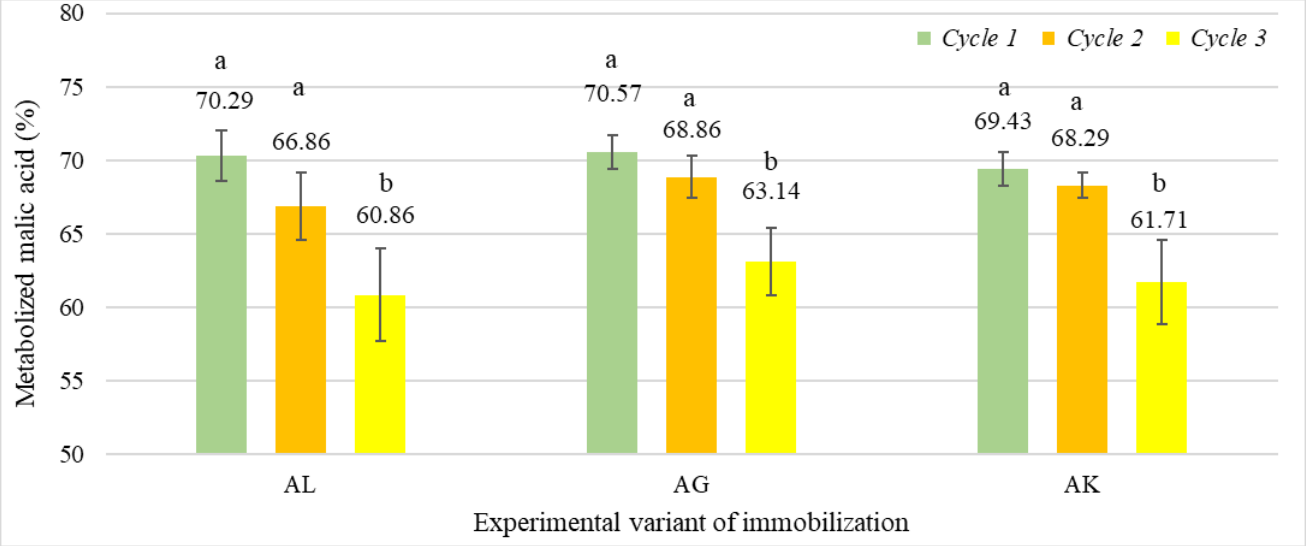


Figure 4. The percentage of malic acid metabolized in successive fermentation cycles by immobilized lactic acid bacteria (AL – alginate; AG – alginate/gellan gum; AK – alginate/k-carrageenan) / *Procentul de acid malic metabolizat în cicluri succesive de fermentație de către bacteriile lactice imobilizate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

ACKNOWLEDGMENTS

This paper was published under the frame of the grant number 3311/08.12.2022, funded by the Academy of Agricultural and Forestry Sciences "Gheorghe Ionescu-Sisesti" Romania (state budget).

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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.

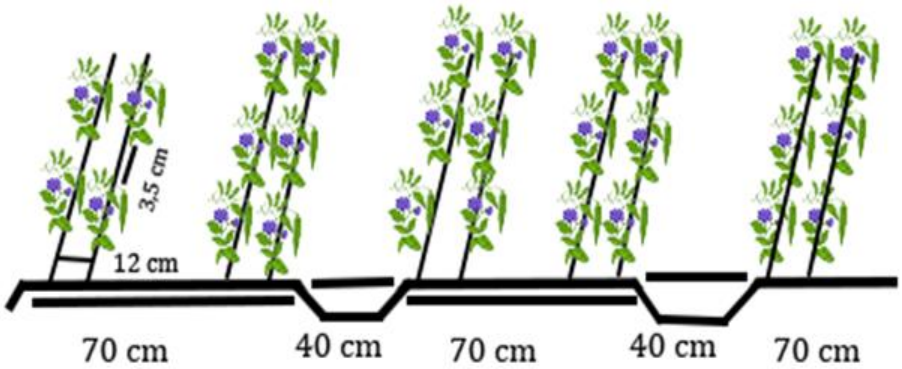


Figure 1. Sowing scheme of the studied accessions / Schema de semănat a liniilor studiate

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.

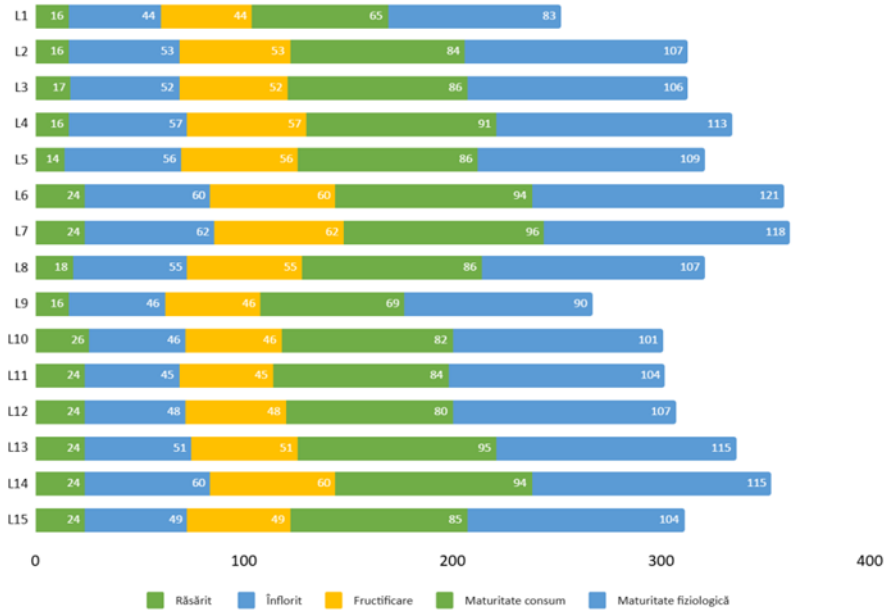


Fig. 2. The reproductive phenogram of pea accessions for the year 2020 / Fenograma reproductivă a liniilor de mazăre pentru anul 2020

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

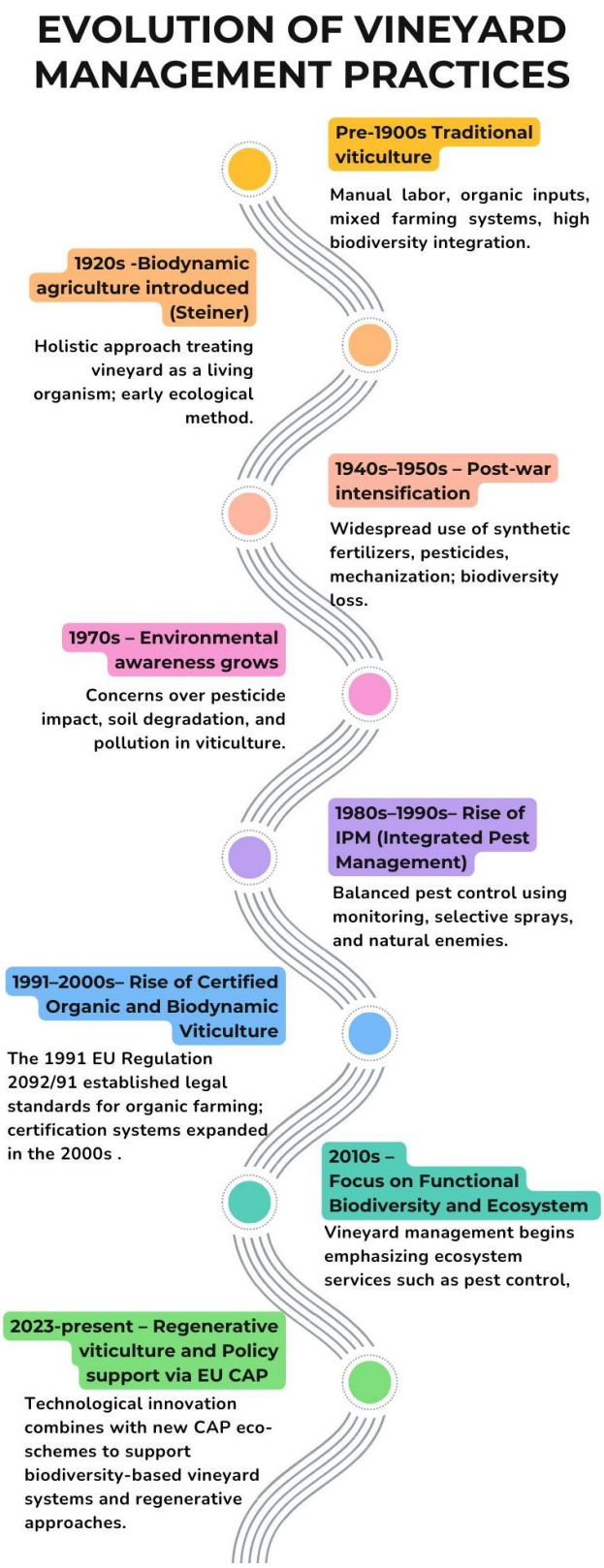


Figure 1. Timeline of milestones in the evolution of vineyard management practices (O'Brien *et al*., 2025; Turinek *et al*., 2009; Winter *et al*., 2018; Geldenhuys *et al*., 2021).

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).

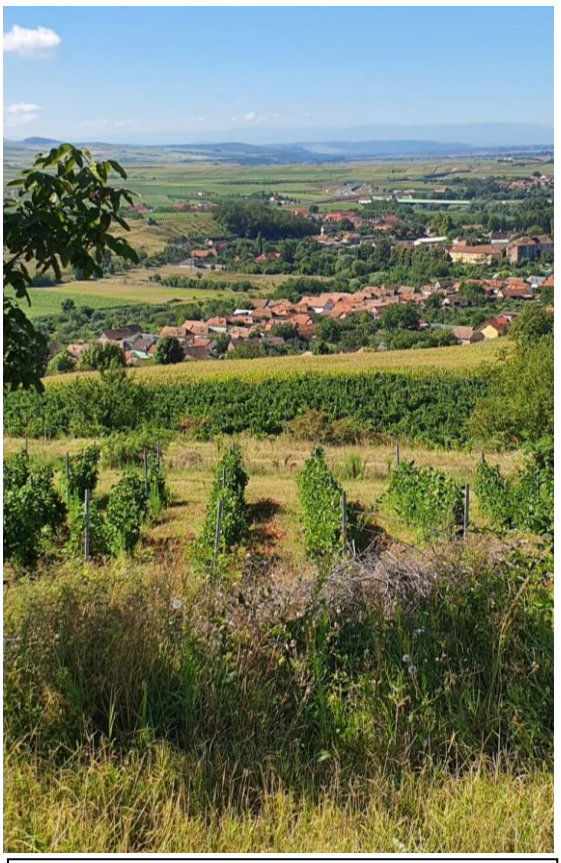


Figure 2. Vineyard landscape in Ciumbrud, Transylvania, an example of biodiversity-friendly viticulture, with diverse inter-row vegetation supporting ecosystem stability.

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.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Romanian Ministry of Agri-culture and Rural Development and was financed by Project ADER 2023-2026 Proiect 5.1.

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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.

ACKNOWLEDGMENTS

The study is part of the Nucleus Program 23.41/2023-2026, Project 01 01 „Procedure of Grapevine Pinot gris virus elimination under conditions of economic efficiency, in order to increase the biological value of viticultural propagation material" which is supported jointly by Ministry of Research, Innovation and Digitalization.

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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.

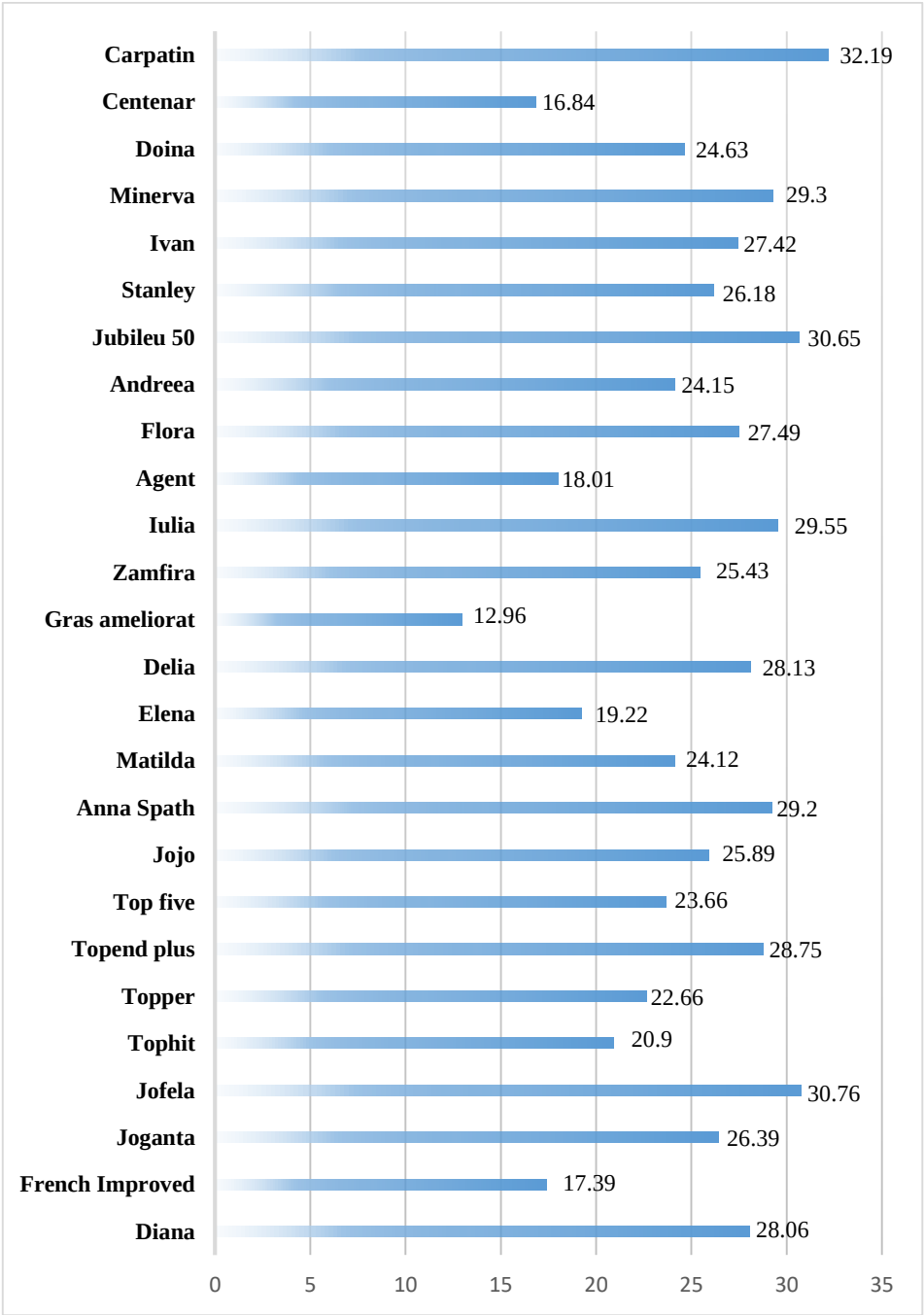


Fig. 2. The damage degree (%) caused by hail on 26 plum cultivars in 2024 / Fig. 2. Gradul de deteriorare (%) cauzat de grindină la 26 de soiuri de prun în 2024

Indirect effects of Monilinia spp. i*nfection*

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.

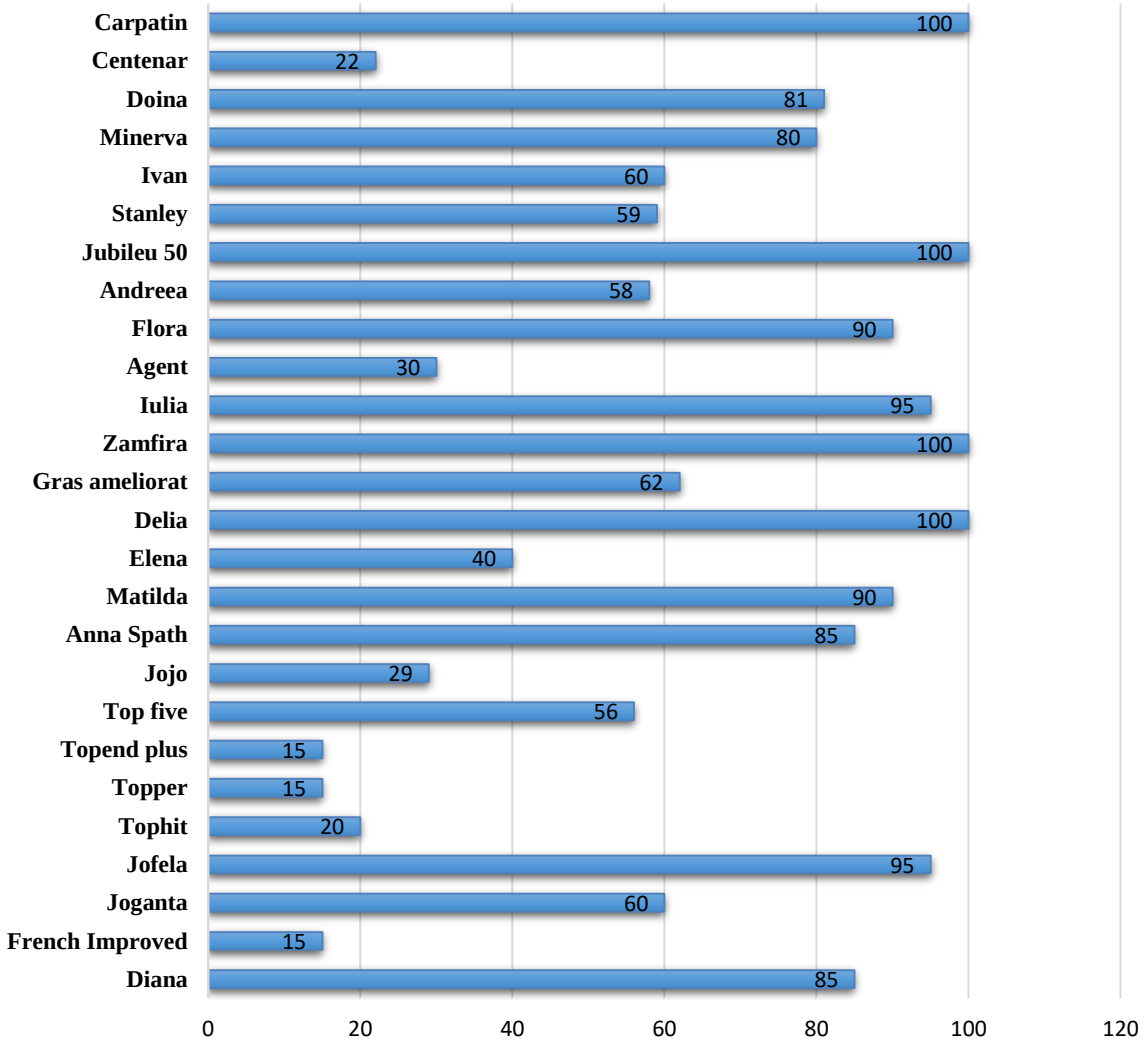


Fig. 3. The frequency of infections with *Monilinia* spp. on the fruits of the plum cultivars / Fig. 3. Frecvența infecțiilor cu *Monilinia* spp. pe fructele soiurilor de prun

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.

ACKNOWLEDGEMENTS

We are grateful to Fruit Research & Development Station Bistrita, Romania, for the generous help and assistance. The study is part of the project CDI-ASAS no. 2183/2023.

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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.

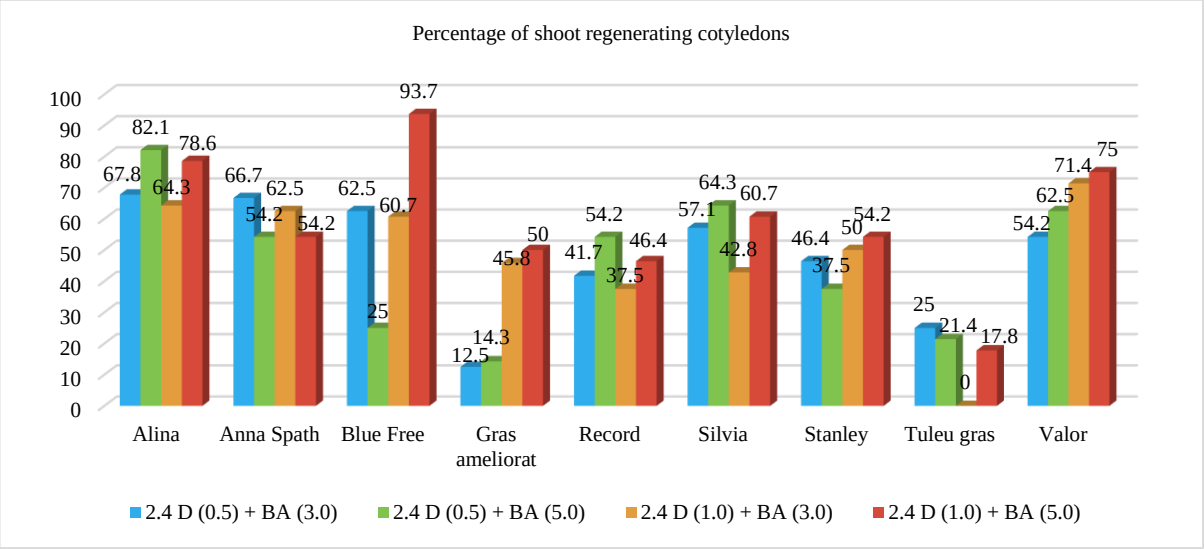


Figure 1. The potential of indirect organogenesis from cotyledons in plum varieties / Potențialul organogenetic al cotiledoanelor de prun în organogeneza indirectă

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).

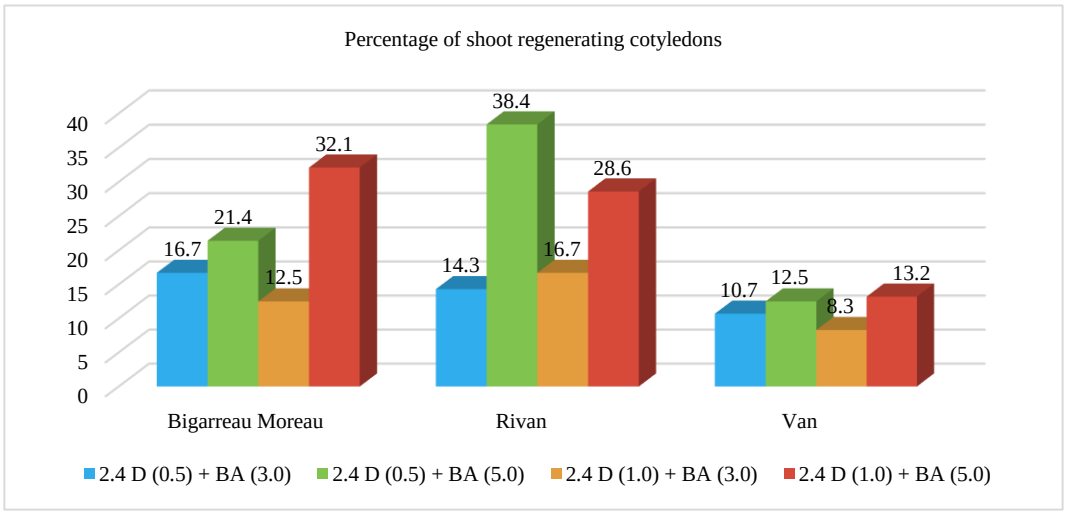


Figure 2. The potential of indirect organogenesis from cotyledons in cherry varieties / Potențialul organogenetic al cotiledoanelor de cireș în organogeneza indirectă

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.

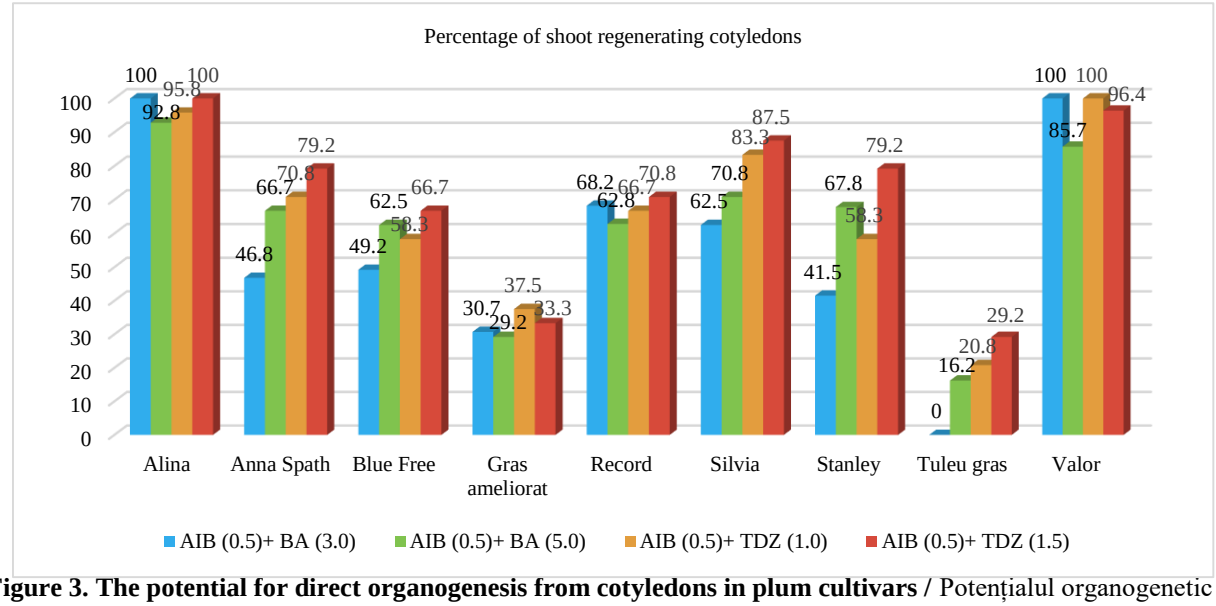


Figure 3. The potential for direct organogenesis from cotyledons in plum cultivars / Potențialul organogenetic al cotiledoanelor de prun în organogeneza directă

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).

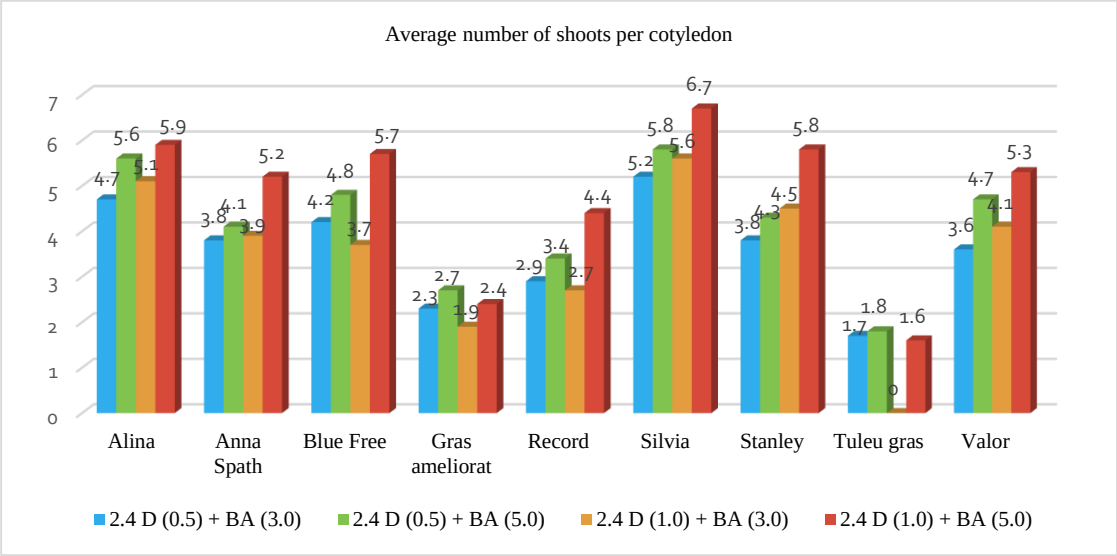


Figure 4. The average number of shoots regenerated per cotyledon by indirect organogenesis in plum cultivars / Numărul mediu de lăstari regenerați pe cotiledon prin organogeneză indirectă la soiurile de prun

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).

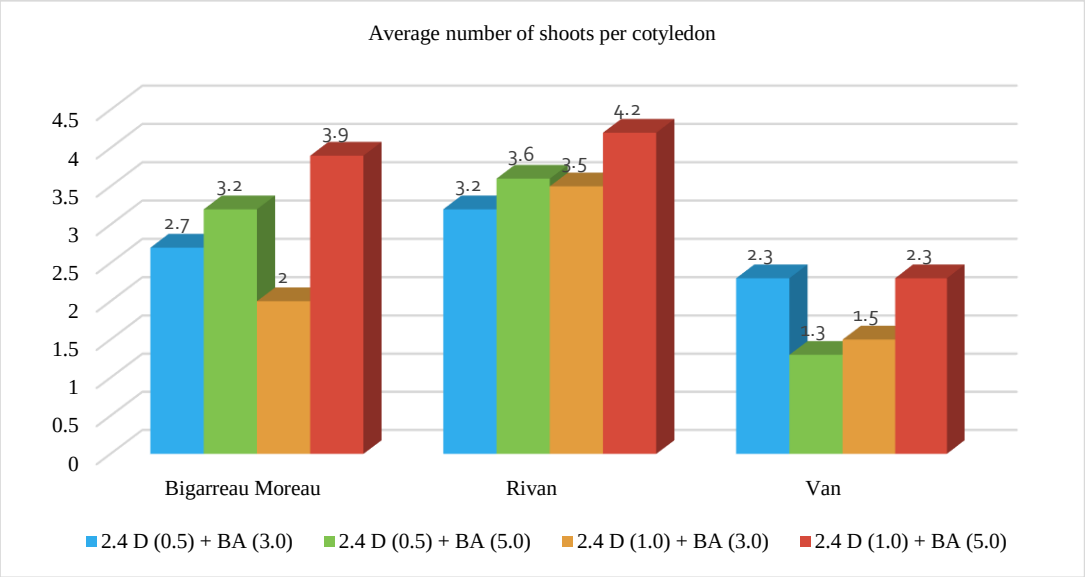


Figure 5. The average number of shoots regenerated per cotyledon by indirect organogenesis in sweet cherry cultivars / Numărul mediu de lăstari regenerați pe cotiledon prin organogeneză indirectă la soiurile de cireș

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.

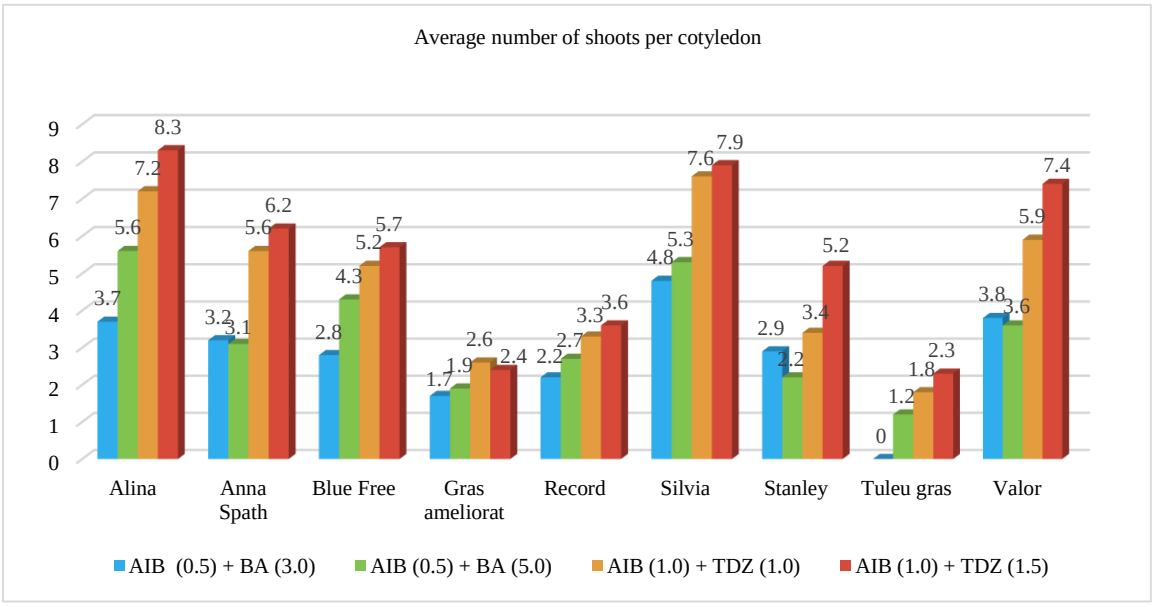


Figure 6. The average number of shoots regenerates per cotyledon by direct organogenesis in plum cultivars / Numărul mediu de lăstari regenerați pe cotiledon prin organogeneză directă la soiurile de prun

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).

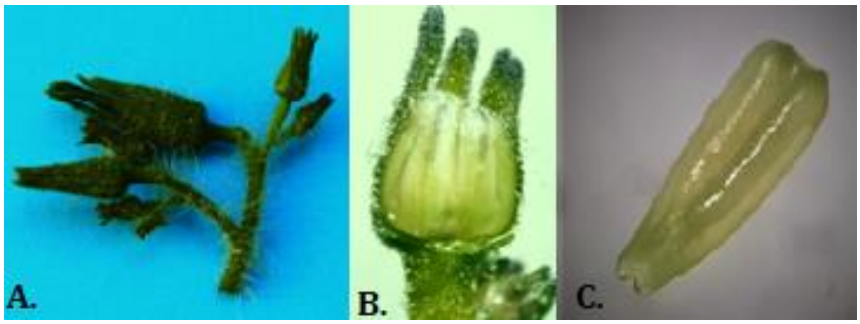


Fig. 1. Developmental stages of tomato floral buds for efficient anther culture: optimal bud selection, carpel elimination, and anther isolation - A. Buds at the optimal harvest stage; B. Removal of carpels; C. Excised anthers / *Fig. 1. Stadiile de dezvoltare ale mugurilor florali de tomate pentru cultura 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).

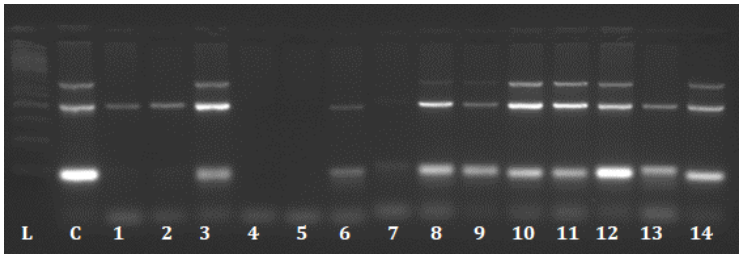


Fig. 3. Electrophoretic profiles of DNA fragments. L = molecular weight ladder; C = donor plant of the cultivar Costate 21; lanes 1–14 represent distinct regenerants obtained through in vitro anther culture / *Fig. 3. Profile electroforetice ale fragmentelor de ADN. L = marker de greutate moleculară; 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.

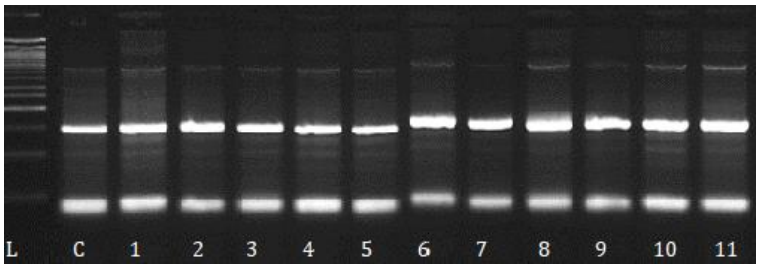


Fig. 4. Electrophoretic analysis of SSR amplified products from second-year plants derived from seeds obtained through in vitro regeneration; L = molecular weight marker; 1-9 = plants; C21 = control, Costate 21 / *Fig. 4 Analiza electroforetică a produselor amplificate SSR din plantele de al doilea an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

ACKNOWLEDGEMENTS

The authors thank all past and present researchers of RSFG Baneasa for their dedication to conserving fruit tree genetic resources and for their pioneering work in breeding apricot and peach cultivars. This synthesis was made possible by the collective efforts and institutional knowledge developed at RSFG Baneasa, with the support and coordination of the "Gheorghe Ionescu-Șișești" Academy of Agricultural Sciences (ASAS). We are also grateful to the University of Agronomic Sciences and Veterinary Medicine of Bucharest for its continued contribution to training qualified human resources in horticultural sciences.

We acknowledge with appreciation the financial and logistical support provided by SCDP Baneasa and ICDP Pitesti-Maracineni through the Thematic Plan for the Implementation of the ASAS Strategy on Research Development–Innovation in Pomology for the period 2021-2027. This work is part of Theme I: Genetic Resources and the Improvement of Fruit Tree Cultivars and Rootstocks. 1.1. Conservation and Sustainable Use of Pomological Biodiversity. We also extend our thanks to the national network of Fruit Growing Research Stations for their ongoing collaboration and support.

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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).

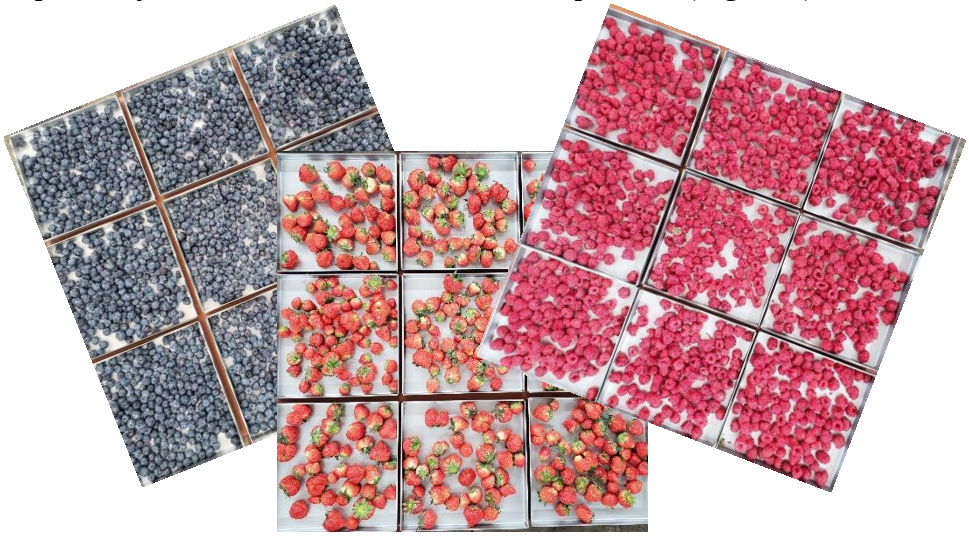


Fig. 1. Species of berries used in the experimentation */ Specii de fructe de pădure utilizate la experimentare*

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.

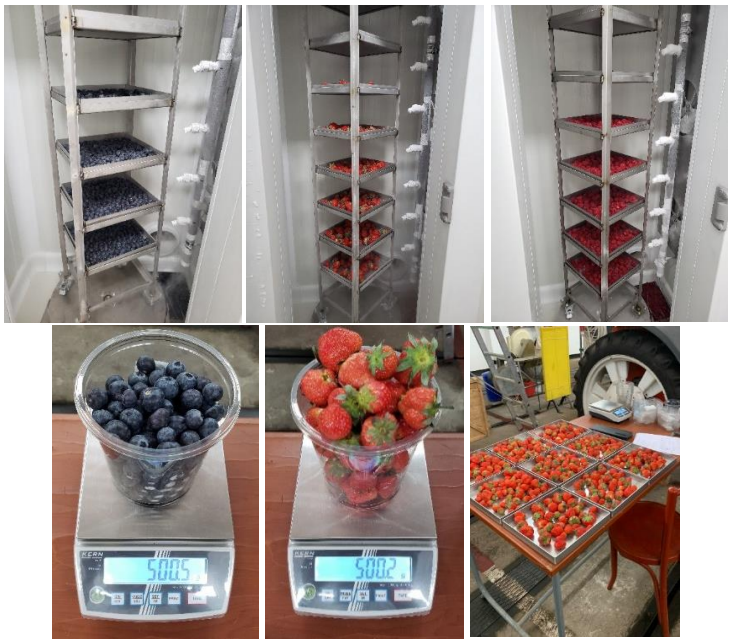


Fig. 4. Preparation of samples for experimentation / *Pregătirea probelor pentru experimentare*

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.

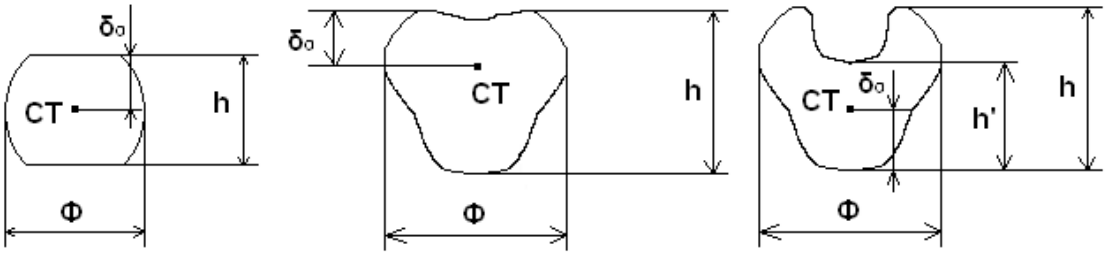


Fig. 5. Dimensional characteristics according to the geometric shape of the analyzed fruits */ Caracteristicile dimensionale în funcție de forma geometrică a fructelor analizate*

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

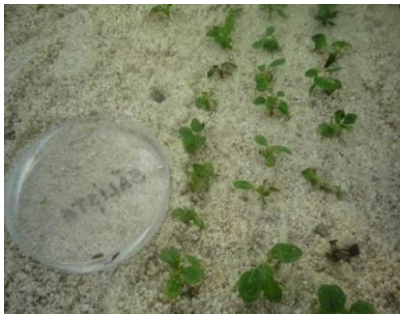


Figure 18. Săliște - aclimatization/
Săliște – aclimatizare

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.

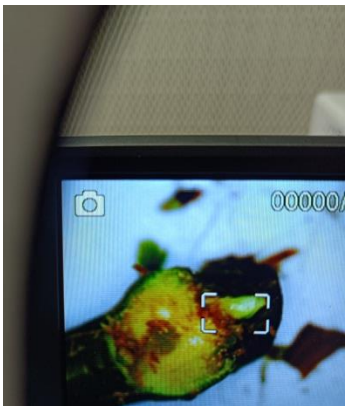


Figure 22. Meristem Miroper/ meristem **Miroper**



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*.

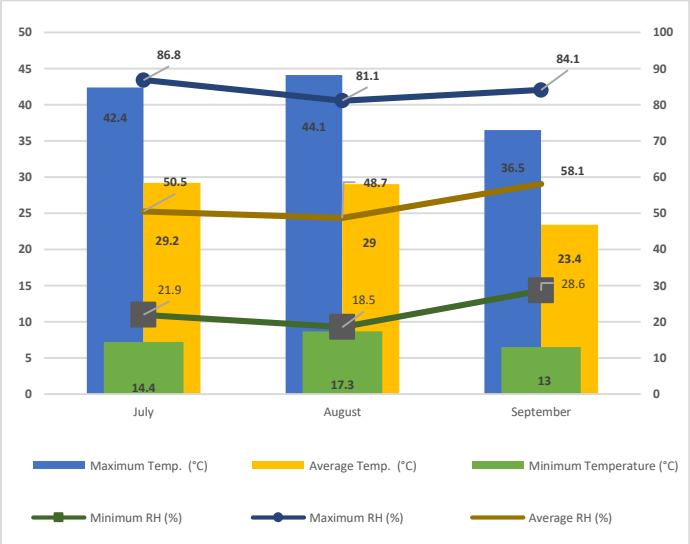


Fig. 1 Greenhouse climate data
for the period July – September 2024 / Datele climatice din seră, în perioada iulie – septembrie 2025

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).

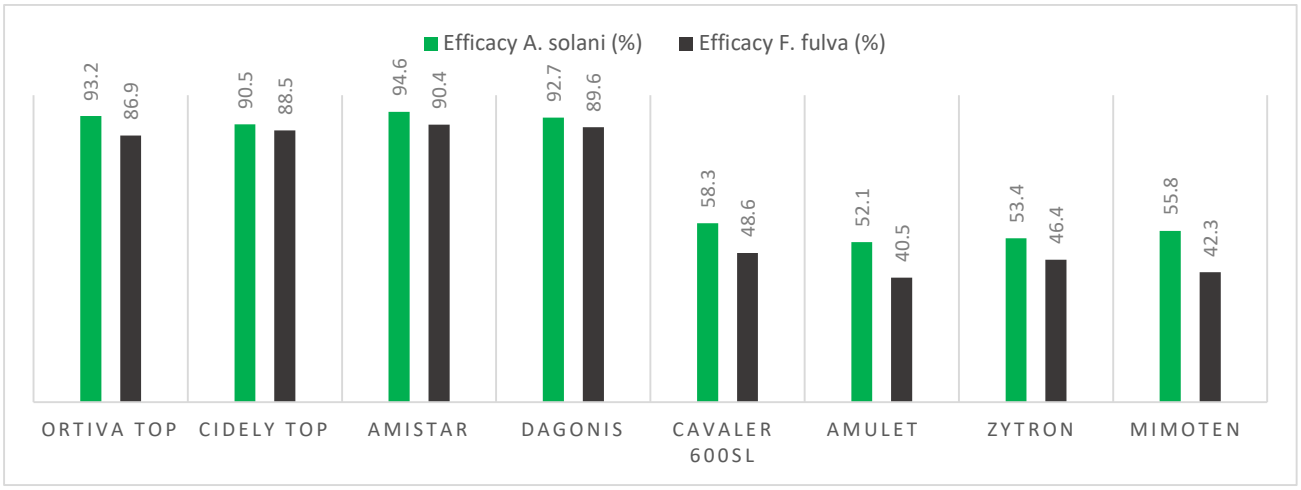


Fig. 2 The efficacy of conventional and biological products on *A. solani* and *F. fulva* / Eficacitatea produselor convenționele și biologice asupra *A. solani* și *F. fulva*

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.

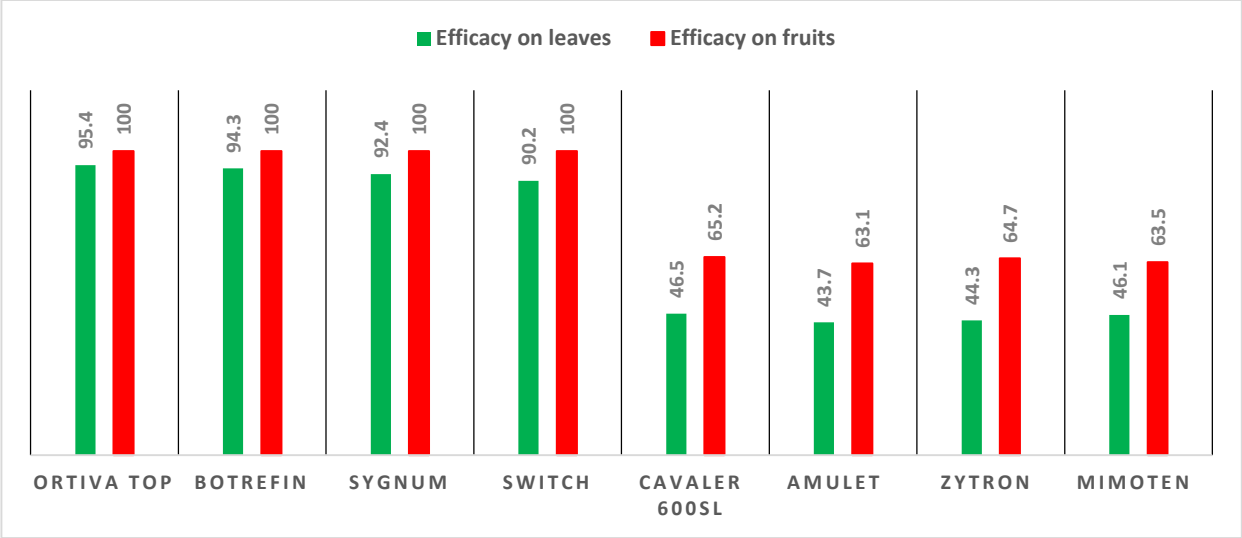


Fig. 3 The efficacy of conventional and biological products on *B. cinerea* / / Eficacitatea produselor convenționele și biologice asupra *B. cinerea*

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).

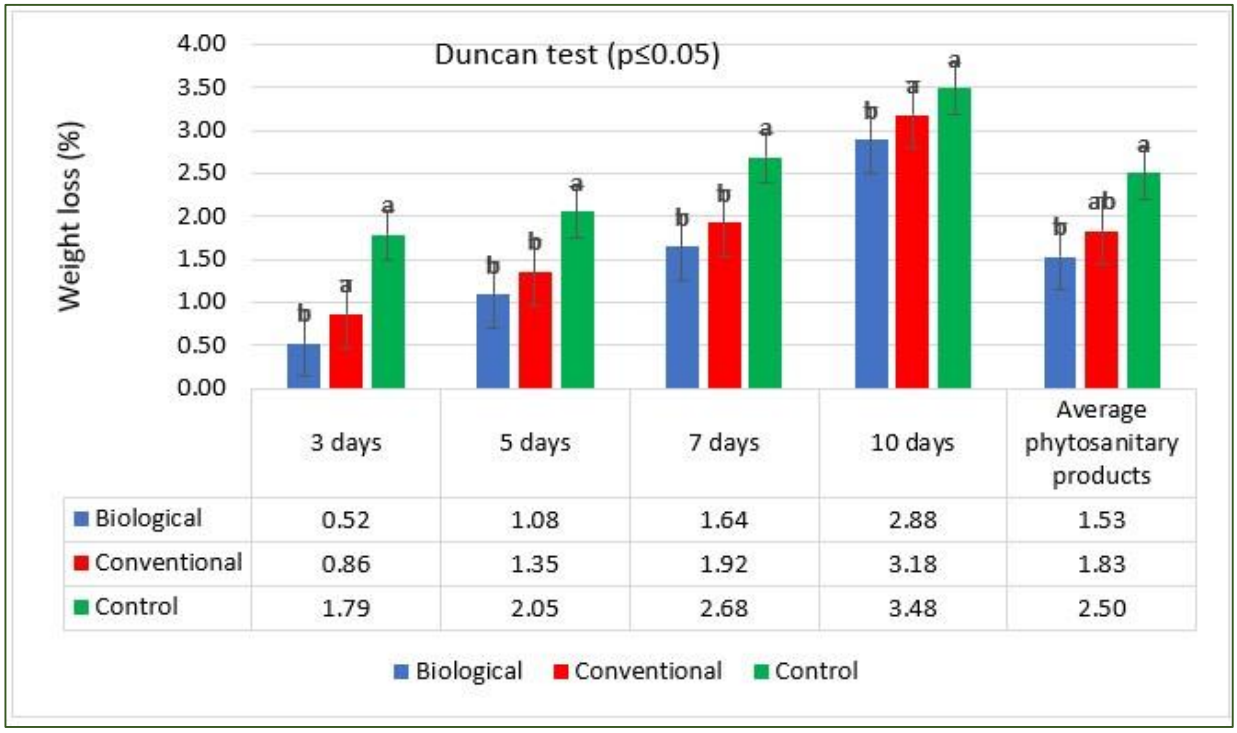


Fig. 4. Influence of phytosanitary control on tomato weight loss (%) depending on the storage period at 6°C / Influența controlului fitosanitar asupra pierderii în greutate a tomatelor în funcție de perioada de depozitare la 6°C

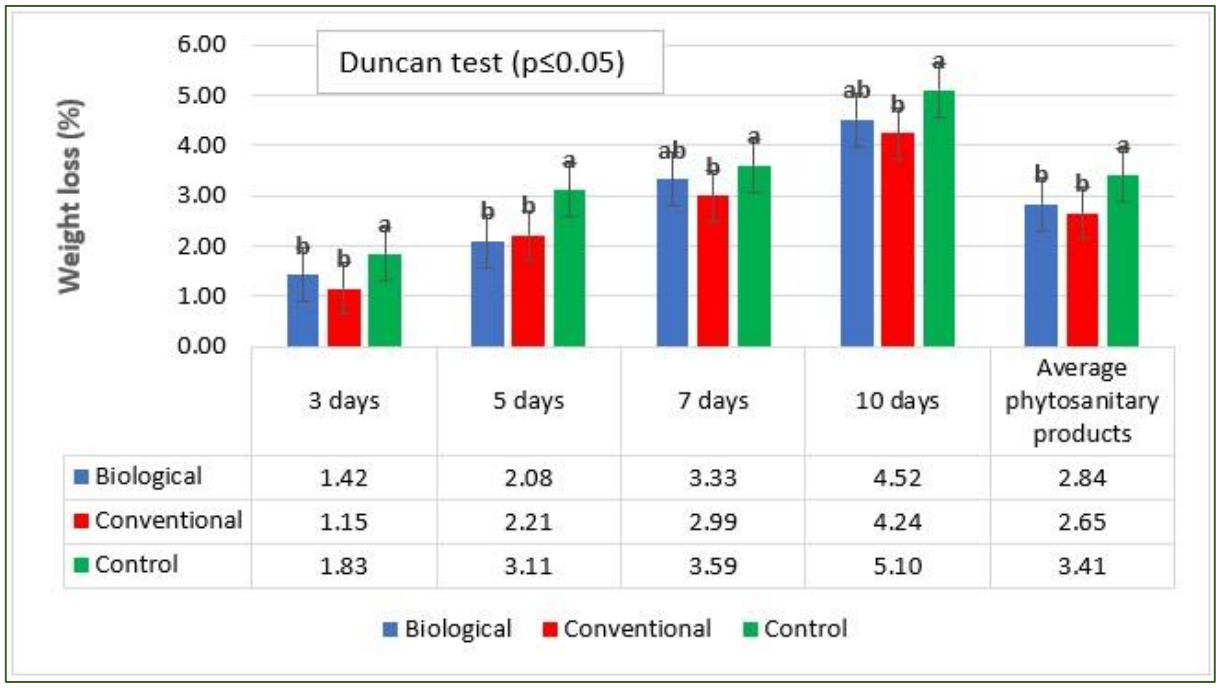


Fig. 5. Influence of phytosanitary control on tomato weight loss (%) depending on the storage period at 22°C / Influența controlului fitosanitar asupra pierderii în greutate a tomatelor în funcție de perioada de depozitare la 22°C

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.

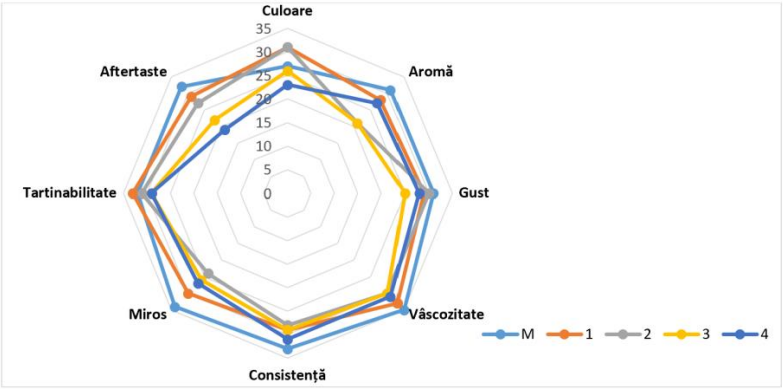


Figure 1. Sensorial evaluation of samples / Evaluarea senzorială a probelor

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.

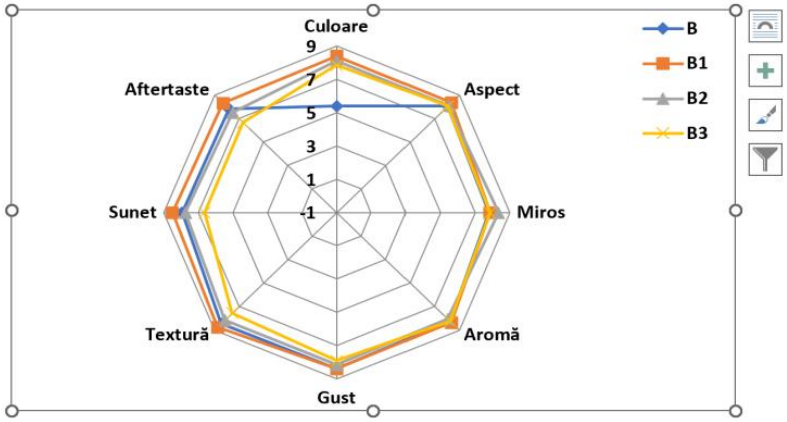


Figure 2. Sensorial evaluation of samples / Evaluarea senzorială a probelor

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.

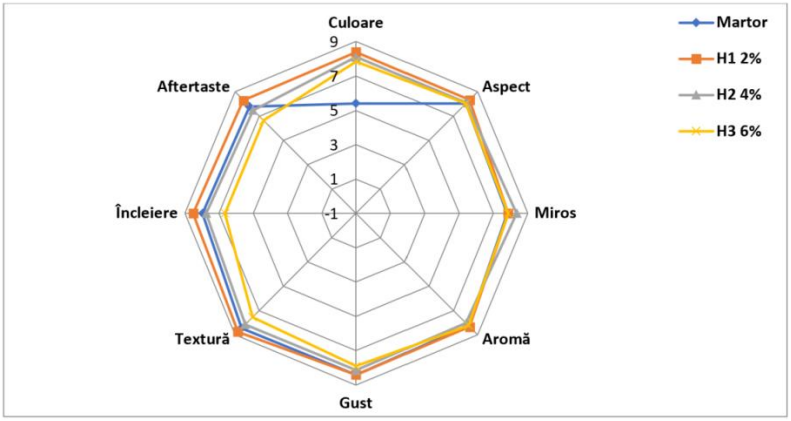


Figure 3. Sensorial evaluation of samples / Evaluarea senzorială a probelor

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).

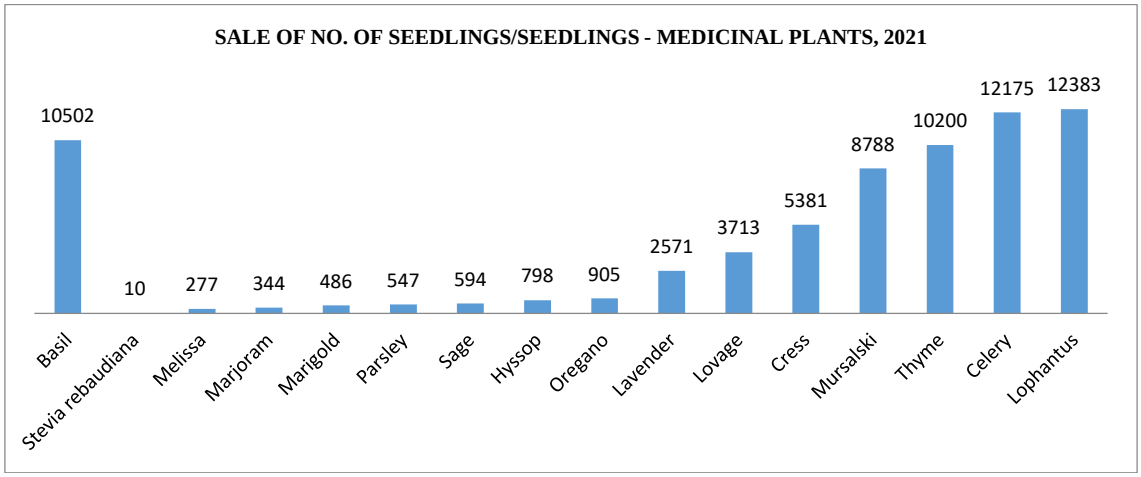


Figure 4. Dominance of classics and low presence of rare plants (2021) / Dominanța clasicelor și prezența scăzută a plantelor rare (2021)

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).*

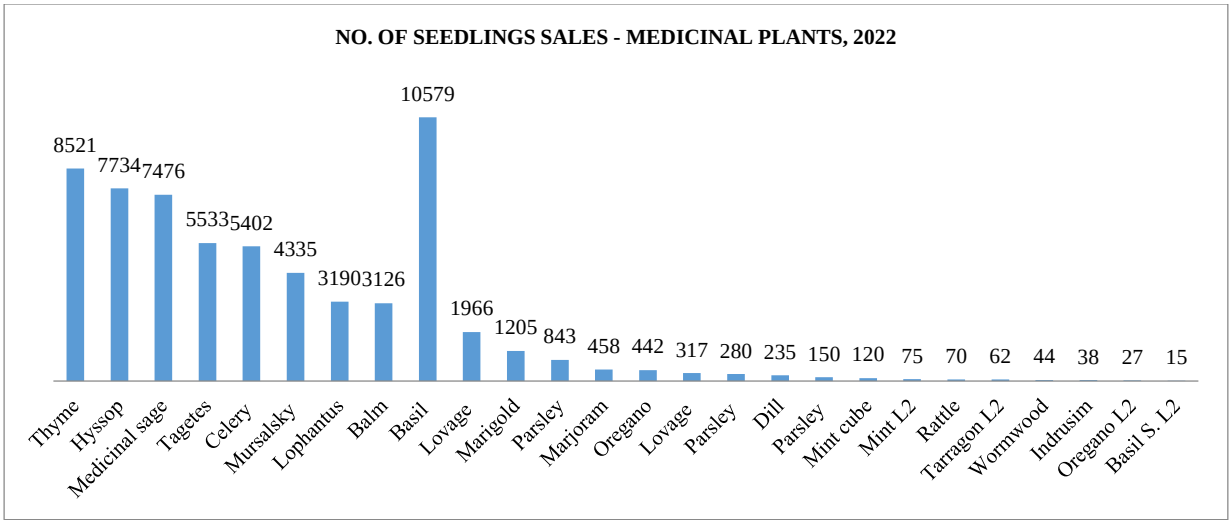


Figure 5. Significant diversification, beginning of the transition to specialized medicinal plants (2022) / Diversificare semnificativă, începerea tranziției la plante medicinale specializate (2022)

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.

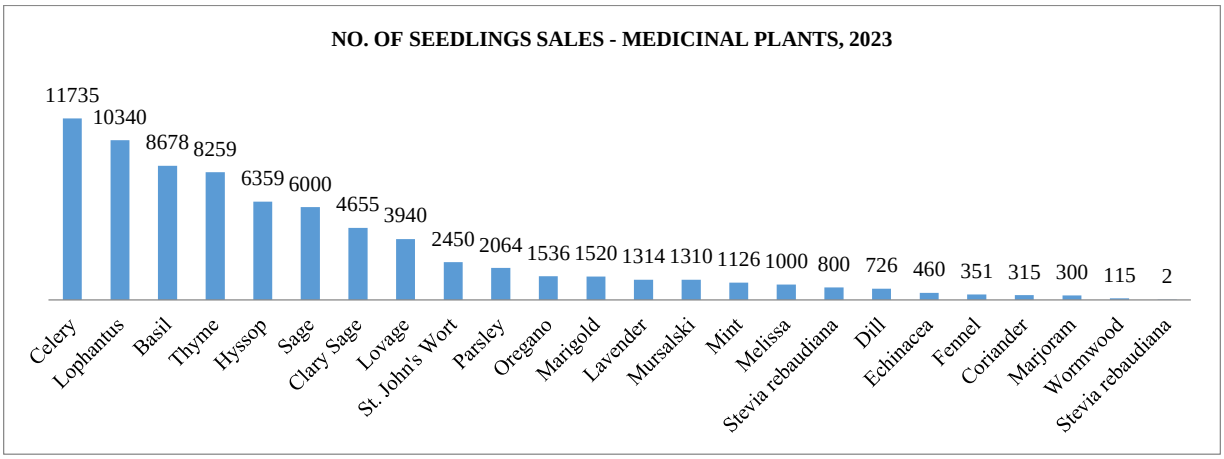


Figure 6. Specialization and balance between culinary, aromatic and medicinal plants (2023) / Specializare și echilibru între plante culinare, aromatice și medicinale (2023)

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.

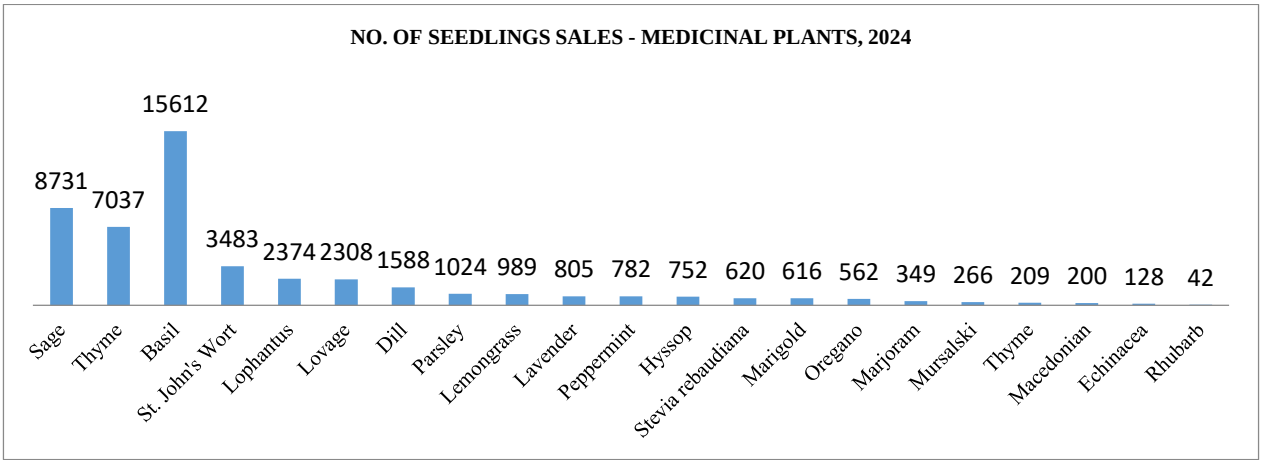


Figure 7. Market maturation and acquisition of rare/valuable species (2024) / Maturizarea pieței și obținerea unor specii rare/valoroase (2024)

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

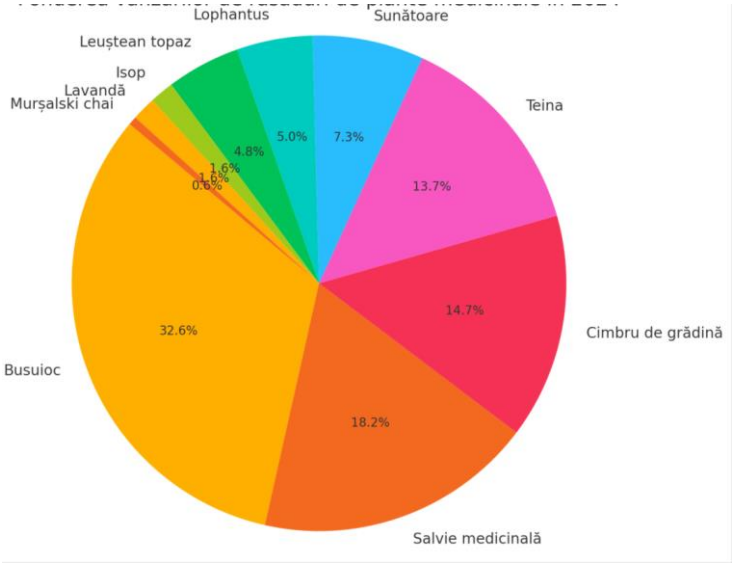


Figure 8. Comparative evolution of sales in the period 2021–2024 / Evoluția comparativă a vânzărilor în perioada 2021–2024

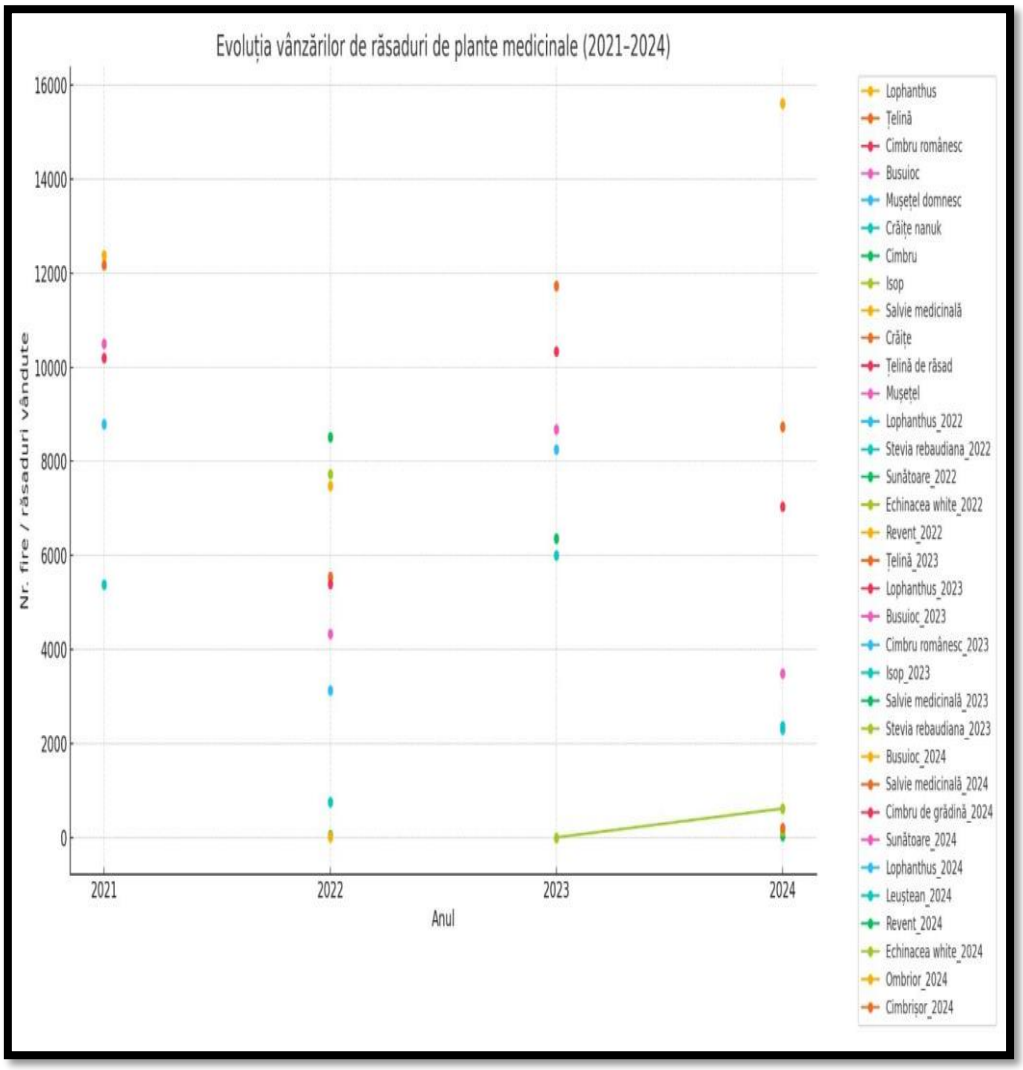


Figure 9. Evolution of seedlings sales of from the medicinal plant collection (2021-2024) / Evoluția vânzărilor de răsaduri din colecția de plante medicinale (2021-2024)

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

Thanks to Buzău Vegetable Research and Development Station for facilitating the access to information (data, photographs, analysis bulletins).

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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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ACTA AGRICOLA ROMANICA

Volume 7, Year 7, No. 7
August 2025

Animal Sciences, Veterinary Medicine and Food Science

BUCHAREST

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The magazine is edited by the Sections of
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The magazine appears annually, in the second semester of the year
ISSN 2784 – 0948 ISSN – L 2784 – 0948

ACCESSORY CORPUS LUTEUM IN CATTLE ASSISTED REPRODUCTION

CORPUL LUTEAL ACCESORIU ÎN REPRODUCEREA ASISTATĂ A BOVINELOR

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Abstract

In dairy cows, current methods for managing reproduction still need improvement. Future advancements will require new strategies to minimize additional interventions and maintain acceptance among veterinarians. As a result, the development of new therapies in dairy cows' reproduction acts for a significant challenge for improving reproductive performances. In recent years, there has been an increasing interest in inducing accessory corpus luteum (aCL) formation in dairy cows, but the results have been inconsistent. It is still uncertain whether this strategy, of injecting gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin (hCG) early in the luteal phase following artificial insemination (AI), can be utilized as a herd management tool to enhance reproduction. Overall, our research supports the idea that giving GnRH agonists during the days 7–14 after AI to repeat breeder (RB) cows increases their fertility and farm profitability. The GnRH agonist treatment improved the odds that pregnant cows would develop an aCL, which could improve their fertility. The UW-DairyRepro$ decision support tool utilized in our study demonstrated that by implementing this approach, the net present value can be increased by US dollars (US$)30.2/RB cow/year. Therefore, our work suggests that implementing this strategy on the farm is feasible only for repeat-breeder dairy cows with low genetic merit for fertility. However, in the assisted reproductive technologies this strategy seems to improve reproduction in recipient heifers.

Keywords: accessory corpus luteum, dairy cows, embryos, reproductive biotechnologies.

Rezumat

La vacile de lapte, metodele actuale de gestionare a activității de reproducere necesită încă îmbunătățiri. Progresele viitoare presupun noi strategii pentru a minimiza intervențiile terapeutice și pentru a fi unanim acceptate de medicii veterinari. Ca urmare, dezvoltarea de noi strategii terapeutice în reproducerea vacilor de lapte reprezintă o provocare pentru îmbunătățirea performanțelor reproductive. În ultimii ani, a existat un interes din ce în ce mai mare pentru inducerea formării corpului galben accesoriu în reproducerea asistată a vacilor de lapte, dar rezultatele sunt controversate. Este încă incert dacă această strategie ce presupune injectarea de GnRH sau hCG la începutul fazei luteale, după inseminare artificială, poate fi utilizată ca instrument de management al efectivelor de taurine pentru a îmbunătăți reproducerea. În general, cercetarea noastră susține ideea că administrarea de agoniști GnRH în zilele 7-14 după inseminare artificială a vacilor infertile, crește fertilitatea și profitabilitatea fermei. Tratamentul cu agonist GnRH a îmbunătățit șansele ca vacile gestante să dezvolte un corp luteal accesoriu, care le-ar putea îmbunătăți fertilitatea. Programul de evaluare economică UW-DairyRepro$, utilizat în studiul nostru a demonstrat că prin implementarea acestei strategii, valoarea actualizată netă poate crește cu 30,2 USD/vacă infertilă/an. În concluzie, studiile noastre sugerează că implementarea acestei strategii în ferme este fezabilă numai pentru vacile de lapte infertile. În biotehnologiile de reproducere asistată, această strategie pare să îmbunătățească activitatea de reproducere doar a junicilor receptoare de embrioni.

Cuvinte cheie: biotehnologii, corpul luteal accesoriu, vacă de lapte, embrioni.

INTRODUCTION

In recent decades, new technologies and approaches have been developed to address reproductive performance issues in high-yielding dairy farms, stemming from a deeper understanding of contemporary reproductive physiology in these cows (Thatcher, 2017; Cardoso et al., 2021).

Progesterone deficiency is a significant factor contributing to embryo loss, which is a major cause of low reproductive performance in dairy cows. High-yielding cows experience increased metabolism, resulting in a higher rate of progesterone degradation and lower concentrations of progesterone in peripheral blood (Wiltbank et al., 2006; Robinson et al., 2008).

The increase in progesterone (P4) after ovulation is crucial for establishing and maintaining pregnancy (Forde et al., 2011; Lonergan and Sánchez, 2020), as it creates an optimal uterine environment for embryo elongation (Clemente et al., 2009).

Progesterone (P4) concentrations exceeding 1 ng/mL during the first five days following artificial insemination (AI) are significant indicators in cattle reproduction. Additionally, an increase in P4 levels after day 7 in recipient cows is strongly correlated with a higher likelihood of successful pregnancy. Monitoring these hormone levels is critical for predicting reproductive outcomes and can help optimize breeding practices in livestock management (Starbuck et al., 2001; Kenyon et al., 2013).

Low levels of circulating progesterone (P4) are strongly associated with diminished fertility in dairy cows, as noted by Lonergan (2011). To counteract the negative effects of low progesterone after AI, contemporary fertility programs often use a gonadotropin-releasing hormone (GnRH) injection to induce a surge of luteinizing hormone (LH). This surge is critical as it not only stimulates the initiation of a new follicular wave, which is essential for the development of new ovarian follicles, but it also triggers ovulation. Furthermore, this process promotes the formation of an accessory corpus luteum (aCL), which plays a vital role in increasing progesterone production. By enhancing progesterone concentrations, these fertility interventions aim to improve overall reproductive efficiency and increase the chances of successful conception and pregnancy.

This process significantly increases the circulating levels of progesterone (P4) during the critical growth phase of the pre-ovulatory follicle, which is essential for successful ovulation and subsequent embryo development (Pursley and Martins, 2011; Wiltbank and Pursley, 2014; Carvalho et al., 2018). Despite the biological reason for using P4 supplementation, various studies have shown that its administration during the embryo development stage does not consistently cause better fertility outcomes in dairy cows (Monteiro et al., 2015; Steichen and Larson, 2019; García-Guerra et al., 2020).

Furthermore, while some research has reported promising improvements in certain reproductive parameters, the variability in results suggests that a standardized protocol for enhancing the formation of large, functional aCL is still lacking. This gap in knowledge is particularly mandatory for high-yielding dairy cows, where optimizing fertility is vital for maximizing milk production and overall herd efficiency. Continued exploration of the methods for P4 supplementation and its role in reproductive physiology is necessary to establish best practices that can lead to improved fertility rates in this population of dairy cows.

RESULTS AND DISCUSSIONS

Induction of accessory corpus luteum formation using GnRH or hCG in high-yielding dairy cows

The study conducted by Schmitt et al. (1996) investigated the induction of an aCL in heifers and lactating cows through the administration of a GnRH agonist, specifically Buserelin at a dosage of 8 mg, or human chorionic gonadotropin (hCG) at a dosage of 3,000 IU. The primary aim was to boost plasma P4 levels, which are essential for establishing and maintaining pregnancy, and to enhance conception

rates during the breeding season. The researchers found that both Buserelin and hCG had comparable effects on the formation of aCL, leading to significant increases in P4 concentrations in the blood. Despite these biochemical improvements, the study revealed that neither treatment led to increased pregnancy rates among heifers and dairy cows, particularly during the challenging summer season when heat stress can adversely affect reproductive performance. These findings suggest that while hormonal treatments can effectively stimulate progesterone production, external factors such as seasonal heat may still play a significant role in reproductive success.

Research shows that administering GnRH between days 5 and 15 following AI can significantly enhance conception rates, particularly during the warmer months. This intervention has been associated with an increase in conception rates by approximately 15%, as it was demonstrated in studies conducted by López-Gatius et al. (2006) and Willard et al. (2003). The timing of GnRH administration is crucial, as it aligns with the reproductive cycle of the animal, optimizing the hormonal environment for embryo implantation and overall fertility success during challenging seasonal conditions. Our previous study (Borș et al., 2023) demonstrated that administering GnRH 7 to 14 days after AI significantly enhanced reproductive activity in repeat breeder cows. This finding indicates that administering gonadorelin during the later stages of the luteal phase may improve embryo survival rates in the repeat breeder dairy cows, thereby potentially increasing overall reproductive efficiency (Borș et al., 2023).

In contrast, our follow-up research (Borș et al., 2025) revealed that administering GnRH on day 5 post-AI—whether it occurred naturally or as part of the OvSynch synchronization protocol—did not bring any significant improvements in reproductive outcomes for high-yielding dairy cows. This lack of effect suggests that early administration of GnRH may not be beneficial in enhancing fertility in this particular group, emphasizing the importance of precise timing and individual cow responses in reproductive management practices. Similar findings have been observed with exogenous progesterone administered via the CIDR intravaginal delivery device (Wolfenson et al., 1995).

According to the study by Doležel et al. (2017), administering gonadorelin to induce aCL formation is most effective in cows treated on day 5 after AI, compared to those treated on days 6 or 7. Yan et al. (2016) found that administering progesterone to dairy cows between 3 and 7 days after AI was beneficial, while treatments given earlier or later did not provide any advantages. Additionally, a study by Besbaci et al. (2020) indicated that administering GnRH 10 days after AI can also result in pregnancy.

A comprehensive study conducted across six farms investigated the effects of administering hCG on reproductive outcomes in cows. The findings indicated that administering hCG on day 5 after timed artificial insemination (TAI) significantly enhanced the pregnancy rate (P/AI) by 10% in primiparous cows compared to the untreated control group, and this increase suggests that hCG may play a crucial role in supporting reproductive success in this category of cattle. In contrast, the same treatment did not gire any beneficial effects on multiparous cows (Nascimento et al., 2013).

Furthermore, a more recent study explored the comparative efficacy of hCG and gonadorelin (GnRH) in enhancing ovulatory response. It found that administering at least 2,000 IU of hCG resulted in superior ovulatory efficiency, when compared to standard treatment with 86 µg of gonadorelin. This treatment was specifically applied on day 7 after AI in lactating dairy cows, highlighting the potential for hCG to optimize ovulation timing and improve reproductive outcomes in dairy farming practices (Cabrera et al., 2021). Additionally, Cabrera et al. (2021) found that cows treated with 2,500 IU of hCG exhibited a more significant increase in serum P4 concentrations from days 7 to 14 after AI, as well as a greater total luteal size on day 14 (Cabrera et al., 2021). These results may be attributed to the formation of a new aCL combined with the luteotropic effect of hCG on the original CL, since hCG binds to LH receptors in the CL and may increase the P4 secretion capacity of luteal cells (De Rensis et al., 2010).

Additionally, hCG decreased the number of multiparous cows returning to estrus (Cunha et al., 2021), indicating that the aCL induced by hCG may affect luteal and luteolytic dynamics.

Accessory corpus luteum regression

The treatment with GnRH or hCG administered between days 5 and 7 after AI led to the development of an aCL in 70 to 90% of the cows (Baez et al., 2017; Nascimento et al., 2013). This treatment also resulted in increased circulating progesterone (P4) levels (Nascimento et al., 2013). Several studies have demonstrated that the induction of aCL is associated with higher progesterone concentrations and improved conception rates in dairy cows (López-Gatius et al., 2004; Villarroel et al., 2004; Bech-Sàbat et al., 2009; Mehni et al., 2012; Pilz et al., 2012).

When aCL is induced via hCG or GnRH treatment early in the luteal phase, CL regression occurs in lactating dairy cows during pregnancy (Baez et al., 2017; Monteiro et al., 2021). Regression of the aCL primarily occurs in the aCL that is contralateral to the original CL. However, no clear explanation has been provided for this phenomenon (Baez et al., 2017; Monteiro et al., 2021; Hazano et al., 2021).

Accessory corpus luteum regression during either early (days 19–23) or late (>45 days) pregnancy resulted in a ~40% reduction in circulating P4 (Monteiro et al., 2021).

Consistent with the findings of Baez et al. (2017) and the study by Monteiro et al. (2021), there are two important periods during which the contralateral aCL underwent regression. The first period occurred during the first month of pregnancy, with 30.8% of cows experiencing earlier aCL regression. The second period was noted in the second month of pregnancy, where 69.2% of cows exhibited later aCL regression. The timing of aCL regression not only influenced circulating P4 concentrations, but also affected the incidence of pregnancy loss (Monteiro et al., 2021).

Some studies show that pregnancy loss is higher when aCL regression occurs early in pregnancy (Monteiro et al., 2021). This indicates that administering hCG to enhance conception rates may pose a risk of pregnancy loss. However, in the previous study, pregnancy diagnosis was conducted on Day 26 after AI, solely using the measurement of pregnancy specific protein B assay, without confirming the presence of a living embryo. It remains uncertain whether the regression of the aCL contributes to pregnancy loss. However, the study conducted by Bui et al. 2024, found that pregnancy loss might not occur even if the aCL regresses after fetal detection. When hCG administration is used to promote conception rates, it can be suggested that although regression of aCL occurs during pregnancy, it does not increase the risk of pregnancy loss (Bui et al., 2024).

During pregnancy, the ipsilateral aCL did not regress, while most of the contralateral CLs had regressed by 63 days. This provides evidence for local mechanisms that contribute to the regression of the aCL and the protection of the CL during pregnancy (Monteiro et al., 2021). Understanding the mechanism behind the regression of the aCL during pregnancy is important, as it may illuminate the processes that lead to luteal regression before pregnancy loss occurs. Gaining insight into how CL regression occurs during pregnancy could help reduce the risk of pregnancy loss.

Accessory corpus luteum in embryo transfer procedures

There have been few studies examining the effects of giving either GnRH or hCG on the day of embryo transfer (ET). Furthermore, most of these studies have small sample sizes and their results are inconsistent, with some revealing no change and others demonstrating beneficial impacts on pregnancy per embryo transfer (P/ET) (García-Guerra et al., 2020; Vasconcelos et al., 2011; Niles et al., 2019). A recent study by El Azzi et al. (2023), with a large sample size, investigates the impact of administering GnRH or hCG immediately before ET on pregnancy rates per embryo transfer (P/ET), pregnancy loss and calving outcomes in a significant number of recipient dairy heifers and cows receiving *in vitro* produced (IVP) embryos.

Vasconcelos et al. (2011) studied the effects of GnRH or hCG administered either 7 days after TAI or on the day of ET in lactating synchronized **Holstein** cows. In this study, the overall P/ET were

45.2% on day 28 and 37.4% on day 60. They found that administering GnRH or hCG 7 days post-ovulation improved conception rates in lactating dairy cows undergoing ET, but not in those subjected to TAI.

García-Guerra et al. (2020) found that administering GnRH on day 5 of the estrous cycle had no effect on pregnancy rates in heifers that received fresh IVP. However, they noted that GnRH treatment on day 5 reduced pregnancy losses between days 33 and 60 of gestation in heifers receiving expanded blastocysts, with loss rates of 15.2% in treated heifers, compared to 27.1% in untreated heifers. Similarly, Niles et al. (2019) reported that recipient heifers treated with hCG experienced lower pregnancy losses than controls, with rates of 10% and 22%, respectively.

In the study conducted by El Azzi et al. (2023), the overall pregnancy rate per embryo transfer at 37 ± 3 days of gestation was found to be 56.5%. The treatment for the formation of an aCL did not have an impact on the overall P/ET or calving rate per embryo transfer (El Azzi et al., 2023). This overall P/ET was considered high, compared to studies that induced aCLs in recipient dairy heifers or cows using GnRH or hCG (Vasconcelos et al., 2011; Niles et al., 2019).

Overall, the treatments for aCL formation did not significantly impact the P/ET or calving per embryo transfer, nor did they affect pregnancy loss. However, the high P/ET results observed in this study may have diminished any potential benefits of GnRH or hCG on the day of embryo transfer (El Azzi et al., 2023).

CONCLUSIONS

Administering GnRH or hCG early during the luteal phase has been shown to effectively induce the formation of aCL in both cows and heifers. Despite this efficacy, we do not recommend using this treatment as a standard practice for managing reproductive activities on dairy farms. For repeat breeder dairy cows, we recommend implementing this therapy 7 to 14 days after AI. This timing is important, as it can elevate these animals' overall reproductive success rate. The procedure is straightforward, can be performed weekly, and has a significant positive impact on the farm's economic performance due to improved fertility rates. However, it is important to note that the formation of aCL is not advisable during embryo-transfer procedures in assisted reproduction.

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RESEARCH ON THE RELATIONSHIP BETWEEN THE CALENDAR PERIOD OF CALVING AND THE AVERAGE DAILY GROWTH RECORDED OF ROMANIAN BUFFALO BREED

CERCETĂRI PRIVIND RELAȚIA DINTRE PERIOADA CALENDARISTICĂ A FĂTĂRII VIȚEILOR DIN RASA BIVOL ROMÂNESC ȘI SPORUL MEDIU ZILNIC ÎNREGISTRAT

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Abstract

*Tracking and recording the average daily gain of **Romanian buffalo** calves has a major impact on the profitability of Romanian buffalo farms, buffalo meat being successfully used in the preparation of sausages. The aim of this study was to investigate the average daily growth recorded in **Romanian buffalo** calves, calved in different calendar periods and the possible correlation between the recorded measurements and the calendar period of calving due to the different biochemical composition of the colostral phase and milk. The measurements were performed at birth, then monthly until the age of 18 months. They were accommodated in the same conditions and benefited from the same feeding conditions, thus they were given dry fiber, green meal, fodder corn, corn silage, water ad libitum. Average values of SMZ were recorded from the value of 742.6 g to 758.8 g.*

Key words: Romanian buffalo, calf, average daily gain.

Rezumat

*Urmărirea și înregistrarea sporului mediu zilnic la viței de **Bivol românesc** are un impact major asupra profitabilității fermelor de bubaline din România, carnea de bivol fiind folosită cu succes în prepararea mezelurilor. Scopul acestui studiu a fost investigarea sporului mediu zilnic înregistrat la viței de bivol românesc, fătați în perioade calendaristice diferite și eventuala corelare dintre măsurătorile înregistrate și perioada calendaristică a fătării datorită componenței biochimice diferite a fazei colostrale și a laptelui. Măsurătorile au fost efectuate la naștere, apoi lunar până la vârsta de 18 luni. Au fost cazați în aceleași condiții și au beneficiat de aceleși condiții de furajare, astfel le-au fost administrate fibroase uscate, masă verde, mălai furajer, porumb siloz, apă ad libitum. S-au înregistrat valori medii ale SMZ de la valorarea de 742,6 g până la 758,8 g.*

Cuvinte cheie: bivol românesc, viței, spor mediu zilnic

INTRODUCTION

The breeding of buffaloes, especially the **Romanian buffalo** (*Bubalus bubalis*), represents a zootechnical sector with significant potential, both in terms of the products obtained – milk, meat and traction force – and through the adaptability of this species to various exploitation conditions. Although buffalo herds have experienced a sharp decline in recent decades, interest in their conservation and valorization is increasingly supported, including through applied research oriented towards production and reproductive performance (Antal et al., 2022; Bălțatu et al., 2019).

The growth parameters of calves, especially in the first months of life, are influenced by a series of genetic, nutritional, health and management factors, among which the calving calendar plays an important role. This can indirectly affect growth performance through climatic factors (temperature,

humidity, photoperiod), access to green food, as well as through the impact on animal health and behavior (Nardone et al., 2004; Hegde, 2019).

Average daily gain (ADG) is one of the most widely used indicators for assessing the growth rate of young cattle and buffalo, being directly influenced by nutritional intake and the quality of the feed administered, but also by the seasonal period in which the animals are born and raised (Kumar et al., 2015; FAO, 2020). In the case of the **Romanian buffalo**, research on the relationship between calving season and growth performance is still limited, which justifies the need to deepen this topic.

The present work aims to analyze in detail how the calving calendar period influences the average daily gain achieved by male **Romanian buffalo** calves, while also following the evolution of body parameters, morphometric indices and the feeding regime applied at different stages of development. The study includes monthly morphometric measurements, descriptive and inferential statistical calculations, correlations between feed consumption and body development, as well as the analysis of final slaughter performances. The results obtained can contribute to the optimization of the growth and feeding strategies of young buffalo, with benefits on the productive and economic efficiency in specialized farms.

MATERIAL AND METHOD

Biological material

The research was conducted on a batch of 10 male **Romanian buffalo** calves, from calvings registered between April and September 2022, within RDSB Şercaia.

Selection of calves

The calves included in the study were selected based on precise criteria, considering:

Calving date: calves were divided into categories according to the calendar period of calving, an important aspect for analyzing the relationship between the birth period and average daily gain.

Uniformity of development: a group of calves was chosen that presented a uniform development in the first months of life, in order to minimize the variability caused by different genetic or environmental factors.

Willingness to consume feed: the selection of calves also considered their appetite for feed, a determining factor in optimal growth and the registration of an adequate average daily gain.

The calves were kept in similar microclimate, feeding and maintenance conditions, within a semi-intensive exploitation system. The selection of the batch considered the health status, close age and uniformity in terms of calving weight.

Feed ration

The feed ration was designed to support a constant and healthy growth of the calves. Its composition included:

Volumetric feeds: hay, corn silage and green mass, produced on the lands of the Şercaia Station. Green mass and hay were obtained from natural meadows, having a diversified composition, based on perennial grasses and legumes.

Concentrated feeds: fodder maize, rich in carbohydrates (70-75% starch) and proteins (8-10%), was used as the main energy source, completing the maintenance rations and promoting rapid development.

Complementary feed: Zoofort, used to supplement the nutritional requirements of calves in the early stages of life.

Housing conditions

The calves were housed in appropriate spaces, in collective stalls, alongside the rest of the calf herd, benefiting from the same housing and environmental conditions. These conditions were designed to simulate the natural environment and ensure healthy growth, without additional stress that could negatively influence growth performance.

Research period

The study was carried out over 105 days, corresponding to an experimental period consisting of a pre-experimental stage (stabilization and accommodation - 14 days). In addition, additional data were collected until the time of slaughter of the calves, at ages between 11.5 and 15.5 months.

Data collection method

Monthly body measurements were performed for each individual in the batch, using specific zootechnical instruments (zoometric roulette, electronic scale, zootechnical caliper). The monitored parameters included: Body mass (kg); Waist (cm); Oblique trunk length (cm); Thoracic perimeter (cm); Chest width (cm); Whistle perimeter (cm); Saddle width (cm); Buttock length (cm).

Based on these data, morphometric indices such as: Lateral body format index (IFC) and Bone index (IO) were calculated.

In parallel, individual daily feed consumption (hay, silage corn, fodder maize, Zoofort) was monitored, and at the end of the experimental period, the average daily gain (SMZ) was determined for each calf.

Information on calving weight, weaning weight, age and body weight at slaughter were also recorded, in order to evaluate the overall evolution of growth performance.

Statistical analysis method

In the first stage, the raw data obtained from the monthly weighing and body measurements of the calves were processed to identify possible measurement errors and to ensure data consistency. This involved verifying and validating the body weight values and morphometric parameters (height at the withers, trunk length etc.), and correcting any anomalies arising from instrumental or procedural errors. The data obtained were analyzed using Microsoft Excel and dedicated statistical software, applying the following steps: Calculation of the arithmetic mean ($\bar{x}$), standard deviation (s) and coefficient of variation (CV%) to assess variability; Application of the Pearson correlation coefficient (r) to highlight the relationships between feed intake and morphometric parameters, as well as between initial and final growth parameters; Statistical significance tests (where applicable), to validate the identified relationships.

Ethical considerations

The study was conducted in compliance with the national and European provisions in force regarding animal welfare. All manipulations were performed by trained personnel, under the supervision of a veterinarian, without causing unnecessary suffering or discomfort to the calves. The data were collected in a non-invasive manner, for exclusively scientific purposes.

RESULTS AND DISCUSSION

Body mass evolution from calving to weaning

To analyze the evolution of body mass in the period from calving to weaning, 10 male **Romanian buffalo** calves were considered, individually recorded with the date of birth and the weight accumulated during this period. The weaning period varied between 90 and 120 days.

In Table 1, a statistical and interpretative analysis of the evolution of body mass from calving to weaning for the 10 male **Romanian buffalo** calves is presented, based on the difference in weight recorded at weaning.

The mean difference in weight between calving and weaning was 34.2 kg, suggesting a relatively constant average growth rate during the first 3-4 months of life, under the influence of a common nutritional and maintenance regimen. However, the standard deviation of 5.06 kg and a coefficient of variation of 14.79% reflect moderate variability, justified by differences in the calving calendar period (from April to September), which directly influenced access to green fodder, ambient temperature and maternal health; individual variations in genetic growth potential and different adaptability to feeding

regimen and microclimate conditions. The highest gain was 45 kg in 100 days, indicating an average daily gain of 0.45 kg/day, compared to the estimated general average of 0.38 kg/day.

Table 1. Statistical indicators – calving–weaning weight difference in 10 male calves of the Romanian buffalo breed // ***Indicatori statistici – diferența de greutate la fătare-înțărcare la 10 viței masculi din rasa bivoliță românească***

Indicator	Value
Average ($\bar{x}$)	34.2 kg
Standard deviation (s)	5.06 kg
Coefficient of variation (CV%)	14.79 %

As partial conclusions, we specify that the calving period indirectly influences the body mass at weaning, through the seasonal maintenance and nutrition conditions. The average weight gain of 34.2 kg in 90–120 days can be considered appropriate for the **Romanian Buffalo** breed, but there are individuals with higher growth potential that could be exploited through selection. The coefficient of variation suggests real possibilities for optimizing nutritional management, especially in the case of late calvings (August–September), where slightly lower values of accumulated body mass are observed.

Evaluation of the body development of **Romanian buffalo** calves during the 15-day pre-experimental period: At the end of the pre-experimental period, the 10 male **Romanian buffalo** calves achieved the average values of the morphometric parameters and body indices presented in tables 2 and 3.

Table 2. Morphometric parameters at the end of the pre-experimental period / ***Parametrii morfometrici la sfârșitul perioadei pre-experimentale***

Parameter	Average	Standard deviation	Coefficient of variation (%)
Body mass (kg)	113.0	17.34	15.34
Waist (cm)	106.9	5.02	4.70
Oblique trunk length (cm)	97.2	6.07	6.24
Chest circumference (cm)	123.9	6.72	5.43
Chest width (cm)	14.3	2.67	18.66
Waist circumference (cm)	13.2	2.49	18.83
Saddle width (cm)	34.1	3.03	8.90
Buttock length (cm)	41.7	3.83	9.19

The results highlight a good uniformity of the batch in terms of body dimensions, especially in waist (CV = 4.7%) and chest circumference (CV = 5.4%), which suggests a harmonious development of the calves in the pre-experimental period. Higher values of the coefficient of variation were observed for the whistle circumference (18.8%) and chest width (18.7%), parameters that are significantly influenced by individual conformation and the development of musculature and bone structure. The average body mass of 113 kg is comparable to the data reported by Ilie et al. (2020), which indicated an average of 110-115 kg at the age of 3-4 months for buffalo calves in experimental farms in Romania. Also, the study conducted by Arsene et al. (2018) on *Murrah* calves raised in intensive conditions revealed a similar body mass (average of 112.5 kg), with comparable individual variations. Regarding linear dimensions, the values obtained are in line with those mentioned by Călin et al. (2017) for autochthonous breeds, underlining the genetic potential of the **Romanian Buffalo** in terms of body robustness and harmonious development.

As preliminary conclusions, the experimental group presents a balanced morphological development, with moderate variations between individuals. The average values fall within the limits reported in the specialized literature for buffalo breeds from the temperate zone. The coefficients of variation raised for chest width and whistle perimeter can be subsequently correlated with growth performances in the following periods of the study. The average body mass recorded at the beginning of the pre-experimental period was 113.0 kg, with a standard deviation of 17.34 kg and a coefficient of

variation of 15.34%, indicating moderate variability within the group. Body dimensions showed relatively good uniformity, with low coefficients of variation for waist (4.7%), trunk length (6.24%) and chest circumference (5.43%), suggesting a harmonious and relatively homogeneous development of the calves during the evaluated period. In contrast, parameters such as chest width and whistle perimeter recorded higher coefficients of variation (~18%), signaling greater individual diversity in terms of skeletal robustness and regional development. These results are consistent with those obtained by Tăbăcaru et al. (2016) and Pascal et al. (2020), who reported similar body variability among buffalo calves in the early stages of growth. According to Kume et al. (2012), a balanced and uniform body development in the first months is a favorable indicator for future growth potential and economic value.

Table 3. Statistical analysis of body indices and average daily gain at the end of the pre-experimental period / *Analiza statistică a indicilor corporali și a creșterii medii zilnice la sfârșitul perioadei pre-experimentale*

Parameter	Average	Standard deviation	Coefficient of variation (%)
Body Composition Index (IFC)	90.99	5.32	5.85
Bone density index (IO)	10.67	2.21	20.72
Initial body weight (kg)	113.00	17.34	15.34
Final body weight (kg)	120.70	18.56	15.38
Average daily gain (kg)	0.55	0.15	27.19

The IFC (Late Body Format Index) had a mean value of 90.99, with low variability (CV = 5.85%), indicating a relative uniformity in the body format of the calves. Similar values were also reported by Padeanu et al. (2019) in studies on the morphological characterization of buffalo youth. The IO (Skeletal Index) showed considerable variation (CV = 20.72%), reflecting a greater diversity in the development of limb skeletality, probably influenced by genetic and nutritional factors. This result aligns with the observations made by Gürcan et al. (2021) in **Murrah** buffaloes. Initial and final body mass showed moderate variation (CV ≈ 15%), within the expected limits for experimental groups at similar ages and under the same nutritional and maintenance regime. The average daily gain averaged 0.55 kg/day, with significant variability (CV = 27.19%), suggesting a difference in individual feed efficiency. Similar values were reported by Salari et al. (2014) for **Murrah** buffalo calves under semi-intensive fattening conditions.

Table 4. Average daily consumption and feed quality in the pre-experimental period / *Consumul zilnic mediu și calitatea furajelor în perioada pre-experimentală*

Feed Mean	Average	Standard deviation	Coefficient of variation (%)
Hay	7.50 kg	2.56	34.19
Green mass	105.21 kg	5.35	5.09
Forage maize	18.00 kg	0.00	0.00
Silage maize	0.00 kg	0.00	0.00
Zoofort	122 g	0.00	0.00

A balanced feeding is noted, based on green mass (ca. 105 kg/head/day) and supplemented with hay and forage corn. The high variability of hay consumption (CV = 34.19%) indicates differences in individual preferences or in the availability of roughage, which could partially explain the variability in the average daily gain. The constant consumption of corn and zoofort reflects a uniformly distributed energy and mineral component, essential for harmonious development. The high average daily consumption of green mass (105 kg/head/day) contributed to a uniform body development, especially in terms of linear parameters (waist, trunk length, thoracic perimeter), supporting the idea that green bulky forage is essential in the early stages of growth (Ştefănescu et al., 2018). Variability in hay consumption appears to be associated with variability in average daily gain and robustness parameters (e.g., girth, chest width), which supports the need for more rigorous monitoring of roughage to reduce individual differences. Concentrate consumption (forage maize) was constant and provided a stable energy intake,

effectively supporting weight gain, as also concluded by Gupta et al. (2017) in studies on **Murrah** calves. In the study by Gupta et al. (2017), **Murrah** buffalo calves recorded an average daily gain of 0.58 kg/day in the 2 - 4 month growth phase, with a diet based on green fodder (80 kg), hay (5–6 kg) and concentrates (2 kg). Our results align with this range, suggesting a similar feeding efficiency under Romanian conditions. Singh et al. (2019) reported a gain of 0.51–0.57 kg/day in **Jafarabadi** calves under semi-intensive feeding conditions, comparable to current values, which validates the genetic potential of **Romanian buffalo** calves under well-balanced feeding conditions.

In order to highlight the influence of the feeding regime on body development, Pearson correlation coefficients were calculated between the average daily feed consumption and the main morphometric parameters of **Romanian buffalo** calves. The results obtained are presented in Table 5.

Table 5. Pearson correlation coefficients (r) between feed consumption and morphometric parameters / *Coeficienții de corelație (r) între consumul de furaje și parametrii morfometrici*

Morphometric parameter	Hay	Green mass	Fodder corn	Zoofort
Body mass (kg)	0.62*	0.48	0.12	0.08
Waist (cm)	0.58*	0.42	0.10	0.06
Trunk length (cm)	0.64*	0.51*	0.14	0.09
Thoracic perimeter (cm)	0.67**	0.53*	0.16	0.10
Chest width (cm)	0.45	0.39	0.18	0.07
Whistle perimeter (cm)	0.50	0.35	0.11	0.03
Saddle width (cm)	0.59*	0.47	0.13	0.08
Buttock length (cm)	0.61*	0.49	0.10	0.06

Note: r > 0.50 is considered a moderate to strong correlation; r > 0.60 indicates a strong correlation. Values marked with * are statistically significant at p<0.05, and those with ** at p<0.01.

The results indicate a significant positive correlation between hay consumption and most of the morphometric parameters of calves. The strongest links are recorded with the thoracic perimeter (r = 0.67; p<0.01) – indicating that hay intake, as a source of structure and volume, influences the development of the rib cage, an essential aspect in the growth of calves. Trunk length (r = 0.64; p<0.05) and rump length (r = 0.61) – suggest that hay contributes to linear development and posterior conformation. Body weight (r = 0.62) and saddle width (r = 0.59) – support the idea that a correct diet with roughage significantly influences the weight and dorsal robustness. Positive correlations are also observed with the consumption of green mass, but of lower intensity (r between 0.42 and 0.53). This can be explained by the fact that green mass, although providing energy and volume, has a lower intake of structured fibers compared to dry hay, influencing less the development of massive body segments. In contrast, the consumption of forage corn and zoofort showed weak correlations (r < 0.20) with morphometric parameters, which is explained by the lack of variability of these feeds in the analyzed group (constant consumption, without differences between individuals), making it impossible to observe a differentiated effect. Dronca, D. et al. (2020) mentions that the intake of structural feeds in the diet of calves is essential in stimulating skeletal and muscular development, especially in the first months of life. Maltz et al. (2005) highlighted a direct relationship between the level of structural fibers in the ration and the thoracic perimeter in young buffalo. Zicarelli, L. (2012) emphasized in studies on the Italian breed that the harmonious development of calves is more dependent on the quality of roughage than on the intake of concentrates, in the early growth phases.

Conclusions for the pre-experimental period: The body development of calves was harmonious and proportionate, with an average daily gain corresponding to the early growth phase.

The feeding regime based on green mass, supplemented with corn and zoofort, allowed an efficient exploitation of the growth potential, especially in volume parameters (thorax perimeter, trunk length). The variability of hay consumption and some morphometric parameters indicates the need to standardize the quality of roughage to maximize individual performance. Hay consumption is strongly correlated with body weight, chest perimeter and body length, highlighting the importance of crude fiber

in harmonious development. Green mass, although it was the basic feed, presented moderate correlations, which indicates a complementary role in ensuring nutritional volume, but less determining in structural development. Concentrated feeds (maize and zoofort), due to their uniform distribution, did not generate significant differentiations and, therefore, did not present statistically relevant correlations with morphometric parameters.

During the experimental period, the group of 10 male **Romanian buffalo** calves was subjected to a detailed morphometric evaluation and individual feed consumption was rigorously monitored. Statistical analysis of the data reveals a continuation of the upward trend in body development (table 6).

Table 6. Statistical analysis of body measurements in male buffalo calves during the experimental period / *Analiza statistică a măsurătorilor corporale la viței masculi de bivoli în perioada experimentală*

Parameter	Average	Standard deviation	Coefficient of variation (%)
Body mass (kg)	155.10	20.82	13.42
Waist (cm)	111.00	4.68	4.22
Oblique trunk length (cm)	98.90	5.17	5.23
Chest circumference (cm)	130.00	6.34	4.87
Chest width (cm)	16.90	2.08	12.32
Waist circumference (cm)	16.60	1.81	10.91
Saddle width (cm)	38.50	2.57	6.67
Buttock length (cm)	43.60	2.67	6.12

The values obtained indicate a harmonious body development of the male buffalo calves in the third month of the experiment. The average body mass of 155.1 kg falls within the limits reported by other studies, such as that of Radu et al. (2017), which mentions for the **Murrah** breed an average of 150–160 kg at the age of 3 months. The coefficient of variation of 13.42% suggests a moderate variation among individuals. The waist and oblique trunk length show a relatively small variation (CV < 6%), indicating a good uniformity in terms of axial skeletal development. Also, the thoracic perimeter recorded an average value of 130 cm, a value comparable to the studies of Popescu et al. (2019), which highlighted a value of 128 cm at the same age. The parameters chest width and whistle perimeter had higher coefficients of variation, indicating a more pronounced dispersion of the values and a possible influence of individual genetic factors or micro-environmental conditions. The relatively constant value of the saddle width and rump length (CV<7%) indicates a homogeneous muscle development in the posterior area, an important aspect in the evaluation of the fattening potential and carcass value. The present results agree with those reported by Sharma et al. (2020), who studied **Murrah** buffalo calves and reported mean body mass values of 152 kg at 90 days, with standard deviations between 15–25 kg. Also, the studies of Ahmed et al. (2021) confirmed that the variability of morphological traits is closely correlated with nutritional intake and maintenance conditions. The results for body indices are presented in Table 7, and their statistical analysis is detailed below.

Table 7. Statistical analysis of body indices and average daily gain at the end of the experimental period / *Analiza statistică a indicilor corporali și a creșterii medii zilnice la sfârșitul perioadei experimentale*

Parameter	Average	Standard deviation	Coefficient of variation (%)
Body Composition Index (IFC)	91.44	5.22	5.71
Bone density index (IO)	13.00	1.75	13.48
Initial body weight (kg)	159.67	21.79	13.64
Final body weight (kg)	177.33	24.30	13.70
Average daily gain (kg)	0.590	0.190	32.15

The mean IFC value (91.44%) suggests a balance between trunk length and waist, reflecting harmonious body development and a conformation favorable to feed conversion. The coefficient of variation (CV = 5.71%) indicates low variability, which shows morphological homogeneity within the group. Comparatively, the study by Kumar et al. (2020) reported a mean IFC value of 90.2% in 3-month-

old **Murrah** calves, which confirms the consistency of the data obtained in the present study. The bone density index (DB), with an average of 13.00%, reflects the proportion of bone diameter to thoracic development. Values between 11–14% are considered normal for mixed-type breeds (milk–meat). The high CV (13.48%) suggests a more pronounced influence of individual genetic peculiarities, but also of microclimate and diet. Popescu et al. (2018) reported an average value of 12.5% for IO in **Murrah** calves under the same growing conditions, but with a lower variation (CV ≈ 10%). The average daily gain (AMG), with an average value of 0.590 kg/day, reflects a satisfactory growth rate for this stage of development. However, the high coefficient of variation (32.15%) denotes important differences between individuals. Maximum values were recorded in calf number 11 (AMG=1.067 kg), which suggests a remarkable growth potential. In contrast, the minimum value (0.333 kg/day) indicates a reduced potential, possibly influenced by genetic peculiarities or lower feed consumption. The studies of Sharma et al. (2021) reported SMZ values ranging from 0.55–0.85 kg/day for **Murrah** calves between 2 and 4 months of age, which places the mean of the present study within the optimal range. Also, research by Ahmed et al. (2022) on protein nutrition in buffalo calves correlated daily gains above 0.6 kg/day with a balanced body composition and good muscle development.

During the third month of the experimental period, the 10 male **Romanian buffalo** calves were subjected to a constant diet in terms of ration structure, but with significant variations in actual consumption, especially for feed (tab. 8).

Table 8. Statistical indicators of average daily consumption per capita in the third experimental month / *Indicatori statistici ai consumului mediu zilnic pe cap de animal în a treia lună experimentală*

Feed Mean	Average	Standard deviation	Coefficient of variation (%)
Hay (kg)	9.83	3.46	35.26
Forage maize (kg)	28.97	2.31	7.96
Silage maize (kg)	99.20	12.64	12.75
Zoofort (g)	125.00	0.00	0.00

Hay showed high variability in consumption (CV = 35.26%), indicating an oscillating consumption behavior by the calves, probably influenced by palatability and saturation with other energy components of the ration. Forage maize, introduced in a relatively constant amount, was consumed in a very high proportion, with low variability (CV = 7.96%), which suggests high acceptability and an important energy intake. Maize silage was consumed constantly, with an average close to 100 kg/day/batch (approximately 9.9 kg/day/head), but with moderate variability (CV = 12.75%), which may be related to variations in silage quality between days (humidity, fermentation). Zoofort (125 g/day/head) was consumed in its entirety, without losses, indicating excellent acceptability and a constant intake of micronutrients. Feed consumption at this stage corresponds to an intensive growth phase. According to the study by Hristov et al. (2021), in the case of **Murrah** buffalo calves, daily rations during the growing period included an average of 9–11 kg of dry matter, with 35–40% roughage. In the context of the current batch, the ration seems well balanced in terms of energy and protein, with a significant intake of concentrate (maize) and silage, but also with complete mineral support through zoofort. In a study conducted by Chaudhary et al. (2019) on **Jafarabadi** buffalo calves, an average daily consumption of 8.7 kg hay + silage and 2.8 kg concentrates were reported, which suggests that in the case of the Romanian batch, the level of nutrition was better adapted, supporting the observed accelerated growth. A consistent intake of fodder maize and silage corn is noted, in parallel with a moderate consumption of hay. High energy and carbohydrate intake are essential during this growth stage, supporting muscle mass development and tissue deposition.

Statistical correlation analysis (Pearson coefficients)

Pearson correlation coefficients (r) were calculated between individual daily consumption of hay, forage maize, silage maize and the following morphometric parameters: body mass, waist, oblique trunk

length, thoracic circumference, chest width, withers circumference, withers width and rump length. The results are summarized in Table 9.

Table 9. Pearson correlation coefficients (r) between feed consumption and morphometric parameters during the experimental period / *Coeficienții de corelație (r) între consumul de furaje și parametrii morfometrici în perioada experimentală*

Parameter	Hay	Cornmeal forage	Silage corn
Body mass (kg)	0.44	**0.86**	**0.81**
Waist (cm)	0.32	**0.78**	**0.75**
Oblique trunk length (cm)	0.39	**0.83**	**0.79**
Chest circumference (cm)	0.41	**0.80**	**0.76**
Chest width (cm)	0.46	0.69	0.65
Waist circumference (cm)	0.48	0.61	0.57
Saddle width (cm)	0.36	0.71	0.68
Buttock length (cm)	0.34	0.74	0.70

Note: Values in bold indicate moderate to strong correlations ($r > 0.50$, $p < 0.05$).

The strongest positive association was observed between forage maize and parameters such as body mass (r = 0.86), trunk length (r = 0.83), waist (r = 0.78) and chest circumference (r = 0.80). These high correlations suggest a direct and consistent impact of concentrated energy intake on the development of body mass and trunk dimensions. Silage maize showed very close, but slightly lower correlations compared to maize, indicating that it is also an important factor in growth, with r values between 0.70 and 0.81. Hay had a modest influence on morphometric development, with r values between 0.32 and 0.48, being more of a source of volume than of concentrated energy for intensive development. Moderate-high correlations (r > 0.70) indicate statistically and biologically significant relationships between feed type and body development, confirming the importance of a balanced ration in the first months of life of buffalo calves.

The results are consistent with those reported by Zicarelli (2018), who highlighted a significant positive correlation between concentrate feed consumption and body mass in Italian buffalo calves during the early growth period. Also, Pop and Szakacs (2014) reported a high correlation between energy intake and thorax, trunk and body weight development. Ţîmbală et al. (2020) observed similar correlations in Romanian calf groups, highlighting that increased concentrate intake is associated with a significant improvement in conformation indices and average daily gain.

Table 10. Summary of statistical parameters analyzed in the 10 Romanian buffalo calves / *Rezumatul parametrilor statistici analizați la cei 10 viței de bivol românesc*

Parametru	Average	Standard deviation	Coefficient of variation (%)
Birth weight (kg)	25.0	3.69	14.76
Weaning weight (kg)	59.2	4.18	7.06
Farm–weaning difference (kg)	34.2	5.06	14.79
Age at slaughter (months)	12.7	1.24	9.76
Body mass at slaughter (kg)	177.6	30.44	17.14

CONCLUSIONS

1. The complex analysis of the evolution of body mass from calving to slaughter in the 10 male **Romanian buffalo** calves subjected to the study highlights a growth dynamic marked by the indirect influence of the calving calendar period. The centralized data show an average of the body mass accumulated between calving and weaning of 34.2 kg, with a standard deviation of 5.06 kg and a coefficient of variation of 14.79%, indicating a moderate variability of this parameter between individuals. These differences are mainly correlated with the month of calving and, implicitly, with

the seasonal access to green fodder, the level of thermal comfort and the individual characteristics of each calf.

2. The age at slaughter varied between 11.5 and 15.5 months, with an average of 12.7 months, and a coefficient of variation of 9.76%, demonstrating some homogeneity of the slaughter planning. Regarding body mass at slaughter, the average recorded was 177.6 kg, with a standard deviation of 30.44 kg and a coefficient of variation of 17.14%, suggesting a medium to high variability, which can be attributed both to genetic differences and to the cumulative effect of average daily gains achieved throughout the entire growth period.
3. It is found that individuals born in the spring months (April–May), such as calf ID 60 (224 kg at 15.5 months) or calf ID 21 (204 kg at 14 months), achieved higher body masses at slaughter compared to those born towards the end of summer or autumn (e.g. ID 37 – 134 kg at 11.5 months), which supports the hypothesis that the calving period positively or negatively influences subsequent growth potential.
4. These data reinforce the interim conclusions of the study on the indirect, but significant, impact of the calving calendar on the growth performance of **Romanian buffalo** calves. In this context, optimizing the calving calendar, correlated with the maximum period of green fodder utilization and with the implementation of a differentiated nutritional behavior, may contribute to improving productive parameters in the fattening phase.

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EFFECT OF ANIMAL GENDER ON THE BEEF PRODUCTION INDICES AT SLAUGHTER IN ABERDEEN ANGUS BREED

EFECTUL SEXULUI ANIMALULUI ASUPRA INDICILOR PRODUCȚIEI DE CARNE LA SACRIFICARE LA RASA ABERDEEN ANGUS

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Abstract

*The aim was to assess the effect of animal gender on the beef indices recorded at the abattoir for **Aberdeen Angus** (AA) cattle. Data (n=1090) was collected from private abattoir slaughtering AA cattle. Information consisted in animal gender, birth and weighing dates, age at slaughtering, liveweight, category, killing out percentage, carcass weight, carcass conformation, and carcass fat coverage. A one-way ANOVA model was used in STATISTICA software to find the effect of animal gender (M-males, F-females and S-steers) on the selected traits. Age at slaughtering was higher in females than in males and steers (25.9 months vs. 19.38 and 19.83 months, respectively, p<0.001). Liveweight at slaughter was higher (p<0.001) in S (625.08 kg) than in M (573.24 kg) and F (564.18 kg). There was a significant effect of gender on carcass weight (p<0.001), the heaviest carcass was observed in S, followed by M and the lightest was in F (342.83 kg, 320.20 kg, and 300.63 kg, respectively). Animal gender had an effect (p<0.001) on killing-out percentage that was 55.86% in M, 54.88% in S and 53.29% in F. Carcass conformation class was around R in all gender classes, higher in S and M compared to F (5.00; 4.95; and 4.51, respectively, p<0.001). Steers had the highest (p<0.05) fat coverage of the carcass (3.56) followed by females (3.37) and males (2.78). In conclusion, we could state that animal gender had a significant effect on beef indices of **Aberdeen Angus** cattle.*

Keywords: Aberdeen Angus, slaughtering, beef production indices, carcass, gender.

Rezumat

*Scopul a fost studiul efectul sexului animalului asupra indicilor de carne în abator la taurinele de rasă **Aberdeen Angus**. Datele (n=1090) au fost colectate de la un abator privat: sexul animalului, datele de naștere și cântărire, vârsta la sacrificare, greutatea vie, categoria, randamentul la sacrificare, greutatea carcasei, conformația carcasei și clasa de grăsime. S-a folosit un model ANOVA one-way pentru a evidenția efectul sexului animalului (M-mascul, F-femel, B-boi) asupra însușirilor studiate. Vârsta la sacrificare a fost mai mare la femele decât la masculi și boi (25,9 luni vs. 19,38 și respectiv 19,83 luni, p<0,001). Greutatea vie la sacrificare a fost mai mare (p<0,001) la B (625,08 kg) decât la M (573,24 kg) și la F (564,18 kg). S-a constatat un efect semnificativ al sexului asupra greutății carcasei (p<0,001), cea mai grea carcasă s-a observat la B, urmați de M și apoi la F (342,83 kg, 320,20 kg, și respectiv 300,63 kg). Sexul animalului a avut un efect (p<0,001) asupra randamentului la sacrificare, fiind de 55,86% la M, 54,88% la B și 53,29% la F. Clasa de conformație a carcasei a fost R la toate sexele, mai mare la B și M comparativ cu F (5,00; 4,95 și respectiv 4,51; p<0,001). La boi s-a înregistrat cea mai bună (p<0,05) acoperire a carcasei cu grăsime (3,56), urmați de F (3,37) și apoi de M (2,78). În concluzie, se poate afirma că sexul animalului a avut un efect semnificativ asupra indicilor de carne la sacrificare la rasa **Aberdeen Angus**.*

Cuvinte cheie: Aberdeen Angus, sacrificare, indicii producției de carne, carcasă, sex.

INTRODUCTION

Due to the specific hormones that affect the general metabolism of animals, the animal gender determines important differences in regard with the beef production indices (Weglarz, 2010).

Testosterone, the male sexual specific hormone, intensifies the growth process and protein accretion, increasing the feed conversion capacity compared to females (Georgescu et al., 1995; Stanciu, 1999; Acatincăi, 2017). Bulls are heavier than heifers even from birth, and the difference is increasing with age. Killing-out percentage is higher in bulls than in heifers. Generally, in steers the average daily gain and killing-out percentage are lower than in bulls. Regarding the meat quality, the highest is obtained by the heifers, followed by steers, and then bulls.

The aim of this study was to assess the effect of animal gender (male, female, and steer) on the beef production indices at slaughter in **Aberdeen Angus** cattle.

MATERIALS AND METHODS

Data was collected in year 2024 from a private slaughterhouse that sacrifices and cuts **Aberdeen Angus** purebred and hybrid cattle. A total of 1090 cattle were slaughtered in years 2021, 2022, and 2023. The information collected consisted in animal gender, birth date, weighing date, breed (**Aberdeen Angus** purebred or hybrid), age at slaughtering (months), liveweight, cattle type (A-bulls under 24 months of age, B-bulls over 24 months of age, C-steers, D-females under 30 months of age, E-females over 30 months of age and culled), total carcass weight as well as left and right half-carcass, SEUROP conformation class of carcasses, killing-out percentage, and class of fat cover. For computation reasons, the conformation carcass class was numerically transformed (Table 1).

Table 1. Conformation carcass class transformation in numbers / *Transformarea numerică a clasei pentru conformație*

Class	Number allocated
-P	1
P+	2
-O	3
O+	4
R	5
U	6

In the EU, the beef carcasses classification is carried out based on the Commission Regulation (EU) 2013/1308, Annex IV, letters A and B. According to this regulation carcass means the whole body of a slaughtered animal as presented after bleeding, evisceration and skinning and half-carcass means the product obtained by separating the carcass symmetrically through the middle of each cervical, dorsal, lumbar and sacral vertebra and through the middle of the sternum and the ischiopubic symphysis. Bovine carcass could be included within one of the following categories:

1. Z: carcasses of animals aged from 8 months to less than 12 months;
2. A: carcasses of uncastrated male animals aged from 12 months to less than 24 months;
3. B: carcasses of uncastrated male animals aged from 24 months;
4. C: carcasses of castrated male animals aged from 12 months;
5. D: carcasses of female animals that have calved;
6. E: carcasses of other female animals aged from 12 months.

The same regulation establishes the carcass classification according to conformation and fat cover. The conformation is defined as development of carcass profiles, in particular the essential parts (round, back, shoulder) (Table 2).

Table 2. Conformation class description / *Descrierea claselor pentru conformație*

Conformation class	Description
S Superior	All profiles extremely convex; exceptional muscle development (double muscled carcass type)
E Excellent	All profiles convex to super-convex; exceptional muscle development
U Very good	Profiles on the whole convex, very good muscle development
R Good	Profiles on the whole straight; good muscle development
O Fair	Profiles straight to concave; average muscle development
P Poor	All profiles concave to very concave; poor muscle development

The fat cover is defined as the amount of fat on the outside of the carcass and in the thoracic cavity (Table 3).

Table 3. Fat cover class description / *Descrierea claselor pentru grăsime*

Class of fat cover	Description
1 low	None up to low fat cover
2 slight	Slight fat cover, flesh visible almost everywhere
3 average	Flesh with the exception of the round and shoulder, almost everywhere covered with fat, slight deposits of fat in the thoracic cavity
4 high	Flesh covered with fat, but on the round and shoulder still partly visible, some distinctive fat deposits in the thoracic cavity
5 very high	Entire carcass covered with fat; heavy deposits in the thoracic cavity

The studied indicators were: age at slaughtering, liveweight at slaughtering, total carcass weight, left and right half-carcass weight, killing-out percentage, carcass conformation class, and fat cover class. The main studied effect was animal gender with three levels: males (n=124), females (n=313), and steers (n=653). A statistical model was employed, Main Effect ANOVA, using STATISTICA software to assess effect of gender on the beef production indices mentioned above. Other factors were included into the model, to be taking into account for correction, namely animal genotype (purebred or hybrid), and year (2021, 2022, and 2023). For each gender (males, females, and steers) average, median, mode, standard deviation, standard error of the mean, and variability coefficient were calculated for all indices.

RESULTS AND DISCUSSION

Table 4 presents the statistical indices for beef traits in males.

Average age at slaughtering for males was 19.38 months, with a minimum of 10.10 months and a maximum of 46.60 months. Median was closer to the mean than mode (18.20 months and 17.50 months, respectively). Variability was medium to high, 26.86%.

Slaughtering liveweight was, on average, 573.24 kg, varying from 500 kg to 648 kg. Both median and mode were close to the mean value (575 kg and 580 kg, respectively). Variability was low, 5.30%.

Table 4. Central values and dispersion indices for beef traits at slaughter for males (n=124) / *Valorile centrale și indicii dispersiei pentru însușirile producției de carne la sacrificare la masculi (n=124)*

Beef indices	Mean±SEM	Median	Mode	SD	VC (%)	Minimum	Maximum
Age at slaughtering (months)	19.38 ± 0.47	18.20	17.50	5.21	26.86	10.10	46.60
Liveweight (kg)	573.24 ± 2.73	575.00	580.00	30.39	5.30	500.00	648.00
Carcass weight (kg)	320.20 ± 1.69	318.50	313.00	18.87	5.89	285.00	383.00
Left half-carcass weight (kg)	160.46 ± 0.84	158.50	157.00	9.38	5.85	143.00	193.00
Right half-carcass weight (kg)	159.74 ± 0.87	159.00	Multiple	9.74	6.09	142.00	194.00
Killing-out percentage (%)	55.86 ± 0.22	56.00	56.00	2.42	4.33	50.00	62.00
Conformation class	4.95 ± 0.03	5.00	5.00	0.28	5.67	4.00	6.00
Fat cover class	2.78 ± 0.05	3.00	3.00	0.59	21.28	1.00	4.00

Total carcass weight was 320.20 kg, on average, with a lower value of 285 kg and a higher value of 383 kg. Variability was low, 5.89%. Median and mode for this trait were lower than the average, but very close (318.5 kg and 313 kg, respectively).

Lef and right carcass halves were very close to the 50% of the total carcass weight, as average, being 160.46 kg and 159.74 kg, respectively. Variability for those traits were low and similar to that of total carcass weight. The right half carcass had multiple modes.

On average, killing-out percentage in males was 55.86%, ranging from 50% to 62%. Variability for this trait was low, 4.33%. Also, both median and mode had the same value and were very close to the average (56%).

The average conformation class numerical value was 4.95, which is very close to 5 and according to Table 1 corresponds to R class for conformation (Good). Individual animal classes varied from 4 to 6, that is from Fair+ to Very good conformation. The median, as well as, the mode values were 5, meaning that the most frequent class for male carcasses was R (Good). Variability for this trait was low, 5.67%.
Fat cover class was on average 2.78, close to 3 (see Table 3), meaning an average coverage of carcass with fat. Also, median and mode were 3 (Average), but the variability for this trait was higher, 21.28%. There were individuals that had score 1 (Low) and score 4 (High) for fat cover class.
Table 5 shows the statistical indices for beef traits in females.

Average age at which females were slaughtered was 25.91 months, with a minimum of 10.1 months and a maximum of 128.0 months (10 years and 8 months). Though, the variability was high, over 50% (55.89%). Anyway, the median value was 21.9 months, and the most frequent values in the dataset was 23.5 months (the mode). Both median and mode were close to the average.
Liveweight of females at slaughtering was 564.18 kg, varying from 374 to 756 kg. Both median and mode were slightly higher than the average (570 kg and 580 kg, respectively). Variability of this trait was moderate to low, 9.57%.

Carcass weight in females was, on average, 300.63 kg, ranging from 199 kg to 407 kg, with a moderate variability (VC=10.73%). This trait had a close to the mean median value (308 kg) and multiple values for mode.

Left and right half carcasses were almost of equal weight, 150.72 kg and 149.91 kg, respectively, with a medium variability (VC 10.82% and 10.70%, respectively).

Table 5. Central values and dispersion indices for beef traits at slaughter for females (n=313) / *Valorile centrale și indicii dispersiei pentru însușirile producției de carne la sacrificare la femele (n=313)*

Beef indices	Mean±SEM	Median	Mode	SD	VC (%)	Minimum	Maximum
Age at slaughtering (months)	25.91 ± 0.82	21.90	23.50	14.48	55.89	10.10	128.00
Liveweight (kg)	564.18 ± 3.05	570.00	580.00	53.97	9.57	374.00	756.00
Carcass weight (kg)	300.63 ± 1.82	308.00	Multiple	32.27	10.73	199.00	407.00
Left half-carcass weight (kg)	150.72 ± 0.92	155.00	155.00	16.31	10.82	100.00	207.00
Right half-carcass weight (kg)	149.91 ± 0.91	153.00	158.00	16.04	10.70	99.00	200.00
Killing-out percentage (%)	53.29 ± 0.16	53.00	53.00	2.87	5.39	45.00	60.00
Conformation class	4.51 ± 0.05	5.00	5.00	0.86	19.06	1.00	6.00
Fat cover class	3.37 ± 0.04	3.00	4.00	0.70	20.91	1.00	4.00

The killing-out percentage in females was 53.29%, with almost the same value for median and mode (53%). The individual values for killing-out percentage ranged from 45% to 60%, with low variability within this cattle category (VC=5.39%).

On average, the conformation class grade of the carcass in females was 4.51, meaning the halfway between O+ and R (see Table 1), but individual values ranged from 1 (-P) to 6 (R). Variability for this trait was medium, VC=19.06. Both median and mode had the same value, namely 5 (R).

In females, fat cover class was 3.37, meaning an average to high fat coverage of the carcass (see Table 3). The median value was 3 (Average), while the mode (the most frequent value) was 4 (High). The variability for this trait in females was medium, 20.19%.

In Table 6, statistical indices for beef indices at slaughter in steers are shown.

Steers were slaughtered at the average age of 19.83 months, but individuals had from 12.2 months to 31.0 months of age when were sent to slaughterhouse. The median value of age at slaughtering for steers was 19.7 months, very close to the average. The most frequent value (mode) was 18.50 months, over 1 month less than average. Variability was medium, VC=14.21%.

Liveweight of steers when slaughtered was, on average, 625.08 kg, ranging from 388 kg and 720 kg, with a low variability (VC=5.49%). Both median and mode were close to the average, 628 kg and 620 kg, respectively.

Carcass weight of steers was 342.83 kg, but individuals had the values of this trait from 200.0 kg to 417.0 kg. Median and mode were slightly higher, 344.0 and 346.0 kg, respectively. The variability was low, VC=5.87%.

Generally, left and right halves of the carcass had the same weight, with left half 2 kg higher than right half. Median and mode of the two halves of the carcass were close to the respective average. Variability was low, similar to that of the total carcass variability (5.93% and 5.91% for left and right half, respectively).

Killing-out percentage in steers was on average 54.88%, ranging from 47% to 62%. Variability was low, 4.1%. Median and mode were close to the average (55% and 54%, respectively).

Table 6. Central values and dispersion indices for beef traits at slaughter for steers (n=653) / *Valorile centrale și indicii dispersiei pentru însușirile producției de carne la sacrificare la boi (n=653)*

Beef indices	Mean±SEM	Median	Mode	SD	VC (%)	Minimum	Maximum
Age at slaughtering (months)	19.83 ± 0.11	19.70	18.50	2.82	14.21	12.20	31.00
Liveweight (kg)	625.08 ± 1.34	628.00	620.00	34.34	5.49	388.00	720.00
Carcass weight (kg)	342.83 ± 0.79	344.00	346.00	20.13	5.87	200.00	417.00
Left half-carcass weight (kg)	172.01 ± 0.40	173.00	174.00	10.21	5.93	99.00	212.00
Right half-carcass weight (kg)	170.82 ± 0.40	171.00	170.00	10.10	5.91	101.00	205.00
Killing-out percentage (%)	54.88 ± 0.09	55.00	54.00	2.25	4.10	47.00	62.00
Conformation class	5.00 ± 0.01	5.00	5.00	0.27	5.31	2.00	6.00
Fat cover class	3.56 ± 0.02	4.00	4.00	0.53	14.90	2.00	5.00

In steers, conformation class had the same value for average, median and mode, namely 5, meaning the carcasses were included in the R class for conformation (see Table 1). Variability was low (VC=5.31%), individual values varying from 2 (P+) to 6 (U).

Carcass fat cover class was, on average, 3.56, value that is halfway between average and high coverage (Table 3). Both median and mode were 4 as value, indicating a high fat coverage of the carcass with fat. Individual values varied from 2 (slight) to 5 (very high), with a medium variability (VC=14.90%).

Results of testing the influence of animal gender on the beef indices of cattle at slaughtering are presented in Table 7.

Age at slaughtering of females was significantly 6 months higher ($p<0.001$) compared to males and steers. There was no significant difference between males and steers regarding this trait (0.45 months, $p>0.05$).

Liveweight at slaughter was significantly higher in steers compared cu males (by 9%, $p<0.001$) and females (by 11%, $p<0.001$). There was a small and non-significant difference between males and females regarding the liveweight at slaughter. Kirkland et al., 2006 showed that, sacrificed at 450 kg, steers had lower carcass gains and higher food conversion ration than bulls. Same authors found out that

steers had lower estimated carcass lean and higher estimated carcass fat concentrations than bulls slaughtered at the same liveweight.

The highest carcass weight was obtained from steers (342.83 kg), which was significantly higher compared to that obtained from males (by 7%, p<0.001) and females (by 14%, 0.001). Also, males had a 6.5% significantly higher (p<0.001) carcass weight than females (300.63 kg).

Steers had lower (*P*<0·001) estimated carcass lean, and higher (*P*<0·01) estimated carcass fat concentrations than bulls slaughtered at the same LW.

Table 7. Differences and their statistical significance for beef traits at slaughter in cattle according to the animal gender / *Diferențele și semnificația statistică a acestora pentru indicii producției de carne la sacrificare la taurine în raport cu sexul animalului*

Beef indices	Averages			Differences and significance		
	Males (M)	Females (F)	Steers (S)	M vs. F	M vs. S	F vs. S
Age at slaughtering (months)	19.38	25.91	19.83	-6.53***	-0.45^{ns}	6.08***
Liveweight (kg)	573.24	564.18	625.08	9.06^{ns}	-51.84***	-60.90***
Carcass weight (kg)	320.20	300.63	342.83	19.57***	-22.63***	-42.20***
Left half-carcass weight (kg)	160.46	150.72	172.01	9.74***	-11.55***	-21.29***
Right half-carcass weight (kg)	159.74	149.91	170.82	9.83***	-11.08***	-20.91***
Killing-out percentage (%)	55.86	53.29	54.88	2.57***	0.98***	-1.59***
Conformation class	4.95	4.51	5.00	0.44***	-0.05^{ns}	-0.49***
Fat cover class	2.78	3.37	3.56	-0.59***	-0.78***	-0.19*

ns - non-significant p>0.05; * - significant p<0.05; ** - distinctly significant p<0.01; *** - very significant p<0.001

The highest value for killing-out percentage was obtained in males (55.86%), followed by steers (54.88%) and females (53.29%). These animal gender differences were statistically significant (p<0.001). Tagliapietra et al., 2018 showed that females calves obtained from different dams and Belgian Blue sires exhibited a very similar carcass conformation compared to males.

Males and steers had a significantly higher (p<0.001) conformation class for carcass than females (4.95 and 5 compared to 4.51, respectively). Thus, males and steers had carcasses classified as R while females were classified between R and O+ (Table 1).

The lowest fat cover of the carcass was observed in males (2.78), that was significantly lower (p<0.001) than in females and steers (3.37 and 3.56, respectively). Also, steers had a significantly higher (p<0.05) fat cover of the carcass than females. Tagliapietra et al., 2018 reported a higher fatness score in heifers than in males at slaughter.

Dimas et al., 2021 obtained higher liveweight at slaughter and carcass weight in steers compared to heifers, while killing-out percentage was the same irrespective of animal gender. Oliveira Cardoso et al., 2014 showed than when fed high grain diets, cattle males and females performed similarly, however females had a better fat coverage.

Mueller et al., 2019 found that heifers had increased fat thickness compared to males and steers, while steers and heifers had higher marbling scores than males.
Sadrettin et al., 2024 found that slaughter traits, carcass characteristics and carcass measurements were affected by sex when compared cattle of different genotypes.

CONCLUSIONS

1. Liveweight at slaughtering of females was similar to those of males, due to the fact that they were 6 months older.
2. Steers were slaughtered at similar age with males, but with higher liveweight. Also, steers had higher liveweight at slaughter than females.
3. Combination of age and liveweight at slaughtering resulted in significant differences between animal gender regarding carcass weight and killing-out percentage.

4. Males and steers produced carcasses classified in class R for conformation, while females' carcasses were classified lower, between R and O+.
5. Animal gender had an effect on fat cover of carcass. The highest fat cover of carcasses was obtained in steers, followed by females and the lowest was observed in males.
6. In conclusion all studied beef traits at slaughtering were significantly influenced by animal gender.

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REPRODUCTIVE INDICES OF COWS FROM INDIGENOUS BREEDS EXPLOITED AT RDSB TARGU MURES

INDICII DE REPRODUCȚIE A VACILOR DIN RASELE AUTOHTONE EXPLOATATE LA SCDCB TÂRGU MUREȘ

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Abstract

Milk and beef represent an important source of protein and essential nutrients worldwide, with a constant demand in both the food industry and international trade. Raising cattle for milk/meat plays an essential role in Romania's agricultural economy, and the efficient use of local resources is crucial for the sustainability of production. In farms for the exploitation of mixed-breed cattle, reproduction plays a special role in the efficiency of milk and meat production; this is achieved by obtaining female and male offspring that will ensure the replacement of reforms and the obtaining of new breeders; and the surplus provides products intended for fattening. The objective of this study was to present the reproduction indices, carried out on the population of cows from RDSB Targu Mures, from the ***Romanian Spotted****,* ***Brown of Maramures*** *and* ***Pinzgauer of Transylvania*** *breeds raised in purebred form.*

Keywords: cattle, indigenous breeds, reproduction

Rezumat

Laptele și carnea de vită reprezintă o sursă importantă de proteine și nutrienți esențiali la nivel mondial, având o cerere constantă atât în industria alimentară, cât și în comerțul internațional. Creșterea bovinelor pentru lapte/carne joacă un rol esențial în economia agricolă a României, iar utilizarea eficientă a resurselor locale este crucială pentru sustenabilitatea producției. În fermele pentru exploatarea taurinelor de rasă mixtă, reproducția are un rol deosebit în eficiența producției de lapte, carne; aceasta se realizează prin obținere de descendenți femeli și masculi care vor asigura înlocuirea reformelor și obținerea de noi reproducători; iar surplusul asigură produșii destinați îngrășării. Obiectivul acestui studiu a fost prezentarea indicilor de reproducție, efectuâdu-se pe populația de vaci de la SCDCB Târgu Mureș, din rasele ***Bălțată Românească****,* ***Brună de Maramureș*** *și* ***Pinzgauer de Transilvania*** *crescute în rasă curată.*

Cuvinte cheie: bovine, rase autohtone, reproducție

INTRODUCTION

Reproduction plays a special role in the perpetuation, multiplication of cattle and a special role in the production of meat and milk. The act of calving is the promoter of milk production but without calving we cannot talk about meat production either.

The potential reproductive capacity in taurine is the biological ability of cows to give birth to a product within a period of time "year and calf ".

Reproduction indicators express in absolute or relative values the level of reproductive activity and with the help of these indicators the reproductive activity is assessed, these values can be compared with established optimal values.

By estimating the value of reproduction indices, the reproduction process on a farm can be managed and monitored, and depending on their level, interventions can be made to eliminate technological deficiencies.

Reproduction in cattle differs greatly from the reproduction of other domestic species, sheep, goats, pigs, the multiplication and numerical growth of the cattle species being much slower than other species of zootechnical interest:

- They are monotocous animals – they give birth to a single product, and very rarely twins are born.
- Sexual maturity differs from a number of factors, but ranges between 12–20 months.
- The gestation period is 280-290 days.
- The generation gap is large.

Since milk production depends on a calving, but meat production is directly influenced by calving, the ideal situation at cattles is one calf a year, with an interval between two calvings of 365 days.

This paper aimed to analyze reproductive indices, providing a comprehensive perspective on the factors that influence fertility and reproductive performance at indigenous breeds.

MATERIALS AND METHODS

The study was conducted on the cow population at the Research and Development Station for Bovine in Târgu Mureș, and the reproductive indices were calculated for the **Romanian Spotted**, **Brown of Maramures** and **Pinzgauer of Transylvania** breeds.

Reproduction at the Research and Development Station for Bovine is ensured through artificial insemination using frozen semen. The improvement of the cow populations is carried out through purebred breeding.

The data processed in this activity were recorded for the years 2023–2024 and grouped by primiparous and multiparous cows for the three breeds.

Reproductive indices were calculated for the entire cow population and expressed as averages for the three indigenous breeds: **Romanian Spotted**, **Brown of Maramures** and **Pinzgauer of Transylvania**.

The data were collected from the farm's records, including: date of mating, date of calving, date of weaning, number of inseminations per gestation, and number of semen doses used per gestation.

By processing the aforementioned data, the calculated values were obtained – the reproductive indices, which are used to assess reproductive performance on the farm.

By comparing these indices with their respective optimal values, the performance level of the farm can be identified, and technological deficiencies can be addressed.

The calculated reproductive indices are:

- Insemination index
- Conception rate
- Service period
- Calving interval
- Gestation duration
- Dry period duration
- Age at first calving

Insemination index – represents the average number of inseminations performed for one gestation and is calculated by dividing the total number of inseminations by the number of pregnant cows. This value is considered very good when it is around 2.

Conception rate – represents the percentage of cows that became pregnant after the first insemination.

The normal value for this index is between 50–70%, and it is influenced by many technological and farm management factors such as: body condition, feeding level, milk production, microclimate

(temperature), reproduction management (monitoring calving, estrous cycle, optimal insemination timing), inseminator skill level, semen fertility, and the reproductive health of the cow.
If the conception rate as a reproductive index is within normal parameters, it positively influences other indices, as the aforementioned factors are correctly managed and controlled.

Service period – the period of uterine rest, expressed in days, between calving and the date of successful insemination (establishment of a new pregnancy).

Optimal values for this index range between 80–120 days. Any deviation above these values negatively impacts milk and meat production, requiring corrective measures to shorten the uterine rest period. Among the many influencing factors are: high milk production (which prolongs the time to conception), calf-at-foot management in beef production (delays estrus), and late return to estrus in lactating cows.

Calving interval – the interval between two successive calvings. It is calculated only for multiparous cows (those that have calved at least twice), either as the time in days between two calvings or by summing the gestation duration and the service period.
The optimal value for this index is under 400 days.

Gestation duration is considered the period from fertilization to parturition (calving). The average gestation length in cows is 285 days. However, many factors can influence this index in one direction or another. Calving is not considered pathological if it occurs between day 240 and day 311.

Weaning duration is a technological indicator and is set at 60 days before the estimated calving date. It can be shortened to 50 days with balanced feeding, or extended to 70–80 days in cases of poor nutrition or record-producing animals.

Age at first calving depends on the breed (precocity) and represents the period during which the animal does not produce. It is desirable to reduce this period without negatively affecting future production. It is calculated as the interval between the date of birth and the date of the first calving.

RESULTS AND OBSERVATIONS

The potential reproductive capacity in cattle is the biological ability of cows to give birth to a product within a period of time, the "year and calf".

Reproduction indices are expressed in absolute or relative values. With these indices we assess the reproductive activity of a population. The values obtained can be compared with the optimal values established as a result of zootechnical research activity.

The reproduction indices were studied and calculated across the entire cow population of the Research Station for the three native breeds: **Romanian Spotted** 200 heads of cattle, **Brown of Maramures** 80 heads of cattle and **Pinzgauer of Transylvania** 40 heads of cattle.

The study was conducted on groups of primiparous and multiparous cows during the period from October 1, 2023, to September 30, 2024, involving the three breeds raised at the Research Station.

The results obtained are presented in the table nr. 1, for each breed separately.

Table 1. Reproduction indices / *Indicii de reproducție*

Specification	Breed		
	Romanian Spotted 200 heads of cattle	Brown of Maramures 80 heads of cattle	Pinzgauer of Transylvania 40 heads of cattle
Age at first calving (months)	31.70	30.20	33.00
Gestation duration (days)	284.00	283.00	284.00
Duration of breast rest (days)	60.00	65.00	89.00
Service period (days)	124.00	143.00	131.00
Calving interval (days)	408.00	426.00	415.00
Seeding index (doses)	1.63	1.84	1.76
Conception rate (%)	63.0	65.0	60.0

The insemination index, namely the number of semen doses used per gestation, is very similar across the breeds: 1.63 for **Romanian Spotted** (B.R.), 1.84 for **Brown of Maramures** (B.M.), and 1.76 for **Pinzgauer of Transylvania** (P.T.).

The conception rate (natality) is 63% for B.R., 65% for B.M., and 60% for P.T.

The calving interval exceeds 400 days in all three breeds: 408 days for B.R., 415 days for B.M., and 426 days for P.T.; this index is correlated with the service period duration, which averages 132 days.

The age at first calving exceeds 30 months in all cases: 31.7 months for B.R., 30.2 months for B.M., and 33 months for P.T. Among the three breeds, **Brown of Maramures** is the most precocious.

According to the data presented in Table 1, the dry period for the indigenous breeds is 60 days for B.R., 65 days for B.M., and 89 days for P.T., due to the latter's shorter lactation period of 230 days compared to 305 days for the other two breeds.



Fig. 1 Romanian Spotted



Fig. 2 Brown of Maramures



Fig. 3 Pinzgauer of Transylvania

CONCLUSIONS

1. The conception rate for the three breeds is very similar, ranging between 60–65%, and the number of inseminations per gestation varies between 1.63 and 1.84.
2. The gestation duration is nearly identical for the three indigenous breeds: Romanian Spotted, Brown of Maramures, and Pinzgauer of Transylvania.
3. The calving interval varies proportionally with the length of the service period, which represents the reproductive inactivity phase.
4. The age at first calving is a reproductive index that falls within the range found in the literature regarding the precocity of mixed-purpose breeds.
5. The dry period duration is a technological indicator, influenced to a small extent by genetic factors.

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EFFECTS OF USING CANNABIS OIL (CBD) ON THE METABOLIC PROFILE OF CALVES DURING WEANNING

EFECTELE UTILIZĂRII ULEIULUI DE CANABIS (CBD) ASUPRA PROFILULUI METABOLIC LA VIȚEI ÎN PERIOADA ÎNȚĂRCĂRII

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Abstract

Cannabis oil is a product obtained from hemp plants and seeds (Cannabis sativa), with anti-inflammatory, analgesic, anxiolytic and anticonvulsant properties due to its high content of cannabidiol (CBD). The aim of this research was to evaluate the effects of CBD on haemato-biochemical profile in dairy calves during weaning. The study was conducted at the Experimental Farm of the Research and Development Institute for Bovine Balotesti, between March-June 2024, using 19 half-sibs ***Romanian Black and White*** *dairy calves, divided into two groups: the experimental group (E: n=10) and the control group (C: n=9). The experimental group received 5 ml of CBD oil/head/day in the first 2 days of experiment (pre-weaning) and 10 ml CBD oil/head/day for a period of 3 consecutive days post-weaning. Blood samples were collected during the weaning day (T_1), at 2 days (T_2) and 5 days (T_3) post-weaning. Mann-Whitney test was used to evaluate the influence of CBD on the blood parameters. The results showed significant differences for the mean corpuscular volume (MCV: p=0.0164) and mean corpuscular hemoglobin (MCH: p=0.0057) in the CBD group compared with the controls at T_1, and significant differences for platelets (PLT: p=0.0050) and alanine-aminotransferase (ALT: p=0.0328) in the CBD group compared to the control group at T_2. At 5 days post-weaning (T_3), the mean corpuscular hemoglobin (MCH: p=0.0018) differed significantly between calves' groups. The obtained results suggest that CBD addition in dairy calves' diets could lead to improvements in the metabolic profile, and as a result, to reducing the negative effects of weaning stress.*

Key word: weaning stress, dairy calves, cannabis oil, metabolic profile.

Rezumat

Uleiul de canabis este un produs obținut din plante și semințe de cânepă (Cannabis sativa), având proprietăți antiinflamatorii, analgezice, anxiolitice și anticonvulsive datorită conținutul ridicat de canabidiol (CBD). Studiul a fost realizat în Laboratorul Experimental Zootehnic al Institutului de Cercetare-Dezvoltare pentru Creșterea Bovinelor Balotești, în perioada martie-iunie 2024, pe un număr de 19 viței din rasa ***Bălțată cu Negru Românească*** *(loturi de semi-frați), grupați în două loturi: lotul experimental (E: n=10) și lotul de control (C: n=9). Lotul E a primit 5 ml ulei CBD/cap/zi în primele 2 zile ale experimentului (pre-înțărcare) și 10 ml ulei CBD/cap/zi timp de 3 zile post-înțărcare. Probele de sânge au fost recoltate în ziua înțărcării (T_1), la 2 zile (T_2) și la 5 zile (T_3) post-înțărcare. Pentru a evalua influența CBD-ului asupra parametrilor sanguini studiați, a fost utilizat testul Mann-Whitney. Rezultatele obținute au relevat diferențe semnificative pentru volumul eritrocitar mediu (VEM: p=0.0164) și hemoglobina eritrocitară medie (HEM: p=0.0057) între loturi, precum și diferențe semnificative pentru trombocite (PLT: p=0.0050) și alanin-aminotranzferaza (GPT: p=0.0328), la două zile post-înțărcare. La 5 zile după înțărcare, hemoglobina eritrocitară medie (HEM: p=0.0018) a înregistrat diferențe semnificative între cele două loturi. În concluzie, rezultatele obținute indică faptul că adăugarea uleiului de canabis în dieta vițeilor poate optimiza anumiți parametri sanguini, în vederea reducerii stresului de înțărcare.*

Cuvinte cheie: stres de înțărcare, viței, ulei de canabis, profil metabolic.

INTRODUCTION

The medical use of cannabis and cannabinoids in humans has increased interest in utilizing them as feed additives for livestock (Kleinhenz et al., 2020). Cannabidiol (CBD) oil, derived from hemp plants and seeds (*Cannabis sativa*), is recognized for its therapeutic properties, including anti-inflammatory, analgesic, anxiolytic, and anticonvulsant properties due to its high content of cannabidiol (Fallahi et al., 2022; Meyer et al., 2022). Authorized hemp varieties registered in the EU's Common Catalogue of Agricultural Plant Species must contain psychotropic delta 9-tetrahydrocannabinol (THC) levels below 0.2–0.3% (Bailoni et al., 2021), adhering to limits of <0.2% THC on a dry matter basis, ensuring safety and compliance (EPSA, 2011; EU Commission Regulation, 2017; EU Commission Regulation, 2023). Cannabinoids, including THC (tetrahydrocannabinol), CBD (canabidiol), cannabichromene (CBC), and cannabigerol (CBG), are plant-derived compounds (phytocannabinoids) synthesized within glandular trichomes of *Cannabis sativa* plants (Farinon et al., 2020; Kicman and Toczek, 2020). THC is the only psychoactive compound, whereas CBD exhibits non-psychoactive therapeutic effects (Atalay et al., 2020).

Hemp seeds and derivatives such as oil, cake and meal, are valuable supplements in ruminant diets, providing essential fatty acids and amino acids (Klir et al., 2019; Semwogerere et al., 2020). Including whole seeds and their co-products can transfer bioactive substances to human food. Incorporating these components into animal feed enhances the fatty acid profile of milk and cheese, increasing n-3 fatty acids and conjugated linoleic acid (Bailoni et al., 2021). Cannabidiol (CBD) oil shows therapeutic potential in reducing stress during weaning and transportation in calves (Austin, 2022), which are critical stress events affecting calves' immune function, growth, and overall performance (Lynch et al., 2019; Meyer, 2022).

This study aims to evaluate the impact of CBD oil supplementation on the haemato-biochemical profile of dairy calves during weaning, highlighting its potential to improve physiological responses to stress.

MATERIALS AND METHODS

All experimental procedures were performed in accordance with the practices and standards approved by Romanian Law no. 43/2014 and the Council Directive 2010/63/EU.

The study was conducted at the Experimental Farm of the Research and Development Institute for Bovine Balotesti, between March-June 2024, using 19 half-sibs **Romanian Black and White** dairy calves, divided into two groups: the experimental group (E: n=10 head/group) and the control group (C: n=9 head/group). The experimental group received 5 ml of CBD oil/head/day in the first 2 days of the experiment (pre-weaning) and 10 ml CBD oil/head/day for 3 consecutive days post-weaning. The cannabis oil was purchased from certified suppliers, with a total cannabinoid content of 1350 mg/100ml.

The calves were weaned at an average age of 90±5 days. Prior to weaning, they were housed in individual hutches. Post-weaning, they were transitioned to group housing, accommodating 15-20 calves/group, which simulated typical social conditions. All calves were fed twice daily with a diet formulated to meet their nutritional requirements at this developmental stage. The diet/head/day consisted of 6 kg milk, quality alfalfa hay *ad libitum*, concentrates *ad libitum*, and water *ad libitum*.

Blood samples were collected from the jugular vein during the weaning day (T_1), at 2 days (T_2), and 5 days (T_3) post-weaning. The amount of blood was collected in vacutainer tubes with K_3EDTA for hemoleukogram (2 ml/tube, and chilled to +4 °C), and vacutainer tubes (9 ml/tube, centrifuged at 6000 rpm for 8 minutes for biochemical examination. Red blood cell (RBC), hemoglobin (HGB), hematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), total white blood cell (WBC), lymphocytes (LY),

monocytes (MO), and neutrophils (NE) were performed using an automated hematology analyzer (Abacus Junior Vet 5). Total protein (TPro), blood urea nitrogen (BUN), creatinine (Cre), and gamma-glutamyltransferase (GGT) were determined using an automated biochemical analyser (Spotchem EZ SP-4430). Statistical analyses were carried out using Minitab Statistical Software (version 17).

The data were expressed as mean±standard error of the mean (SEM), standard deviation (SD), coefficient of variation3 (CV), minimum and maximum values. To test the influence of cannabis oil on the blood parameters studied, the Mann-Whitney test (one-tailed hypothesis) was used. Differences were considered statistically significant at $p \leq 0.05$, and trends $p \leq 0.10$. The correlations between blood parameters across different weaning periods (T_1, T_2, T_3) were estimated using Pearson's correlation coefficient with a statistical significance threshold of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

The descriptive statistics of RBC, HGB, HTC, MCV, MCH, MCHC, and PLT are presented in Table 1. The obtained results showed a significant difference in mean corpuscular volume (MCV: p=0.0164) and mean corpuscular hemoglobin (MCH: p=0.0057) in the E group compared with the C group at T_1, and significant differences for mean corpuscular volume (MCV: p=0.0188), mean corpuscular hemoglobin (MCH: p=0.0232), and platelets (PLT: p=0.0050) in the E group compared to the C group at T_2.

These results suggest that CBD oil influences red blood cell size and haemoglobin content during weaning stress. Cannabinoids are known to modulate oxidative stress, which may impact erythrocyte morphology and oxygen transport efficiency (Radkowska and Herbut, 2014; Tathong et al., 2024). A tendency for platelet suggests that CBD oil may modulate coagulation and platelet activation during stress, as supported by studies on cannabinoid effects on thrombocyte function and reduce inflammatory responses (Atalay and Skrzydlewska, 2019; Kicman and Toczek, 2019).

Table 1. The effects of CBD oil diet addition on the haematological parameters in dairy calves during weaning /
Efectele administrării uleiului de CBD asupra parametrilor hematologici în perioada înțărcării la viței

Variable	Experimental group					Control group					p
	X±SEM	SD	CV	Min	Max	X±SEM	SD	CV	Min	Max	
RBC, T_1	9.60±0.45	1.43	14.90	6.65	12.32	9.72±0.36	1.08	11.13	7.66	11.25	0.3556
RBC, T_2	10.46±0.66	2.11	20.22	8.77	16.15	10.04± 0.20	0.61	6.08	8.84	10.79	0.4840
RBC, T_3	9.68±0.23	0.73	7.56	8.49	10.37	10.35± 0.21	0.63	6.12	9.56	11.47	**0.0951**
HGB, T_1	11.40±0.34	1.09	9.62	10.30	14.00	10.65±0.50	1.51	14.26	7.00	11.90	0.2451
HGB, T_2	11.17±0.22	0.69	6.22	10.10	12.40	10.80± 0.26	0.79	7.38	9.30	11.80	*0.1948*
HGB, T_3	11.00±0.15	0.48	4.41	10.20	11.70	11.01± 0.32	0.97	8.88	9.50	12.20	0.3556
HTC, T_1	30.82±1.42	4.49	14.57	20.38	37.65	30.37±1.11	3.32	10.93	23.10	32.99	0.3557
HTC, T_2	33.48±1.81	5.72	17.09	29.06	49.05	30.98± 0.78	2.34	7.55	26.76	33.71	*0.1949*
HTC, T_3	31.22±0.49	1.55	4.99	28.09	33.98	32.22± 0.51	1.53	4.76	29.51	34.43	0.7630
MCV, T_1	32.30±0.57	1.82	5.66	31.00	36.00	30.00± 0.33	1.00	3.33	29.00	32.00	**0.0016**
MCV, T_2	32.44±0.42	1.34	4.13	30.00	34.36	31.00± 0.44	1.32	4.27	30.00	33.00	**0.0188**
MCV, T_3	32.06±0.51	1.62	5.07	30.66	36.00	31.33± 0.28	0.86	2.76	30.00	33.00	0.2177
MCH, T_1	12.08±0.62	1.97	16.36	10.80	17.50	10.74± 0.21	0.63	5.91	9.20	11.40	**0.0057**
MCH, T_2	10.91±0.42	1.35	12.41	7.300	12.00	10.78±0.18	0.54	5.00	10.10	11.90	**0.0232**
MCH, T_3	11.57±0.18	0.57	4.97	10.90	12.40	10.72± 0.18	0.54	5.04	10.00	11.50	**0.0180**
MCHC, T_1	37.73±2.18	6.90	18.28	34.60	57.20	36.04±0.88	2.66	7.39	30.50	41.00	0.2709
MCHC, T_2	33.84±1.14	3.59	10.62	24.20	37.00	34.92±0.26	0.79	2.27	33.70	36.20	0.2327
MCHC, T_3	35.37±0.59	1.86	5.27	32.20	37.80	34.80±0.33	0.99	2.86	32.40	35.50	0.3557
PLT, T_1	383.3±36.2	114.6	29.89	191.0	575.0	450.9± 45.9	137.7	30.53	216.0	619.0	*0.1736*
3, T_2	374.0±52.6	166.3	44.47	139.0	697.0	526.4±21.9	65.6	12.45	409.0	607.0	**0.0050**
PLT, T_3	423.6±36.1	114.1	26.94	276.0	583.0	452.8±39.0	116.9	25.81	319.0	682.0	0.2980

RBC=red blood cells (10^3/µl), HGB=hemoglobin (g/dl), HTC=hematocrit (%), MCV=mean corpuscular volume (fl), MCH=mean corpuscular hemoglobin (pg), MCHC=mean corpuscular hemoglobin concentration (g/dl), PLT=platelet (10^3/µl); T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; Reference values – RBC: 5-8$10^3$/µl, HGB: 9-11 g/dl, HTC: 32-38 %, MCV: 40-60 fl, MCH: 11-17 pg, MCHC: 30-36 g/dl, PLT: 100-800 10^3/µl; $p \leq 0.05$, trends $p \leq 0.10$.

At T_3 intervals, mean corpuscular hemoglobin (MCH: p=0.0018) differed significantly between groups. A tendency for red blood cell (RBC: p=0.0951) at T_3 between groups was also recorded. This indicates potential long-term effects of CBD oil on erythropoiesis, aligning with findings on cannabinoid effects on hematopoiesis (Neary et al., 2024).

For hemoglobin (HGB), hematocrit (HTC), and mean corpuscular hemoglobin concentration (MCHC), no significant differences were observed for these parameters across T_1, T_2, and T_3 ($p>0.05$), showing that CBD oil did not affect these haematological indicators between the studied groups. The obtained values have been found to be in contrast with values obtained by Austin (2022), who reported no statistically significant differences for these haematological parameters studied in 27 beef calves during weaning stress.

The descriptive statistics for white blood cells (WBC), lymphocytes (LY), monocytes (MO), and neutrophils (NE) are summarized in Table 2. At T_2 (2 days post-weaning), the E group showed significantly lower variability (CV of 15.66 %) and a lower mean WBC count compared to the control group (10.33±0.51 10^3/µl vs. 11.83±0.62 10^3/µl; p = 0.0050). This suggests that CBD may stabilize immune responses during stress periods, aligning with findings that cannabinoids modulate immune function (Tathong, 2024).

Across T_1, T_2, and T_3 intervals, no statistically significant differences between the experimental and control groups ($p>0.05$) were observed for LY, MO, and NE. However, trends in MO percentages suggest that cannabinoids may suppress monocyte activation during stress (Cabral et al., 2015; Maggirwar and Khalsa, 2021).

In contrast, Austin (2022) reported significant differences for monocyte count ($p<0.0001$) in a pilot study that investigated 27 beef calves during weaning stress.

Table 2. The effects of CBD oil diet addition on the withe blood cell and leucocytes formula in dairy calves during weaning / ***Efectele administrării uleiului de CBD asupra leucogramei în perioada îngrășării la viței***

Variable	Experimental group					Control group					p
	X±SEM	SD	CV	Min	Max	X±SEM	SD	CV	Min	Max	
WBC, T_1	9.79±0.73	2.33	23.80	7.30	15.56	10.96± 1.39	4.17	38.06	2.96	16.78	*0.1736*
WBC, T_2	10.33±0.51	1.61	15.66	6.77	13.45	11.83±0.62	1.87	15.83	9.320	14.62	**0.0050**
WBC, T_3	10.38±0.38	1.22	11.76	8.68	12.53	11.10±0.69	2.07	18.66	8.650	14.45	0.2980
LY, T_1	70.42±1.99	6.31	8.96	58.70	77.70	68.80±3.88	11.65	16.94	53.50	83.80	0.4840
LY, T_2	67.73±2.28	7.22	10.66	55.90	81.00	64.00±3.04	9.13	14.26	46.20	76.80	0.2709
LY, T_3	63.62±1.77	5.60	8.81	52.60	72.10	63.94±3.49	10.48	16.38	44.20	78.90	0.4524
MO, T_1	2.31±0.88	2.80	121.37	0.60	9.90	1.48±0.48	1.45	97.93	0.50	5.20	0.2578
MO, T_2	1.92±0.440	1.39	72.54	0.80	5.10	1.61±0.36	1.08	67.17	0.60	3.50	0.2061
MO, T_3	1.86±0.40	1.27	68.30	0.50	3.80	2.65±0.93	2.81	105.99	0.50	9.70	0.3263
NE, T_1	27.30±2.34	7.41	27.16	16.10	40.30	29.87±4.00	12.01	40.22	11.00	44.40	0.3859
NE, T_2	30.36±2.17	6.86	22.60	17.80	43.10	34.39±3.05	9.15	26.61	21.30	52.90	*0.1736*
NE, T_3	34.51±1.96	6.21	18.00	25.30	46.70	33.43±2.78	8.35	24.96	20.60	46.10	0.4840

WBC=total white blood cells (10^3/µl), LY=lymphocytes (%), MO=monocytes (%), NE=neutrophils (%); T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; Reference values – WBC: 6.5-9.5 10^3/µl, LY: 45-61 %, MO: 0-4 %, NE: 15-41 %; $p \leq 0.05$, trends $p \leq 0.10$.

The descriptive statistics of serum biochemical parameters are presented in Table 3. Serum biochemical analysis found a tendency for gamma-glutamyltransferase (GGT) levels at T_2 and T_3 (p=0.0822 and p=0.0764), suggesting potential liver function modulation by CBD oil. Cannabinoids are known to influence hepatobiliary function and reduce oxidative stress in liver tissues (Mboumba Bouassa et al., 2021; Hassan et al., 2023; Huang et al., 2025). No significant differences were observed between experimental and control groups ($p>0.05$), however, trends in BUN levels suggest improved nitrogen metabolism in the experimental group.

Table 3. The effects of CBD oil diet addition on the biochemical parameters in dairy calves during weaning / *Efectele administrării uleiului de CBD asupra parametrilor biochimici în perioada înțărcării la viței*

Variable	Experimental group					Control group					p
	X±SEM	SD	CV	Min	Max	X±SEM	SD	CV	Min	Max	
TPro, T_1	6.16±0.11	0.35	5.69	5.70	6.90	6.26±0.14	0.42	6.72	5.70	6.90	0.2980
TPro, T_2	6.19±0.14	0.45	7.40	5.20	6.70	6.13±0.10	0.30	5.03	5.70	6.70	*0.1948*
TPro, T_3	6.08±0.10	0.31	5.25	5.60	6.60	6.211±0.05	0.16	2.72	6.00	6.50	*0.1356*
BUN, T_1	11.20±0.61	1.93	17.25	8.00	14.00	10.55±0.55	1.66	15.79	9.00	13.00	0.2177
BUN, T_2	10.40±0.37	1.17	11.29	9.00	13.00	10.33±0.62	1.87	18.10	7.00	13.00	0.4840
BUN, T_3	9.50±0.76	2.41	25.42	6.00	15.00	10.66±0.86	2.59	24.36	8.00	15.00	*0.1840*
Cre, T_1	3.28±1.63	5.15	156.87	0.70	14.00	0.82±0.03	0.09	11.82	0.60	0.90	*0.1538*
Cre, T_2	2.78±1.29	4.08	146.61	0.80	11.00	0.82±0.03	0.10	13.29	0.60	1.00	*0.1190*
Cre, T_3	2.26±0.95	3.02	133.87	0.80	8.00	0.87±0.03	0.09	11.07	0.80	1.10	0.4207
GGT, T_1	30.40±3.51	11.11	36.54	22.00	60.00	30.22±1.35	4.06	13.42	25.00	37.00	*0.1948*
GGT, T_2	30.40±3.94	12.45	40.94	20.00	62.00	30.22±1.15	3.46	11.44	26.00	36.00	**0.0822**
GGT, T_3	28.60±3.42	10.82	37.85	18.00	54.00	29.22±0.83	2.48	8.52	25.00	32.00	**0.0764**

TPro=total protein (g/dL), BUN=blood urea nitrogen (mg/dL), Cre=creatinine (mg/dL), GGT=gama-glutamyltransferase (IU/L); T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; Reference values –TPro: 5.8-8.5 g/dl, BUN: 10-25 mg/dl, Cre: 0.4-2.1 mg/dl, GGT: 10-39 IU/L; $p \leq 0.05$, trends $p \leq 0.10$.

Literature review indicates that cannabinoids can influence protein metabolism and nitrogen excretion (Landi et al., 2018; Saloner and Bernstein, 2022). Creatinine levels in the E group at T_1 and T_2 may reflect muscle metabolism adaptations during stress. Studies suggest that cannabinoids can modulate renal function and creatinine clearance (Di Salvo et al., 2024; Młynarska et al., 2024). In general, the experimental group showed more stability in serum biochemical indicators compared with the control group, suggesting better metabolic adaptation.

Table 4, presents Pearson correlation coefficients, showing the relationship between TPro levels and WBC count, LY, MO, and NE, across T_1, T_2, and T_3 intervals for the experimental group and the control group.

Total protein levels, which reflect nutritional and immune status, may correlate with WBC counts and leukocyte distribution. Higher total protein could indicate an immune response or inflammation, which is reflected in changes in leukocyte formula (neutrophils or lymphocytes).

For the E group, moderate positive correlations between TPro vs. MO at T_2 (r=0.558, p=0.094), as well as moderate negative correlations between TPro vs. WBC (r=-0.529, p=0.116), and TPro vs. NE (r=-0.532, p=0.113) at T_2 were found, suggesting potential relationships, even they are not statistically significant.

Table 4. Pearson correlation coefficient between WBC and leucocytes formula vs. TPro in dairy calves across T_1, T_2, and T_3 point time / *Coeficientul de corelație Pearson între WBC și formula leucocitară vs. TPro la viței*

Blood indicators	TPro		TPro		TPro	
	T_1		T_2		T_3	
	Experimental group					
	r	p	r	p	r	p
WBC	*-0.014*	0.970	-0.529	*0.116*	-0.345	0.329
LY	*0.049*	0.894	0.401	0.251	0.034	0.926
MO	0.273	0.445	0.558	0.094	-0.035	0.923
NE	-0.147	0.685	-0.532	*0.113*	-0.024	0.948
Control group						
WBC	-0.130	0.738	-0.006	0.987	-0.095	0.807
LY	-0.279	0.468	0.004	0.991	0.609	*0.082*
MO	0.083	0.832	0.542	*0.132*	-0.490	*0.181*
NE	0.154	0.693	-0.070	0.857	-0.598	*0.089*

WBC=total white blood cells, LY=lymphocytes, MO=monocytes, NE=neutrophils, TPro=total protein; T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; r=Pearson correlation coefficient; p-values.

For the C group, the correlation between TPro and LY (r=0.609, p=0.082) at T_3 indicated a potential link between total protein levels and lymphocyte activity under baseline conditions. These

results highlight the interaction between total protein levels and immune indicators, particularly under stress or experimental conditions.

Blood urea nitrogen (BUN) is a critical marker of protein metabolism and renal function, and its correlation with hemoleucogram parameters like hemoglobin (HGB) levels and red blood cell (RBC) counts provides valuable information about metabolic efficiency and oxygen transport. Higher BUN levels may indicate excessive protein intake or inefficient utilization, which can impact these haematological parameters.

The Pearson correlation coefficients between BUN vs. RBC and HGB levels are summarised in Table 5.

For the E group, a positive moderate correlation between BUN and RBC at T_2 (r=0.601, p=0.066) suggests that BUN may influence RBC dynamics during this period, potentially reflecting adaptive metabolic responses. Additionally, the moderate positive correlation between BUN and HGB at T_3 (r=0.542, p=0.102) indicates some influence on hemoglobin levels, highlighting the connection between protein metabolism and oxygen transport.

For the C group, moderate negative correlations of BUN with RBC at T_2 (r=-0.509, p=0.162) and T_3 (r =-0.551, p=0.124) suggest a trend of inverse effects on red blood cell dynamics, possibly reflecting differences in protein utilization efficiency under baseline conditions.

Table 5. Pearson correlation coefficient between RBC and HGB vs. BUN in dairy calves across T_1, T_2, and T_3 point time / *Coeficientul de corelație Pearson între RBC și HGB vs. BUN la viței*

Blood indicators	BUN		BUN		BUN	
	T_1		T_2		T_3	
	Experimental group					
	r	p	r	p	r	p
RBC	- 0.218	0.545	0.601	*0.066*	- 0.041	0.911
HGB	0.073	0.840	0.044	0.905	0.542	*0.102*
Control group						
RBC	0.075	0.847	- 0.509	*0.162*	- 0.551	*0.124*
HGB	- 0.048	0.902	- 0.184	0.635	- 0.387	0.303

RBC=red blood cells, HGB=hemoglobin, BUN=blood urea nitrogen; T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; r=Pearson correlation coefficient; p-values.

Table 6 illustrates the relationship between Cre levels and WBC count, LY, MO, and NE. Creatinine may correlate with WBC counts and leukocyte formula, especially under stress or metabolic strain. Higher creatinine levels could indicate renal stress, which might also affect immune function.

Table 6. Pearson correlation coefficient between WBC and leucocytes formula vs. Cre in dairy calves across T_1, T_2, and T_3 point time / *Coeficientul de corelație Pearson între WBC și formula leucocitară vs. Cre la viței*

Blood indicators	Cre		Cre		Cre	
	T_1		T_2		T_3	
	Experimental group					
	r	p	r	p	r	p
WBC	0.550	*0.099*	0.518	*0.125*	0.324	0.361
LY	- 0.509	*0.133*	- 0.479	0.161	0.125	0.730
MO	- 0.279	0.435	- 0.380	0.279	- 0.481	0.159
NE	0.536	*0.110*	0.577	*0.081*	- 0.014	0.970
Control group						
WBC	0.566	*0.112*	- 0.233	0.547	-0.312	0.414
LY	- 0.248	0.519	0.127	0.746	0.180	0.642
MO	0.355	0.349	0.235	0.543	0.463	0.210
NE	0.211	0.585	**0.020**	0.959	0.643	*0.062*

WBC=total white blood cells, LY=lymphocytes, MO=monocytes, NE=neutrophils, Cre=creatinine; T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; r=Pearson correlation coefficient; p-values.

Creatinine levels show moderate positive correlations with WBC (r=0.550, p=0.099) and NE (r=0.536, p=0.110) at T_1 and T_2 (WBC: r=0.518, p=0.125; NE: r=0.577, p=0.081), suggesting potential links between renal function and immune activity during stress periods for the experimental group.

In contrast, the control group findings at T_3 highlight baseline immune activity, with neutrophils showing a moderate positive correlation with creatinine levels (r=0.643, p=0.062). These results underscore the complex relationship between renal function and immune activity, emphasizing the importance of monitoring creatinine levels in stress conditions.

Gamma-glutamyltransferase (GGT) often shows a correlation with red blood cell (RBC) and hemoglobin (HGB) levels (Table 7), as it reflects liver function and metabolic processes. GGT levels are an indicator of hepatic health. Changes in GGT levels may impact the synthesis and breakdown of hemoglobin in the liver, thereby influencing RBC and HGB levels. The liver plays a central role in iron storage and release, essential for RBC production and HGB synthesis. Elevated GGT levels can signal metabolic stress, which may directly or indirectly affect RBC and HGB values. These correlations suggest that GGT could serve as a marker for monitoring not just liver function but also the overall metabolic health, especially in contexts like disease, stress, or dietary interventions.

For the experimental group, RBC and GGT showed a strong negative correlation (r=-0.699) with statistical significance (p=0.025) at T_1, indicates a significant inverse relationship, suggesting metabolic stress affecting RBC dynamics. At T_2, a strong positive correlation (r=0.807) with high statistical significance (p=0.005) was found, highlights a significant direct relationship, reflecting adaptive changes in liver function and RBC levels under experimental conditions. HGB and GGT showed a moderate positive correlation (r=0.554), without statistically significant (p=0.096) at T_3, suggesting a potential relationship.

For the control group, RBC and GGT showed a moderate positive correlation (r=0.651), with statistical significance (p=0.058) and a moderate positive correlation (r=0.637) for HGB and GGT, without statistical significance (p=0.065) at T_1. Moderate positive correlations at T_1 for both RBC and HGB suggest potential baseline relationships between these parameters and liver function, even though statistical significance. These observations highlight how GGT might serve as a marker for liver function and its interplay with RBC and HGB levels under different conditions.

Table 7. Pearson correlation coefficient between RBC and HGB vs. GGT in dairy calves across T_1, T_2, and T_3 point time / *Coeficientul de corelație Pearson între RBC și HGB vs. GGT la viței*

Blood indicators	GGT		GGT		GGT	
	T_1		T_2		T_3	
	Experimental group					
	r	p	r	p	r	p
RBC	- 0.699	**0.025**	0.807	**0.005**	0.204	0.572
HGB	- 0.027	0.940	0.197	0.586	0.554	**0.096**
Control group						
RBC	0.651	**0.058**	- 0.187	0.630	- 0.149	0.701
HGB	0.637	**0.065**	- 0.132	0.736	- 0.171	0.661

RBC=red blood cells, HGB=hemoglobin, GGT= Gamma-glutamyltransferase; T_1=weaning day, T_2=2 days post-weaning, T_3=5 days post-weaning; r=Pearson correlation coefficient; p-values.

CONCLUSIONS

1. The obtained results suggest that CBD oil diet supplementation improves haematological and biochemical parameters stability in dairy calves during the weaning stress period, when both immune and metabolic markers are concerned.
2. Significant improvements in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), platelet (PLT), and total white blood cells (WBC) at specific time points (T_1, T_2, and T_3) highlight the

potential use of CBD oil as a natural and effective strategy to mitigate weaning stress and improve overall calf health during this critical transition phase.

3. Further research is necessary to explore the long-term effects of CBD oil supplementation, its impact on other physiological parameters, and its applicability across different dairy farming conditions. These future investigations could contribute to the understanding of CBD's effects and expand the benefits of CBD oil in livestock management for improving the overall animal health and welfare.

ACKNOWLEDGEMENTS

This study has received funding from the Research and Development Institute for Bovine Balotesti.

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MOLECULAR EPIDEMIOLOGICAL STUDY ON THE CIRCULATION OF PROTOZOAN *TOXOPLASMA GONDII* IN WILDLIFE FROM ROMANIA

STUDIU EPIDEMIOLOGIC MOLECULAR PRIVIND CIRCULAȚIA PROTOZOARULUI *TOXOPLASMA GONDII* ÎN FAUNA SĂLBATICĂ DIN ROMÂNIA

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Abstract

Toxoplasmosis is an important worldwide protozoan disease caused by coccidia belonging to the Toxoplasmidae family. The only species of the genus is Toxoplasma gondii*, a significant zoonotic parasite that can cause severe illness in immunocompromised humans. It parasitizes various organs and tissues in over 350 species of vertebrates, making it perhaps the most widespread protozoan in this regard. Wild animals play a significant role in the epidemiology of toxoplasmosis, particularly when contact with domestic animals is possible, through which humans can acquire infection with* T. gondii*. The aim of the study was to investigate the potential circulation of the protozoan* T. gondii *in wild hosts collected from various hunting grounds across eight counties in Romania. Tissue samples (heart, brain, liver, spleen, and muscle) from 96 animals, specifically 13 wild host species, were processed by artificial digestion with trypsin and analyzed by real-time PCR for parasite detection.* Toxoplasma gondii *was identified in 57.29% (55/96) of the examined wild animals. The present study provides important information on the circulation of* T. gondii *in Romania's wildlife and highlights the potential risk of human infection, especially in anthropized areas where domestic and wild animals may interact.*

Keywords: T. gondii, wildlife, Romania

Rezumat

Toxoplasmoza este o protozooză importantă la nivel mondial produsă de coccidii ce aparţin familiei Toxoplasmidae. Singura specie a genului este Toxoplasma gondii, un parazit zoonotic important care poate provoca boli severe la persoanele imunocompromise. Parazitează în diverse organe şi ţesuturi la peste 350 specii de vertebrate, fiind din acest punct de vedere, poate, cel mai răspândit protozoar. Animalele sălbatice joacă un rol important în epidemiologia toxoplasmozei, atunci când contactul cu animalele domestice este posibil, iar prin acestea, omul poate dobândi infecţia cu T. gondii*. Studiul a avut ca scop investigarea circulaţiei potenţiale a protozoarului* T. gondii *la gazde sălbatice colectate din diverse fonduri de vânătoare, situate în opt judeţe din România. Probe de ţesut (cord, tesut cerebral, ficat, splină şi musculatură) rezoltate de la 96 de animale, mai exact 13 gazde silvatice au fost efectuate prin digestie artificială cu tripsină şi analizate prin PCR în timp real pentru detectarea parazitului. Parazitul* T. gondii *a fost identificat la 57,29% (55/96) din animalele sălbatice examinate. Studiul prezent oferă informații importante despre circulația T. gondii în fauna sălbatică din România și subliniază riscul potențial de infecție umană, în special în zonele antropizate, unde animalele domestice și sălbatice pot interacționa.*

Cuvinte cheie: T. gondii, faună sălbatică, România

INTRODUCTION

The medical and veterinary significance of *T. gondii* has established this protozoan as one of the most extensively studied parasites. It is widely used as a model organism for studying apicomplexan cell biology due to several advantageous characteristics. *Toxoplasma gondii* is a protozoan of a size suitable for visualization under a light microscope, can be cultured in virtually any cell line, maintained indefinitely in mice and cell cultures, and consists of a single species capable of infecting all host types. Moreover, this parasite is highly amenable to genetic manipulation, with well-established protocols for classical genetics and high efficiency of experimental infections [11].

Toxoplasmosis is a systemic protozoan disease caused by coccidia belonging to the family Toxoplasmidae. The parasite infects various organs and tissues in more than 350 species of vertebrates, making it arguably the most widespread protozoan in this regard. The infection typically progresses asymptomatically in its definitive hosts - domestic and wild felids. However, in intermediate hosts, it can lead to abortions or even death. The resulting economic losses are significant, primarily due to reduced birth rates and abortions in economically important livestock species, particularly sheep and pigs. From a public health perspective, toxoplasmosis is of major importance, as it is one of the most critical zoonotic diseases, potentially causing congenital malformations or fetal loss [4, 12, 25].

Wild animals play an important role in the epidemiology of toxoplasmosis, particularly when contact with domestic animals is possible, thereby creating a potential route for human infection with *Toxoplasma gondii*.

Studies on the occurrence of toxoplasmosis in Romania indicate a seroprevalence (ELISA) ranging between 42.5% and 80.5% in cats [6, 14, 17], 6.5% in lambs slaughtered for Easter, and 65.7% in adult sheep. In pigs, infection rates range between 0.8% and 46.8% [28].

Research on human toxoplasmosis has been conducted by Coroiu Z. et al., 2009 [4]; Dubey J.P. et al., 2014 [10]; Mederle N. et al., 2008 [23]; Olariu T.R. et al., 2015 [25]; Radbea N. et al., 2006 [31], reporting a seroprevalence ranging from 10.3% to 59.5% in the human population.

Studies concerning the presence of *T. gondii* in wild animals in Romania remain scarce. Șuteu O. et al., 2014 [35], reported a seroprevalence of 6% in foxes, while in 2016, Morariu S. et al. [24] recorded a prevalence of 22.22% in European bison from the Armeniș-Plopu reserve.

Contact between necrophagous wild species (such as jackals, bears, and wild boars) and the carcasses of carnivores, including felids (e.g., the wildcat and lynx), occurs frequently and directly contributes to the non-trophic transmission of parasites in the wild. This aspect is particularly relevant in the study and analysis of diseases with zoonotic potential [13].

Considering the limited bibliographic data available in Romania and recognizing the role of wildlife in the life cycle of the protozoan, as ecological indicators in anthropogenic ecosystems and as sentinels for a broad range of zoonotic infections, the present study aimed to update current knowledge on the circulation of *T. gondii* in Romanian wildlife using real-time PCR (qPCR).

MATERIALS AND METHODS

The research was conducted over a two-year period on a total of 96 wild animals (jackal, fox, badger, wildcat, weasel, otter, pine marten, stone marten, European polecat, muskrat, wild rabbit, grey plover, and raccoon dog), originating from various hunting grounds across eight counties in Romania (Arad, Timiș, Bihor, Vâlcea, Olt, Ialomița, Iași, and Tulcea). The animals were either found dead (roadkill) or were legally hunted by the annual harvest quotas established by the Ministry of Environment, Waters and Forests. The hunting activities through which these wild animal specimens were collected were carried out in compliance with Law No. 407/2006 (regarding hunting and the protection of wildlife) [27] (Figure 1).



Figure 1. The map shows the counties where wild animals were collected / *Harta arată județele de unde au fost recoltate animalele sălbatice*

The study was conducted in collaboration with the École nationale vétérinaire d'Alfort (ENVA), France, specifically with the reference laboratory UMR BIPAR ANSES "Foodborne Parasites", under the coordination of Professor Radu Blaga and Dr. Sandra Thoumire.

The first step of the research involved the collection of tissue samples of heart, brain tissue, liver, spleen, and muscle - carried out at the Parasitology and Parasitic Diseases Clinic of the Faculty of Veterinary Medicine in Timisoara/ University of Life Sciences ”King Mihai I” from Timisoara.

The sample collection process included the following procedures:

- necropsy examination performed using standard necropsy techniques;
- skinning of the carcass;
- opening of the carcass;
- sampling of each tissue.

The tissue samples used in the present study (heart, brain tissue, liver, spleen, and muscle) are by the recommendations of national and international authors. Specifically, brain and lung samples collected from European beavers and wildcats [15]; brain tissue from hedgehogs [16]; cardiac tissue from small rodents, foxes, jackals, and wolves [18, 38]; heart, brain, liver, spleen, and lung tissues collected from black-backed jackal, African wildcat, and rusty-spotted genet [29]; brain samples from foxes [35].

Following the collection of tissue samples, trypsin artificial digestion and molecular analyses for *T. gondii* DNA were performed at the UMR BIPAR ANSES “Foodborne Parasites” reference laboratory, within ENVA, France.

Trypsin artificial digestion

A total of 200 grams of tissue comprising heart, brain, liver, spleen, and muscle from each jackal, fox, badger, wildcat, weasel, otter, pine marten, stone marten, European polecat, muskrat, wild rabbit, grey plover, and raccoon dog were blended and incubated (90 minutes at 37°C, 200 RPM on a shaking plate) in trypsin solution (Trypsin (1:250), powder, Gibco™, Scotland, final concentration of 4 g/L). The mixture was filtered through a double layer of gauze, transferred to 50 mL sterile centrifuge tubes, and centrifuged at 1800 g for 10 minutes. The formed pellet was washed twice of leftover trypsin using a saline solution (0,9% NaCl, Sigma-Aldrich, US). The weight of the final pellet was recorded and samples were stored at - 20°C until DNA extraction and subsequent qPCR analysis (Figures 2, 3).



Figure 2. The mixture was filtered through a double layer of gauze into 50 ml tubes / *Filtrarea amestecului printr-un strat dublu de tifon in tuburi de 50 ml*



Figure 3. The final result of the trypsin digestion / *Rezultatul final al digestiei cu tripsină*

Molecular analyses of T. gondii DNA

DNA was extracted from 80mg of digested pellet, using NucleoSpin DNA RapidLyse, Mini kit for rapid DNA purification (Macherey Nagel, France), according to the manufacturer's instructions. Extracted DNA was cleaned of PCR inhibitors, concentrated using ethanol precipitation protocol [41], and resuspended in 30µL of Milli-Q water. The detection and quantification of *T. gondii* DNA in each sample was performed in duplicate by amplification of a sequence within the 529 bp repetitive element, according to Opsteegh M. et al. (2010) [26] with minor modifications (25 µL total reaction mixture volume, 2X Premix Ex Taq™ (TakaraBio, Japan) and 5 µL of DNA as template) and competitive internal amplification control (CIAC) probe modification by Deng H. et al. (2021) [7], using LightCycler® 480 System 96-plate thermocycler (Roche, Germany) (Figure 4).

Genomic DNA from a suspension of 106 cultured RH-strain (Type I, ToxoDB Genotype #1) (ToxoDB, n.d.) was extracted using NucleoSpin DNA RapidLyse, Mini kit (Macherey-Nagel, France), according to the manufacturer's instructions. A standard curve was prepared with ten-fold serial dilutions of the genomic DNA, corresponding to 100 to 104 tachyzoites/µL [37]. Positive and negative controls, distilled H2O samples, and a standard curve were included in each qPCR run.

The qPCR results were analyzed using LightCycler® 480 System software, Version 1.2.9.11 (Roche, Switzerland) (Figure 5).



Figure 4. Molecular Biology Analyses / *Analize de biologie moleculară*

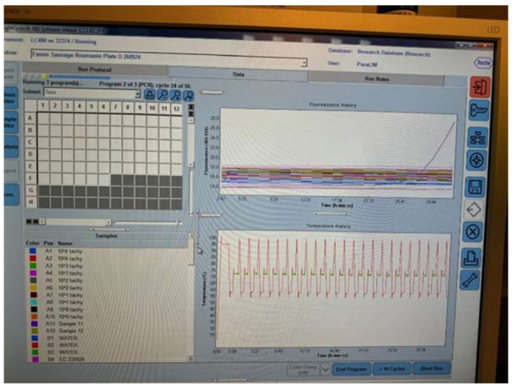


Figure 5. Software LightCycler® / *Software LightCycler®*

RESULTS AND DISCUSSION

From the collected tissues (heart, brain, liver, spleen, and skeletal muscle) originating from 13 wild host species (jackal, fox, badger, wildcat, weasel, otter, pine marten, stone marten, European polecat, muskrat, wild rabbit, grey plover, and raccoon dog) and analyzed using the real-time PCR, 57.29% (55/96) were found to be infected with the protozoan *T. gondii* (Figure 6, Table 1).

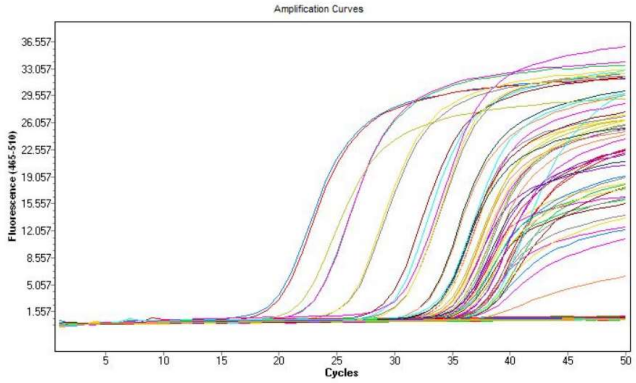


Figure 6. Amplification curves of positive samples derived from the examined wild animals / *Curbele de amplificare ale probelor pozitive provenite de la animalele sălbatice examinate*

Table 1. Prevalence of infection for *T. gondii* in wild animals examined / *Prevalența infecției cu T. gondii la animalele sălbatice examinate*

Host	Origin of positive samples [a]	Infected/examined (%)
Red fox (*Vulpes vulpes*)	AR, TM, IS, TL	31/49 (63.26)
Golden jackal (Canis aureus)	AR, TM	3/10 (30)
Badger (*Meles meles*)	AR, BH	3/8 (37.5)
European wild cat (*Felis silvestris*)	AR, TM	8/10 (80)
Weasels (*Mustela nivalis*)	AR, TM, BH	¾ (75)
Otters (*Lutra lutra*)	-	0/2
Pine marten (*Martes martes*)	AR, VL	2/2
Stone marten (*Martes foina*)	AR, TM	2/2
European polecat (*Mustela putorius*)	AR	2/2
Muskrat (*Ondatra zibethicus*)	AR	1/3 (33.33)
Wild rabbit (*Lepus europaeus*)	-	0/2
Grey Plover (*Pluvialis squatarola*)	-	0/1
Raccoon dog (*Nyctereutes procyonoides)*	-	0/1

[a] AR: Arad; TM: Timiș; IS: Iași: TL: Tulcea; BH: Bihor; VL: Vâlcea

Epidemiological research highlights that *T. gondii* is regarded as an unusual parasite due to its wide host range and the existence of a single species within the genus [11]. Toxoplasmosis is a globally significant zoonosis with potentially severe consequences for human health, as well as for certain domestic and wild animal species. The World Health Organization has recommended the collection of epidemiological data on *T. gondii*. However, despite the well-established links between infection in wildlife, domestic animals and humans, the prevalence of the parasite in wild animals is rarely monitored [3]. Consequently, studies concerning the presence of *T. gondii* in wildlife remain notably scarce.

There are reports of seroprevalence in foxes from Poland (85.3%) and Portugal (40%) [21, 39]. Additional studies have investigated seroprevalence in wildcats, with reported rates of 67% in France, 83.3% in Portugal, and 84.7% in Spain [1, 11, 39]. In the present study, by real-time PCR confirmed prevalence of *T. gondii* infection was 63.26% in foxes and 80% in wildcats.

PCR-RFLP analysis using nine genetic markers indicated that Type II *T. gondii* is the most common genotype infecting wildcats and beavers in Germany [15].

Similar results were reported by the authors of a study aimed at confirming the presence of the parasite in the wildlife of Serbia. Uzelac A. et al. (2019) [38] performed multiplex PCR-RFLP testing followed by genotyping on cardiac tissue from three species of wild canids: foxes, jackals, and wolves. The overall prevalence was 27.3%. The *T. gondii* population structure in wild canids from Serbia showed notable genetic diversity between foxes and jackals, with *T. gondii* genotype II being predominant.

Wild boars and wolves from the Apennine region of Italy can be considered important contributors to the transmission of toxoplasmosis in both peri-urban and wild areas, with a reported prevalence of 22.6% in wild boars and 26.6% in wolves. No statistically significant differences in seropositivity were observed based on age, sex, or geographic origin of the animals [9].

Similar findings were reported by researchers in southern Italy, who conducted a three-year molecular surveillance study and found a prevalence of 21.8% in examined wild mammals (including wolves, foxes, wild boars, badgers, and roe deer). The authors did not identify statistically significant differences in prevalence based on trophic level or host age, thereby rejecting the hypothesis that *T. gondii* infection is more prevalent in top predators and adult individuals [5].

In Slovakia, Kik M. et al. [20] reported a prevalence of 16.6% in red squirrels. Seltmann A. et al. (2020) [33] reported ELISA-based seroprevalence rates of 93.2% in African lions, 52.4% in cheetahs, and 10% in wildebeest from Namibia.

The Sabin-Feldman Dye Test, considered the gold standard in serological diagnostics, was used to monitor the spread of *T. gondii* in the United Kingdom, with the Eurasian otter - an important sentinel species for freshwater environments - serving as the intermediate host. This first spatial distribution study of *T. gondii* in UK wildlife reported a seroprevalence of 39.5%, with significantly higher infection rates in the eastern region and among adult animals. The findings suggest widespread fecal contamination of freshwater ecosystems with oocysts. Moreover, monitoring *T. gondii* infection in the Eurasian otter is of particular value for conservation research, given its status as a "near threatened" species [3].

In Romania, the present study reports the absence of the protozoan in tissue samples collected from otters and examined using real-time PCR.

Matas Méndez P. et al. (2023) [22] demonstrated that the wildcat and the Iberian lynx are among the most important definitive hosts susceptible to *T. gondii* infection in Spain. Indirect fluorescent antibody testing (IFAT) and molecular analysis revealed a prevalence of 85% in wildcats and 45% in lynxes.

Episodes of acute toxoplasmosis have been diagnosed in Slovak zoos, affecting wolves and zebras, with a prevalence reaching 43% [30].

A well-known mesocarnivore, the Eurasian badger, has been identified as a potential source of toxoplasmosis infection in the Izmir region of Turkey [19].

Similarly, in Romania, the present study indicates a molecular prevalence of 37.5% in badgers.

Hedgehogs, as indicator species in anthropogenic ecosystems and sentinels for a wide range of zoonotic infections, show susceptibility to *T. gondii* infection. PCR-confirmed prevalence of the protozoan ranged from 16.6% in the Northern white-breasted hedgehog (*Erinaceus roumanicus*) to 19.2% in the European hedgehog (*Erinaceus europaeus*) living in close proximity to urban areas in the Czech Republic [16].

Toxoplasma gondii is present in the tissues of wild animals, and the consumption of game meat may represent a potential source of infection for humans. During the 2009/2010 and 2010/2011 hunting seasons in Poland, Witkowski L. et al. (2015) [40] collected meat juice samples from hunted animals, including 552 red deer, 367 wild boars, and 92 roe deer. Using the ID Screen Toxoplasmosis Indirect kit (IDvet, Montpellier, France), the following prevalence rates of *T. gondii* infection were recorded: 24.1% of red deer tested positive, 37.6% of wild boars had *Toxoplasma*-specific antibodies, and 30.4% of roe deer were seropositive for *T. gondii* infection.

Black bears in the United States were tested serologically, with a reported seroprevalence rate of 76% [34].

Dini F.M. et al. (2024) [8] highlighted the crucial role of synanthropic rodents in the life cycle of *T. gondii* in anthropized regions, as well as their function as indicators of environmental contamination with oocysts.

In Romania, comprehensive studies addressing the identification of *T. gondii* in wildlife are lacking. Șuteu O. et al. (2014) [35] reported a seroprevalence of 6% in foxes, while in 2016, Morariu et al. indicated a prevalence of 22.22% in European bison from the Armeniș-Plopu reserve.

In wild animals (felines and ruminants) from a zoological garden in Romania, a high seroprevalence of anti-*Toxoplasma* antibodies was reported—73.1% [10].

Studies have been conducted in Romania to evaluate the influence of various factors—such as season, habitat, and origin (rural/urban areas) on *T. gondii* infection. Among 471 individuals belonging to 20 species of small wild mammals, cardiac tissue analysis by PCR revealed a prevalence of 7.3% positive for toxoplasmic infection. Neither season, habitat type, nor geographic origin had a significant influence on *T. gondii* infection rates, findings that are consistent with those reported by others researchers [18].

The present study, which aimed to identify the protozoan *T. gondii* by PCR in tissue samples collected from 13 host species (fox, jackal, badger, cat, weasel, otter, ferret, muskrat, hare, raccoon dog, pine marten, stone marten, bird of prey), revealed the presence of the parasite in 9 of them (fox, jackal, badger, cat, weasel, ferret, muskrat, pine marten, and stone marten), with prevalence rates ranging from 30% to 100%.

Surface water contaminated with oocysts and consumed by ungulate species, along with the accidental ingestion of contaminated soil during grazing, particularly in wild ruminants such as cervids represent key pathways for the transmission of various parasites to wild carnivores through the consumption of infected ruminant meat [2, 32].

Toxoplasmosis remains a zoonosis with a high risk of transmission to humans, with the golden jackal representing an important vector species for the spread of the parasite within Romania's wildlife. The results of the present study highlight a molecular prevalence of 30% in this key intermediate host involved in the *T. gondii* life cycle.

In comparison, research conducted in Hungary reported a *T. gondii* prevalence of 5% in jackals, based on a two-year study conducted between 2010 and 2012 [36].

The first study on *T. gondii* genotypes in golden jackals was conducted in Serbia in 2019. Given that anthropogenic food sources represent a major reservoir of archetypal strains from clonal lineages II and III of *T. gondii*, the authors suggest that the golden jackal may outcompete the red fox, forcing it to hunt wild mammals and birds for subsistence—thereby increasing its likelihood of acquiring variant *T. gondii* strains [38].

The protozoan has also been detected in tissue samples collected from jackals in Qatar and Senegal [11].

The first genetic characterization of *T. gondii* isolated from wild carnivores on the African continent, and the first report of the Africa 4 lineage (equivalent to RFLP genotype ToxoDB 20) was conducted in the black-backed jackal (*Canis mesomelas*). The study was based on a sample of 80 animals representing 20 species, with *T. gondii* DNA detected in 5% of the tested samples [29].

In comparison, the present study, conducted on a sample of 96 wild animals from 13 species, reports the absence of the protozoan in otters, raccoon dogs, birds of prey, and hares, but confirms the circulation of *T. gondii* in foxes, jackals, badgers, weasels, wildcats, ferrets, muskrats, pine martens, and stone martens.

CONCLUSION

1. Systematic surveillance of wildlife is essential for preventing zoonotic infections that pose a threat to human health while also undermining biodiversity.
2. The real-time PCR (qPCR) was used to detect *Toxoplasma gondii* in tissue samples (heart, brain tissue, liver, spleen, and muscle) collected from nine wild animal species across six counties in Romania.
3. The high circulation rate of *T. gondii* (ranging from 30% to 100%) in jackals, foxes, badgers, wildcats, weasels, pine martens, stone martens, European polecats, and muskrats, underscores the potential risk of human infection, particularly in anthropized areas where domestic and wild animals may come into contact.

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RESEARCH ON THE INFLUENCE OF CALVING SEASON ON MILK YIELD, CHEMICAL COMPOSITION AND SOMATIC CELLS COUNT IN DAIRY COWS

CERCETĂRI PRIVIND INFLUENȚA SEZONULUI FĂTĂRII ASUPRA PRODUCȚIEI DE LAPTE, COMPOZIȚIEI CHIMICE ȘI A NUMĂRULUI DE CELULE SOMATICE LA VACILE DE LAPTE

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Abstract

This study aimed to evaluate the influence of the calving season on cow's milk yield during the first 100 days of lactation (100 DIM) and on milks chemical composition, including fat, protein, lactose, total solids, urea and casein. Additionally, the study assessed the dynamics of the somatic cells count (SCC) in relation to calving season (spring, summer, autumn and winter). The research was conducted at the Research and Development Institute for Bovine Baloteşti on 40 multiparous ***Romanian Black and White*** *cows (10 cows/calving season), kept under identical housing and feeding conditions. Statistical analysis included descriptive statistics and the Mann-Whitney U test to determine seasonal differences. Results indicated that cows calving in spring produced the highest 100DIM yields (2733.0 ± 144.0 kg), followed by summer (2599.2 ± 99.7 kg), whereas the lowest production was recorded in winter (2365.2 ± 82.7 kg) and autumn (2356.0 ± 230.0 kg) calving cows. Statistically significant differences ($p \leq 0.05$) were found between spring and autumn for parity corrected milk yield, as well as between spring and winter. The SCS was significantly higher in summer season ($p \leq 0.05$), while the lowest values were recorded in autumn, suggesting an increased risk of mastitis during periods of heat stress. The fat and protein percentages were not influenced ($p > 0.05$) by the calving season, likely due to the consistent feeding diet applied throughout the year. These findings highlight the effects of the calving season and it's potential to optimize production efficiency and udder health through reproductive planning and management.*

Keywords: calving season, milk yield, chemical composition, somatic cells count

Rezumat

Scopul prezentului studiu a constat în evaluarea influenței sezonului fătării asupra producției de lapte în primele 100 de zile de lactație, precum și asupra compoziției chimice a laptelui, urmărind indicatori precum procentul de grăsime, proteină, lactoză, substanță uscată totală, uree și cazeină. În plus, studiul a urmărit dinamica numărului de celule somatice (NCS) în funcție de sezonul fătării (primăvară, vară, toamnă și iarnă). Cercetările s-au efectuat în Laboratorul Experimental Zootehnic al I.C.D.C.B. Baloteşti, pe un număr de 40 vaci multipare din rasa ***Bălţată cu Negru Românească*** *(10 vaci/sezon), întreținute în condiții identice de furajare și management. Analiza statistică a inclus statistici descriptive și testul Mann-Whitney U. Rezultatele au arătat că vacile care au fătat în sezonul de primăvară au înregistrat cea mai mare producție de lapte (2733,0 ± 144,0 kg), urmate de cele care au fătat vara (2599,2 ± 99,7 kg), cele mai scăzute producții de lapte fiind înregistrate la vacile care au fătat iarna (2365,2 ± 82,7 kg) și toamna (2356,0 ± 230,0 kg). Diferențe semnificative ($p \leq 0,05$) au fost observate între sezonul de primăvară și toamnă pentru producția de lapte, precum și între sezoanele de primăvară și iarnă. Scorul celulelor somatice (SCS) a fost semnificativ mai ridicat în sezonul de vară ($p \leq 0.05$), în timp ce cele mai mici valori au fost înregistrate în sezonul de toamnă, sugerând un risc crescut de mastită în perioadele de stres termic. Conținutul de grăsime și proteină nu a înregistrat diferențe semnificative ($p > 0,05$) între sezoane, cel mai probabil datorită furajării din stoc (monodietă). Aceste rezultate subliniază efectele pe care sezonul fătării îl are asupra eficienței producției de lapte și sănătății ugerului la vacile de lapte.*

Cuvinte cheie: sezonul fătării, producția de lapte, compoziție chimică, număr de celule somatice

INTRODUCTION:

Milk production in dairy cows is influenced by multiple factors, including the individuality, lactation length, photoperiod, cold and heat stress, seasonal changes in forage availability and quality, lactation persistency and the cow's genetic background. It is well-established that approximately 75% of milk yield variation is attributed to environmental factors, while only 25% is due to genetics (Collier et al., 2017). Environmental factors include nutritional and herd management, housing technologies, disease occurrence, seasonal conditions and related climate-induced stressors. However, as milk yield continues to increase through genetic selection, improved nutrition and management, high-producing dairy cows become more vulnerable to thermal stress. Extreme temperatures can severely alter metabolic pathways in dairy cattle, as animals attempt to maintain homeostasis while adjusting their production (Baumgard & Rhoads, 2012).

The thermoneutral zone for dairy cattle ranges between 5 and 18°C, with heat stress signs appearing above 28°C. While adult cows are generally more tolerant of cold than heat, calves under 3 months of age are more sensitive to low temperatures. Furthermore, milk composition is also affected by various intrinsic and extrinsic factors, such as diet (Larsen et al., 2010), breed (Palladino et al., 2010), genetic variation (Soyeurt et al., 2008), lactation stage (Stoop et al., 2009), management (Coppa et al., 2013), calving season (Heck et al., 2009) and cow interactions (Stergiadis et al., 2013). Additionally, the seasonal transition from grazing to indoor housing alters both milk yield and composition. Milk fat, protein, lactose and solids content are all affected by changes in the feeding regime and environmental conditions. The somatic cell count (SCC), a key indicator of udder health, increases in response to intramammary infections and stress, with cows exceeding 200.000-400.000 cells/mL generally considered to have subclinical mastitis. Mineral content in milk (e.g., Ca, Mg, Zn, Cu) also varies with season, diet, housing system and region (Weiss et al., 2017; Orjales et al., 2018; Costa et al., 2021).

While molecular tools have been proposed to identify genes regulating macro- and micronutrient levels in milk, limited progress has been made so far (Bailey et al., 2015; Costa et al., 2021). In the context of climate change, dairy cows are increasingly exposed to heat stress, which negatively affects production, fertility and welfare. Moreover, heat tolerance has a negative genetic correlation with milk production traits (Aguilar et al., 2009; Pryce et al., 2022). A promising adaptation strategy is the SLICK1 mutation, which improves thermotolerance in cattle under heat stress without compromising milk yield (Dikmen et al., 2014). However, the physiological response of SLICK1 carriers to temperate conditions and cold stress, as found in Romania, remains largely undocumented.

The objective of this study was to evaluate the influence of calving season on milk yield and milk composition (fat, protein, lactose, total solids, urea and casein), as well as somatic cell score (SCS), during the first 100 days of lactation, in **Holstein** dairy cows managed under temperate continental conditions.

MATERIALS AND METHOD:

The present study was carried out at the Research and Development Institute for Bovine Balotesti (44°36′46″N 26°4′43″E) Romania, (altitude of site 92 m), where 40 purebred **Romanian Black and White** cows (Holstein-Friesian group, *Bălţată cu Negru Românească* national name) were managed under identical conditions. Cows taken into study were between 1st and 5th lactation, with age and parity balanced within the herd and representing a diverse sampling of genetic lines. Since 1987, the **Romanian Black and White** breed was selected for milk yield and fertility, with weights in the breeding scheme of 90% and 10%, respectively. All animals were included in the performance and recording scheme, being

registered as a nucleus reference breeding herd. Cows were housed in a tie-stall barn (170/85 cm), using wheat straws as bedding, having *ad libitum* access to water, mineral blocks and during warm weather to outside paddocks (8 m^2/head, maximum 12 hours/day) and the feeding line outside ensuring minimum 0.75 cm/head. Cows were milked twice per day in the barn (starting at 5:00 and 17:00), using individual milking machines and received a daily feed ration of 30 kg corn silage, 6 kg of alfalfa hay and 6 kg of concentrates. The cow groups were established according to calving season (10 animals per season): spring (calvings in March–April), summer (calvings in June–July), autumn (calvings in September–October) and winter (calvings in December–January).

Milk yield (kg) during the first 100 days in milk (DIM) was taken from the results of the official performance recordings, according to the standardized International Committee for Animal Recording (ICAR) guidelines (2012). The alternative milking at 4 weeks (28AM/PM) recording intervals method was used for this purpose. The total milk production for the first 100 DIM was standardized for mature equivalent (cow's parity) using correction coefficients (Velea & Marginean, 2012). Milk fat content (% m/m), protein (% m/m), lactose (% m/m), total solids (% m/m), urea (mg/100g) and casein (g/L) were recorded based on the first three test-day records following calving. The somatic cell score (SCS) was calculated using the following formula: $SCS = \log_2 (SCC / 100.000) + 3$, where SCC = Somatic Cell Count (cells per millilitre of milk) to normalize its distribution and reduce skewness in statistical analyses. The transformation converts the typically skewed SCC distribution into an approximately normal distribution, facilitating parametric and non-parametric statistical analysis. An SCS of 4 corresponds to an SCC of approximately 200.000 cells/mL.

RESULTS AND DISCUSSIONS:

As shown in Table 1, calving season exerted a notable influence on milk yield during the first 100 days of lactation, when corrected for the mature equivalent coefficient. Cows that calved in spring recorded the highest average milk yield (2733.0 ± 144.0 kg), with values ranging from 1873.0 kg to 3346.0 kg. These were followed by cows calving in summer, with a mean production of 2599.2 ± 99.7 kg and a narrower range (2023.0–2983.1 kg). In contrast, cows that calved in autumn showed lower performance (2356.0 ± 230.0 kg), but with the widest variability, ranging between 1613.0 and 4081.0 kg. A similar average was observed in winter-calving cows (2365.2 ± 82.7 kg), although this group showed more consistent yields (CV = 11.05%). Statistical analysis using the Mann–Whitney U test revealed that cows calving in spring produced significantly more milk than those calving in autumn ($p \leq 0.05$) and winter ($p \leq 0.05$). Other pairwise comparisons were not statistically significant ($p > 0.05$), although numerical trends indicated a production advantage for cows calving in the warmer months.

Table 1. Calving season influence on milk yield in the first 100 DIM, corrected with mature equivalent coefficient (in kg) / *Influența sezonului fătării asupra producției de lapte în primele 100 de zile de la fătare, corectată cu coeficient echivalent maturitate (în kg)*

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	2733.0±144.0	456.0	16.69	1873.0	2390.0	3154.0	3346.0
Summer	2599.2±99.7	315.2	12.13	2023.0	2325.7	2848.8	2983.1
Autumn	2356.0±230.0	729.0	30.94	1613.0	1813.0	2564.0	4081.0
Winter	2365.2±82.7	261.4	11.05	1902.4	2175.6	2600.6	2734.4
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer		NS (P= 0.4727)		***Summer vs. Autumn***			NS (P= 0.1041)
Spring vs. Autumn		*** (P= 0.0452)**		***Summer vs. Winter***			NS (P= 0.1041)
Spring vs Winter		*** (P= 0.0376)**		***Autumn vs. Winter***			NS (P= 0.3258)

This seasonal pattern may be influenced by ambient temperature, as thermoneutral conditions for dairy cattle (5–18 °C) are generally present during spring, supporting optimal milk synthesis. Conversely,

in colder seasons, a greater portion of dietary energy is diverted toward maintaining body temperature, reducing the energy available for lactation (Debnath et al., 2024). This physiological shift likely contributes to the lower milk yields observed in cows calving during cold seasons.

Table 2 presents the influence of calving season on somatic cell score (SCS) during the first 100 days in milk (DIM), along with descriptive statistics and results of pairwise comparisons using the Mann–Whitney U test. The results indicate that cows calving in summer exhibited the highest average SCS (5.1 ± 0.47), with values ranging from 2.8 to 8.1 and a moderate level of variation (CV = 29.00%). Cows that calved in spring and winter had identical mean SCS values of 4.8, but spring-calving cows showed a higher variability (SD = 1.5; CV = 32.01%) compared to those calving in winter (SD = 1.1; CV = 22.95%). The lowest somatic cell score was recorded in cows calving during autumn, with a mean of 3.5 ± 0.28, lower variability (CV = 25.44%) and a narrower range (2.3 to 5.5). The statistical analysis using the non-parametric Mann–Whitney U test revealed that the difference in SCS between summer and autumn was statistically significant (p = 0.0073), with lower values observed in autumn. A similar pattern was detected between autumn and winter season (p = 0.0091). In contrast, no significant differences were found between spring and any other season, nor between summer and winter, suggesting that autumn calving is more consistently associated with lower somatic cell counts and potentially better udder health during early lactation.

These findings support the hypothesis that seasonal environmental conditions, management practices and feed quality may influence udder health status. Autumn calving might be advantageous due to more favourable climate conditions and less heat stress at the beginning of the lactation, potentially reducing the risk of intramammary infections and inflammation, which are commonly reflected in elevated SCC. For instance, in a study conducted by Marinov et al. (2019) on **Holstein** cows, the authors reported the highest somatic cell count (SCC) during the summer season, while the lowest values were recorded in winter. This similar pattern with elevated SCS during summer could be attributed to heat stress and increased humidity, which compromised the cow's immune system and promoted the growth of mastitis-causing bacteria in the housing environment. These conditions increased the risk of intramammary infections and, consequently, elevate somatic cell counts in milk. These outcomes are most likely explained by the temperature and relative humidity conditions inside the barn, as during winter, cows were housed for extended periods on deep straw bedding in a high-humidity environment. Such conditions favoured the multiplication of environmental pathogens and prolonged contact between the mammary gland and the bedding, particularly immediately after milking, when the teat sphincter remained open and may had further increased the risk of intramammary infections.

Table 2. Calving season influence on somatic cells score in the first 100 DIM / ***Influența sezonului fătării asupra scorului de celule somatice din lapte în primele 100 de zile de lactație***

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	4.8±0.49	1.5	32.01	2.4	3.7	6.1	6.7
Summer	5.1±0.47	1.4	29.00	2.8	4.4	5.8	8.1
Autumn	3.5±0.28	0.9	25.44	2.3	2.9	3.7	5.5
Winter	4.8±0.35	1.1	22.95	3.3	4.1	5.3	7.1
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer		NS (P= 0.9097)		***Summer vs. Autumn***			**** (P= 0.0073)**
Spring vs. Autumn		NS (P= 0.0640)		***Summer vs. Winter***			NS (P= 0.7054)
Spring vs Winter		NS (P= 0.9097)		***Autumn vs. Winter***			**** (P= 0.0091)**

Table 3 shows the effect of calving season on milk fat percentage during the first 100 days of lactation. The highest average fat percentage was recorded in cows that calved in autumn, with a mean value of 4.280%, ranging from 3.557% to 4.783% and a relatively high variability (CV = 11.50%). Cows that calved during winter had an average fat percentage of 4.035% and values ranging between 3.55% and 4.973%. Cows that calved in the spring showed a moderate average fat percentage of 3.854%, with

less variability (CV = 8.46%) and a range from 3.303% to 4.427%. The lowest average fat percentage was observed in cows calving during summer, at 3.718%, with values varying more widely between 2.683% and 5.067% (CV = 16.76%). The statistical analysis (Mann-Whitney U test) revealed no significant differences between any of the seasonal pairs ($p > 0.05$), although the comparison between summer and autumn ($P = 0.0757$) and summer and winter ($P = 0.0890$) approached the threshold of significance.

These results suggest a tendency for higher fat content in milk from cows calving in cooler seasons (autumn and winter), although this trend did not reach statistical significance in the current dataset. Previous research has shown that milk production in cattle tends to decrease with declining ambient temperatures and as milk volume decreases, fat percentage increases, due to a strong negative correlation between these two traits. This physiological relationship may partially explain the observed trend of higher fat percentages in cows calving during autumn and winter.

Table 3. Calving season influence on fat percentage in the first 100 DIM / *Influența sezonului fătării asupra procentului de grăsime din lapte în primele 100 de zile de lactație*

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	3.854±0.103	0.326	8.46	3.303	3.696	4.025	4.427
Summer	3.718±0.197	0.623	16.76	2.683	3.442	3.915	5.067
Autumn	4.280±0.156	0.492	11.50	3.557	3.806	4.731	4.783
Winter	4.035±0.126	0.399	9.90	3.55	3.808	4.237	4.973
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer		NS (P=0.2730)		***Summer vs. Autumn***		NS (P=0.0757)	
Spring vs. Autumn		NS (P=0.1212)		***Summer vs. Winter***		NS (P=0.0890)	
Spring vs Winter		NS (P=0.4727)		***Autumn vs. Winter***		NS (P=0.4727)	

Milk protein percentage during the first 100 days of lactation showed relatively minor fluctuations across calving seasons (Table 4). The highest average was recorded in cows that calved in autumn (3.324%), followed closely by those calving in winter (3.266%) and summer (3.252%), while the lowest average was observed in cows calving in spring (3.212%). All seasons showed a relatively low coefficient of variation (ranging from 5.55% to 10.59%), suggesting a relatively stable distribution of values within each group. Although slight numerical differences were present, no statistically significant variation in protein percentage was detected between seasons ($p > 0.05$ for all pairwise comparisons).

This relative consistency may reflect the greater physiological stability and stronger genetic regulation of milk protein content, as opposed to other milk components such as fat. While environmental factors like nutrition and metabolic stress can still influence protein synthesis, the data suggest that seasonal conditions exert a weaker short-term effect on this trait compared to fat percentage or overall milk yield.

Table 4. Calving season influence on protein percentage in the first 100 DIM / *Influența sezonului fătării asupra procentului de proteină din lapte în primele 100 de zile de lactație*

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	3.212±0.070	0.221	6.89	2.923	2.988	3.355	3.643
Summer	3.252±0.057	0.180	5.55	3.030	3.105	3.380	3.630
Autumn	3.324±0.111	0.352	10.59	2.680	3.070	3.612	3.803
Winter	3.266±0.099	0.313	9.59	2.896	2.997	3.495	3.820
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer		NS (P=0.5967)		***Summer vs. Autumn***		NS (P=0.3847)	
Spring vs. Autumn		NS (P=0.4057)		***Summer vs. Winter***		NS (P=0.9698)	
Spring vs Winter		NS (P=0.7913)		***Autumn vs. Winter***		NS (P=0.6232)	

The influence of calving season on milk lactose percentage during the first 100 days of lactation is summarized in Table 5. The highest average lactose content was observed in cows calving during the

winter season, with a mean of 4.786% and values ranging from 4.483% to 4.916%. This was followed by cows calving in spring (4.722%; min–max: 4.436–4.946%), autumn (4.673%; min-max: 4.350–4.916%) and summer, which recorded the lowest average lactose content at 4.637%, with a narrower range of 4.540% to 4.826%. A statistically significant difference ($p \leq 0.01$) was detected between summer and winter, indicating a higher lactose percentage in milk from cows that calved in the colder season. No other seasonal comparisons reached the threshold of statistical significance ($p > 0.05$).

Previous studies investigating seasonal variation in milk lactose percentage reported similar patterns, with the highest values recorded during winter, followed by a gradual decrease throughout spring, autumn and summer (Bondan et al., 2018). One possible explanation is the increased inclusion of corn silage in winter diets, which contributes to a higher energy intake. Since milk lactose synthesis is tightly linked to energy availability, cows receiving more energy-dense rations during the cold season may exhibit increased lactose levels in early lactation.

Table 5. Calving season influence on lactose percentage in the first 100 DIM / ***Influența sezonului fătării asupra procentului de lactoză din lapte în primele 100 de zile de lactație***

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	4.722±0.048	0.153	3.25	4.436	4.620	4.801	4.946
Summer	4.637±0.0241	0.076	1.64	4.540	4.598	4.650	4.826
Autumn	4.673±0.058	0.183	3.93	4.350	4.540	4.837	4.916
Winter	4.786±0.040	0.128	2.69	4.483	4.710	4.885	4.916
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer		NS (P=0.0890)			***Summer vs. Autumn***		NS (P=0.2899)
Spring vs. Autumn		NS (P=0.5453)			***Summer vs. Winter***		**** (P=0.0091)**
Spring vs Winter		NS (P=0.2265)			***Autumn vs. Winter***		NS (P=0.1509)

Milk total solids percentage during the first 100 days of lactation varied noticeably across calving seasons (Table 6). The highest concentration of milk solids was observed in cows that calved during the autumn, with a mean value of 14.234% and values ranging from 12.510% to 16.043%. This group also showed the widest variation (SD = 1.109, CV = 7.79%), suggesting a more heterogeneous metabolic response during this period. Cows calving in summer had the second-highest average (12.995%), followed closely by those calving in winter (12.847%) and spring (12.669%). Compared to autumn, the other seasons presented more homogeneous data distributions, with coefficients of variation between 3.28% and 4.58%. The Mann-Whitney U test revealed statistically significant differences between several seasonal pairs. Specifically, autumn-calving cows had significantly higher solids content than those calving in summer ($p = 0.0173$), spring ($p = 0.0036$) and winter ($p = 0.0082$). No significant differences were observed among the other comparisons ($p>0.05$).

These findings support the idea that autumn calving may enhance milk solids concentration, potentially due to a combination of post-summer nutritional recovery, favourable metabolic conditions, or stage of lactation aligning with cooler months.

Table 6. Calving season influence on solids percentage in the first 100 DIM / ***Influența sezonului fătării asupra procentului de solide din lapte în primele 100 de zile de lactație***

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	12.669±0.131	0.415	3.28	11.773	12.447	13.040	13.213
Summer	12.995±0.176	0.556	4.28	11.883	12.753	13.251	14.053
Autumn	14.234±0.351	1.109	7.79	12.510	13.394	15.172	16.043
Winter	12.847±0.186	0.588	4.58	12.187	12.391	13.220	13.943
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer		NS (P=0.1405)			***Summer vs. Autumn***		*** (P=0.0173)**
Spring vs. Autumn		**** (P=0.0036)**			***Summer vs. Winter***		NS (P=0.4057)
Spring vs Winter		NS (P=0.9698)			***Autumn vs. Winter***		**** (P=0.0082)**

The influence of calving season on milk urea concentration (expressed in mg/100 g milk) during the first 100 days of lactation is shown in Table 7. The highest average levels were recorded in cows that calved in winter (28.90 mg/100 g) and spring (27.77 mg/100 g), with values ranging from 21.33 to 35.00 mg and 16.67 to 34.67 mg, respectively. These were followed by cows that calved in autumn, with a mean value of 23.83 mg/100 g (range: 18.00–29.33 mg) and summer-calving cows, which had the lowest average urea levels, at 18.97 mg/100 g, ranging between 14.00 and 26.00 mg. Statistical analysis revealed several significant differences between seasons. Summer-calving cows had significantly lower milk urea concentrations compared to spring ($p = 0.0025$), autumn ($p = 0.0284$) and winter ($p = 0.0010$). A significant difference was also observed between spring and autumn ($p = 0.0494$) and between autumn and winter ($p = 0.0343$), while no significant difference was found between spring and winter ($p = 0.7337$).

In our study, spring and winter calvings were associated with higher milk urea concentrations during early lactation. Similar findings were reported by Bendelja et al. (2011), who observed significantly higher urea levels in spring and summer compared to autumn and winter. Moreover, elevated milk urea concentrations have been reported in the period July–September, in connection with increased total and crude protein levels, particularly casein, which tend to rise in parallel with milk urea levels. For **Simmental** cattle, lower urea concentrations during summer and higher values in autumn were also observed by Marenjak and Poljicak-Milas (2007), potentially explained by the reduced dry matter intake due to heat stress, as well as greater access to fresh forage on pasture. These findings underline the complex interaction between seasonal feeding regimes, thermal comfort and protein metabolism, which together influence milk urea concentrations, a potential indicator of the protein-energy balance in the cow's diet.

Table 7. Calving season influence on milk urea in the first 100 DIM / ***Influența sezonului fătării asupra cantității de uree din lapte în primele 100 de zile de lactație (mg/100g)***

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	27.77±1.61	5.09	18.34	16.67	25.00	30.83	34.67
Summer	18.97±1.36	4.32	22.76	14.00	15.58	24.17	26.00
Autumn	23.83±1.16	3.67	15.38	18.00	21.17	27.42	29.33
Winter	28.90±1.56	4.94	17.08	21.33	25.83	34.42	35.00
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer		**** (P=0.0025)**		***Summer vs. Autumn***		*** (P=0.0284)**	
Spring vs. Autumn		*** (P=0.0494)**		***Summer vs. Winter***		***** (P=0.0010)**	
Spring vs Winter		NS (P=0.7337)		***Autumn vs. Winter***		*** (P=0.0343)**	

Milk casein content during the first 100 days of lactation was influenced by calving season, with clear variations in average concentrations and statistical significance between certain seasonal groups. The highest mean casein concentration was observed in cows calving during autumn (29.50 g/L), with values ranging from 22.83 to 36.30 g/L and the highest variability among all groups (CV = 13.51%). This was followed by cows calving in summer, which showed a more consistent distribution (mean = 26.52 g/L; CV = 4.50%) and cows calving in spring (25.45 g/L) and winter (25.37 g/L), both with moderate to low variation. Statistical comparisons using the Mann-Whitney U test revealed that autumn-calving cows had significantly higher casein concentrations than those calving in spring ($p = 0.0173$), summer ($p = 0.0452$) and winter ($p = 0.0233$). No significant differences were found between spring and summer, spring and winter, or summer and winter (Table 8).

Table 8. Calving season influence on milk casein in the first 100 DIM / *Influența sezonului fătării asupra cantității de cazeină din lapte în primele 100 de zile de lactație (g/L)*

Season	X̄±SEM	SD	CV	MIN	Q1	Q3	MAX
Spring	25.45±0.54	1.711	6.72	23.46	23.55	26.85	28.43
Summer	26.52±0.37	1.194	4.50	24.70	25.55	27.72	28.30
Autumn	29.50±1.26	3.990	13.51	22.83	26.95	32.26	36.30
Winter	25.37±0.80	2.550	10.05	22.36	23.08	27.45	29.60
Differences with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01; *** p≤0.001							
Spring vs. Summer		NS (P=0.1736)		***Summer vs. Autumn***		*** (P=0.0452)**	
Spring vs. Autumn		*** (P=0.0173)**		***Summer vs. Winter***		NS (P=0.2413)	
Spring vs Winter		NS (P=0.9097)		***Autumn vs. Winter***		*** (P=0.0233)**	

These results align with the previously observed trends in milk urea levels, as elevated urea concentrations are often associated with an increase in total milk protein, particularly casein. The autumn peak in casein could be linked to improved post-summer feeding strategies, enhanced dry matter intake, or altered nitrogen metabolism.

CONCLUSIONS

1. Calving season had a clear impact on early lactation performance. Spring calvings were associated with higher milk yields, while winter and autumn calvings resulted in the lowest production.
2. Autumn-calving cows showed the best udder health, with the lowest somatic cell scores, significantly better than those calving in summer and winter.
3. Milk fat and solids contents were highest in cows calving during autumn and winter, while lactose was significantly higher in winter compared to summer.
4. Overall, autumn calving emerged as optimal for both milk quality and udder health, while spring calving was most favourable for milk yield.

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DYNAMICS OF THE CHEMICAL COMPOSITION OF MILK FROM ROMANIAN BUFFALOES COWS DEPENDING ON THE LACTATION CURVE

DINAMICA COMPOZIȚIEI CHIMICE A LAPTELUI PROVENIT DE LA BIVOLIȚELE DIN RASA ROMÂNEASCĂ ÎN FUNCȚIE DE CURBA DE LACTAȚIE

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Abstract

*The aim of this study was to analyze the dynamics of fat, protein and lactose content in fresh milk from **Romanian Buffaloes** cows according to the different stages of lactation. Samples were collected from 20 buffaloes in four phases of lactation: early lactation (1-30 days), plateau phase (30-50 days), end of plateau (180-200 days) and end of lactation period (200-270 days). The variation of the parameters monitored in this study was also highlighted by following their changes throughout the lactation. The results indicated a decreasing trend of all three parameters as lactation progressed. The fat content decreased slightly but steadily, from 7.87% in early lactation to 7.46% in the last phase. Protein levels followed a similar pattern, decreasing from 4.51% to 4.22%, while lactose content decreased from 4.56% to 4.19%. These results highlight the natural variations in the composition of fresh buffalo raw milk during lactation, with implications for optimizing management, increasing efficiency in buffalo milk production, both for milk processing and for its nutritional value.*

Key words: buffaloes, milk, lactation, chemical composition, lactation curve.

Rezumat

Scopul acestui studiu a fost de a analiza dinamica conținutului de grăsimi, proteine și lactoză din laptele proaspăt de bivoliță românească în funcție de diferitele stadii de lactație. S-au recoltat probe de la 20 de bivolițe în patru faze de lactație: lactație timpurie (1-30 de zile), faza de platou (30-50 de zile), sfârșitul de platou (180-200 de zile) și sfârșitul perioadei de lactație (200-270 de zile). Variația parametrilor monitorizați în acest studiu a fost evidențiată și prin urmărirea modificărilor acestora pe parcursul lactației. Rezultatele au indicat o tendință de scădere a tuturor celor trei parametri pe măsură ce lactația a progresat. Conținutul de grăsime a scăzut ușor, dar constant, de la 7,87% la începutul lactației la 7,46% în ultima fază. Nivelurile de proteine au urmat un model similar, scăzând de la 4,51% la 4,22%, în timp ce conținutul de lactoză a scăzut de la 4,56% la 4,19%. Aceste rezultate evidențiază variațiile naturale ale compoziției laptelui crud proaspăt de bivoliță în timpul lactației, cu implicații pentru optimizarea managementului, creșterea eficienței în producția de lapte de bivoliță, atât pentru prelucrarea laptelui, cât și pentru valoarea sa nutritivă.

Cuvinte cheie: bivolițe, lapte, compoziție chimică, curba lactației.

INTRODUCTION

The domestic buffalo (*Bubalus bubalis*) is a valuable species in animal husbandry, appreciated for its adaptability and for its ability to produce milk with a superior chemical composition, rich in fat, protein and minerals (Borghese, 2005). Buffalo milk is preferred in the dairy industry for obtaining refined and high-quality products, such as mozzarella and matured cheeses.

The chemical composition of milk varies throughout the lactation period. Studies show that in the first weeks after calving, fat and protein levels are higher, gradually decreasing as lactation progresses

(Kashwa, 2016). These variations reflect the physiological changes that occur in the mammary gland and influence both the quality of milk and its economic value.

The lactation curve is essential in production studies, providing a clear picture of how milk production varies during lactation. This includes an initial phase of growth, followed by a plateau and then a final phase of decline. The shape of the curve is mainly determined by physiological factors, such as the activity and number of secretory epithelial cells in the mammary gland (Penchev et al., 2011).

In Romania, studies on the variation of milk composition according to the lactation curve are still limited, especially in the case of the local population. This work aims to analyze the variations of fat, protein and lactose content in **Romanian buffalo** milk, according to the different stages of the lactation curve, thus contributing to a better valorization of this indigenous genetic resource.

MATERIAL AND METHOD

The study was conducted on a batch of 20 buffaloes of the **Romanian Buffalo breed**, in different stages of lactation. All milk samples were taken from buffaloes in lactations between the first and fourth, to allow a balanced assessment of the variation in composition depending on the stage of lactation. The animals were maintained under similar feeding and housing conditions, within the Research and Development Station Buffaloes in Şercaia.

The breeding system applied was semi-intensive. During the summer, the buffaloes were taken out to pasture, and in the cold season they were fed with pickled and coarse fodder.

Milk was collected twice a week, at both milkings (morning and evening), and the samples were analyzed immediately after collection. The determinations were made for the following parameters: fat, protein and lactose.

Physico-chemical analyses were performed using an EKOMILK-Ultra device, and the data obtained were centralized for each lactation phase. The stages analyzed were:

- phase 1: beginning of lactation (1–30 days),
- phase 2: plateau phase (30–50 days),
- phase 3: end of plateau (180–200 days),
- phase 4: end of lactation (200–270 days).

The values obtained were expressed in percentages (%) and statistically processed by calculating means and standard deviations.

RESULTS AND DISCUSSION

FAT

The fat content values in buffalo milk varied according to the stage of lactation, following the profile of the lactation curve. A higher concentration at the beginning of lactation, a period of relative stability and a decrease towards the end of lactation. These changes reflect the physiological changes that occur in the mammary gland during lactation and are influenced by the volume of milk produced, the metabolic state of the animal and the activity of the secretory cells. These are represented by the alveolar epithelial cells, located in the alveoli of the mammary gland, which have the role of synthesizing and secreting the main components of milk.

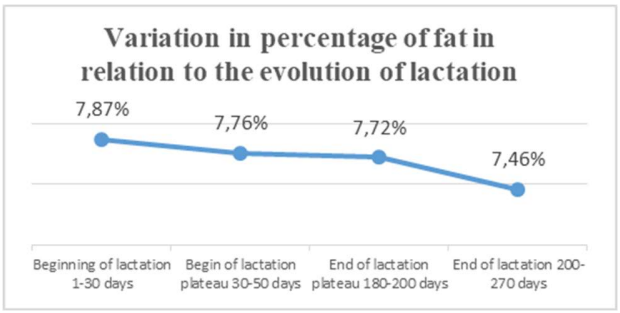


Figure 1. Evolution of fat content (%) according to lactation stage / *Evoluția conținutului de grăsime (%) în funcție de stadiul lactației*

At the beginning of lactation (1–30 days), the highest fat content was recorded with an average of 7.87 ± 0.05%. This increased content is specific to milk from the postpartum period, when the amount of milked milk is reduced, but its composition is rich in concentrated nutritional components, intended to support the development of newborn calves. The activity of alveolar epithelial cells is intense, which are responsible for the synthesis and secretion of the main components of milk, especially fat, proteins and lactose. This stage reflects the ascending phase of the lactation curve, in which the mammary gland activates its physiological mechanisms for optimal milk production. During the plateau period of lactation (30–50 days), the fat content recorded a slight decrease compared to the first phase, with an average of 7.76 ± 0.33%. This decrease is mainly influenced by the increase in the volume of milk produced, a phenomenon that determines the physiological dilution of solid components. However, the fat content remains high, reflecting an efficient secretory activity of the mammary gland during a period of maximum milk production. Studies by Yadav et al. (2013) and Gunathilake et al. (2024) confirm that variations in fat content can be influenced by both the stage of lactation and the breed, but in general, slightly lower values are observed in the phases of intensive production, followed by progressive increases towards the end of lactation. At the end of the lactate curve (180–200 days), fat content continued to decrease slightly, with a mean value of 7.72 ± 0.37%. This decrease can be attributed to the progressive decrease in mammary gland activity, influenced by hormonal changes and physiological adaptations that occur as lactation progresses. As milk production decreases, the activity of alveolar epithelial cells slows down, leading to changes in milk composition, including a slight decrease in fat content. Similar results were reported by Nayak et al. (2017) in **Murrah** buffaloes, where a decreasing trend in fat was observed towards the end of lactation.

In the last phase of lactation (200–270 days), the lowest fat content was recorded, with an average of 7.46 ± 0.07%. This decrease is associated with the end of the lactation curve, a period in which milk production is minimal, and the body redirects metabolic energy towards body maintenance, to the detriment of milk production. The activity of alveolar epithelial cells is significantly reduced, which directly affects fat synthesis. In addition, the decrease in prolactin levels and the increase in progesterone levels towards the end of lactation influence the secretory capacity of the mammary gland. Similar results were reported by El-Tarabany et al. (2016), who observed a decrease in fat content in the last months of lactation in Egyptian buffaloes, under conditions of decreased production and metabolic adaptation. These results highlight the dynamics of fat content depending on the stage of lactation, an aspect analyzed in parallel for the other parameters of milk composition. The decrease in fat content in the advanced stages of lactation is also associated with changes in the fatty acid composition of milk.

Table 1. Evolution of fat content (%) depending on lactation stage / *Evoluția procentului de grăsime în funcție de stadiul lactației*

FAT %			
Beginning of lactation 1-30 days	**Begin of plateau 30-50 days**	**End of plateau 180-200 days**	**End of lactation 200-270 days**
X±Sx	**X±Sx**	**X±Sx**	**X±Sx**
7.87±0.05	7.76±0.33	7.72±0.37	7.46±0.07

During periods of low milk production, studies have shown a tendency to decrease short-chain fatty acids, such as butyric acid, but also essential polyunsaturated acids, such as linoleic acid (C18:2) and alpha-linolenic acid (C18:3). These variations affect not only the nutritional value of milk, but also its behavior in industrial processing, influencing the texture, taste and final quality of dairy products. In addition, the reduction in the total amount of lipids entails a lower intake of unsaturated fatty acids, which may indirectly modify the characteristics of buffalo milk fat in the final stage of the lactation curve.

PROTEINS

The proteins in buffalo milk represent an essential parameter of its nutritional value and its ability to be used in the dairy industry. It is mainly composed of casein and serum proteins, which have a fundamental role in the development of calves in the first days of life, but also in the coagulation of milk for the production of dairy products. The protein content varies during lactation, being influenced by the physiological stage of the animal, nutrition and endocrine activity. As a rule, the values are higher in the early phases of lactation and tend to decrease towards the end of it.

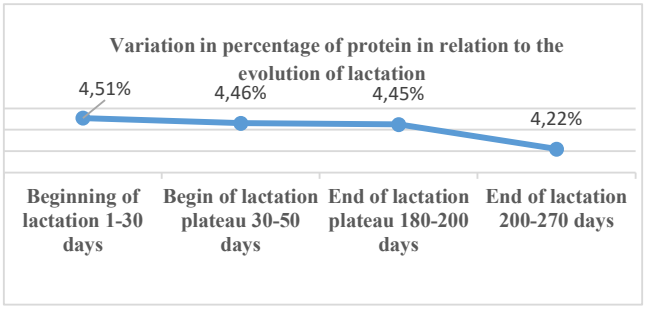


Figure 2. Evolution of protein content (%) depending on the stage of lactation / *Evoluția procentului de proteină în funcție de stadiul lactației*

In the first 30 days of lactation, the protein content recorded the highest value, with an average of 4.51 ± 0.07%. This increased level is characteristic of the beginning of lactation, when the milk has a high nutritional density, with a significant protein intake. These play an essential role in supporting the vital functions of calves, being involved in both nutrition and immunity. The high amount of casein facilitates the absorption of calcium, while serum proteins contribute to protection against infections. These particularities reflect the physiological adaptation of the body to the postpartum period and support the superior quality of milk at this stage. At the beginning of the plateau phase of lactation (30–50 days), the protein content presented an average value of 4.46 ± 0.09%, marking a slight decrease compared to the previous stage. This variation is associated with the metabolic changes that occur with reaching a constant level of milk production. Unlike the onset of lactation, when protein secretion is influenced by intensely stimulating hormonal factors (e.g. prolactin), in this phase milk protein synthesis stabilizes, being regulated more by physiological needs and the efficiency of nutrient use. At the end of the lactation curve (180–200 days), protein content remained at a relatively constant level, with an average value of 4.45 ± 0.36%. Although close to the previous phase, this stage is marked by a slight reduction in secretory activity, along with a gradual decrease in milk production. The greater variation in protein value in this

interval may reflect the influence of individual factors such as the number of calvings, the physiological state of the animal or differences in nutritional intake. Milk protein synthesis remains active, but registers a slightly downward trend, characteristic of the middle to late phases of lactation. At the end of lactation in the decline phase (200–270 days), the protein content recorded the lowest value, with an average of 4.22 ± 0.18%. This reduction is associated with the end of the lactation curve, a period in which milk production decreases significantly. The decrease in protein content can be explained by the reduction in the activity of alveolar epithelial cells in the mammary gland, responsible for the synthesis of milk proteins. In parallel, the body redirects metabolic resources to the maintenance of vital functions, and the lower frequency of milking causes a hormonal decline that affects secretion. All these changes contribute to the progressive decrease in protein concentration, characteristic of the end of lactation.

Table 2. Evolution of protein content (%) depending on the stage of lactation / *Evoluția procentului de proteină în funcție de stadiul lactației*

PROTEIN %			
Beginning of lactation 1-30 days	**Begin of plateau 30-50 days**	**End of plateau 180-200 days**	**End of lactation 200-270 days**
X±Sx	**X±Sx**	**X±Sx**	**X±Sx**
4.51±0.07	4.46±0.09	4.45±0.36	4.22±0,18

Beyond quantitative variations, the quality of the protein in buffalo milk remains high throughout lactation, due to its complete profile of essential amino acids, such as leucine, valine or methionine (Coroian, 2009). These amino acids play an essential role in protein synthesis, supporting the immune system and the growth and regeneration of the body. Even in the final stages of lactation, milk continues to provide important nutritional benefits, which supports its value both from a nutritional and qualitative point of view.

LACTOSE

Lactose is the main carbohydrate present in buffalo milk, being composed of two monosaccharides glucose and galactose. It is responsible for the slightly sweet taste of fresh milk and represents an essential source of energy for calves in the early stages of life. At the same time, lactose contributes to the development of beneficial intestinal flora, having an important prebiotic role. From a technological point of view, lactose is indispensable in fermentation processes, being a substrate for lactic acid bacteria used in obtaining acidic products. Although fluctuations in lactose content during lactation are lower compared to other components, they may indicate metabolic changes in the mammary gland, the health status of the animal or the occurrence of functional imbalances, such as subclinical inflammations (e.g. mastitis) or the redirection of metabolic resources in contexts of stress or inadequate nutrition.

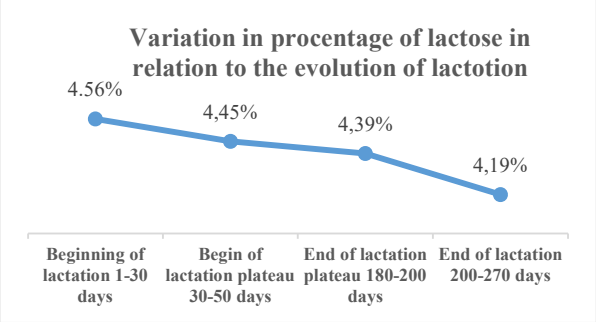


Figure 3. Evolution of lactose content (%) depending on the stage of lactation / *Evoluția procentului de lactoză în funcție de stadiul lactației*

At the beginning of lactation (1–30 days), lactose content reached the maximum value recorded during the study, with an average of 4.56 ± 0.16%. This high concentration can be associated with the physiological specificity of the onset of lactation, when the mammary gland has an intense secretory activity, and the composition of the milk reflects a formula adapted to the initial needs of the maternal organism and the newborn calf. The increased lactose level contributes to maintaining a balanced milk secretion, both in terms of quantity and quality. Higher values were reported by Kashwa (2016) in the case of Egyptian buffaloes, with an average of 5.08% in the first 30 days of lactation. The differences between the two studies can be attributed to the genetic influence (breed), Mediterranean climatic conditions and the intensive feeding system used in that area, compared to traditional conditions in Romania.

In the early stage of the lactation plateau (30–50 days), the lactose content remained at a relatively constant level, with an average of 4.45 ± 0.17%, registering a slight decrease compared to the first stage of lactation. This stabilization reflects the achievement of a functional and constant milk production, characterized by a balance between milk production and its chemical composition..

In the late stage of the lactation plateau (180–200 days), the lactose content continued to decrease slightly, reaching an average value of 4.39 ± 0.07%. This reduction can be explained by the physiological changes characteristic of advanced lactation, which occurs concurrently with gestation. Part of the body's metabolic resources are directed towards supporting fetal development, which causes a gradual decrease in mammary gland activity. At the same time, hormonal changes associated with this period, such as a decrease in prolactin levels and an increase in progesterone, can negatively influence lactose synthesis, by reducing the production of α-lactalbumin, an enzyme directly involved in its formation.

In the last stage of lactation (200–270 days), lactose content recorded the lowest value in the entire analyzed interval, with an average of 4.19 ± 0.03%. This decrease reflects the physiological decline in milk production, at a time when the buffalo's body prioritizes maintaining gestation and conserving metabolic resources. The reduction in the activity of secretory cells in the mammary gland, especially the alveolar epithelial ones, leads to a general decrease in milk synthesis, including lactose. Also, towards the end of lactation, its level can be influenced by structural changes in the mammary tissue, associated with the gradual involution of the gland. Similar results were reported by Kashwa (2016) in the case of Egyptian buffaloes, where lactose content decreased from 5.08% to 4.64% in the seventh month of lactation, confirming the downward trend observed in the present study.

Table 3. Evolution of lactose content (%) depending on the stage of lactation / *Evoluția procentului de lactoză în funcție de stadiul lactației*

LACTOSE%			
Beginning of lactation 1-30 days	**Begin of plateau 30-50 days**	**End of plateau 180-200 days**	**End of lactation 200-270 days**
X±Sx	**X±Sx**	**X±Sx**	**X±Sx**
4.56±0.16	4.45±0.17	4.39±0.07	4.19±0.03

CONCLUSIONS

The lactation curve expresses how milk production and composition change during a full lactation, reflecting the physiological, hormonal and metabolic influences on the mammary gland. In this study, a gradual decrease in fat, protein and lactose content was observed, with maximum values in the first 30 days after calving and minimum values in the late lactation phase (200–270 days). Specifically, fat content ranged from 7.87 ± 0.05% to 7.46 ± 0.07%, protein from 4.51 ± 0.07% to 4.22 ± 0.18%, and lactose from 4.56 ± 0.16% to 4.19 ± 0.03%. These variations are determined by functional changes in the mammary gland, by the redistribution of metabolic resources in the body, but also by the gradual

decrease of hormonal stimuli specific to lactation. Fat content is the first to be affected in the advanced stages of lactation, while protein and carbohydrate content follow a slower but constant decline. The results obtained emphasize the importance of careful monitoring of the stage of lactation in order to maintain milk quality. Adapting feeding and selection strategies according to these variations can significantly contribute to increasing efficiency and to the superior valorization of production in buffalo farms in Romania. In this context, an in-depth knowledge of the lactation curve becomes an essential tool for optimizing productivity and supporting a sustainable breeding system, with high technological and economic results. Deepening these aspects allows the development of modern management strategies, the adaptation of genetic selection programs, the adjustment of feed rations depending on the lactation phase and the careful monitoring of biochemical parameters of milk, all of which contribute to improving the performance of buffalo milk production in Romania.

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PRELIMINARY STUDY OF THE QUALITATIVE PARAMETERS OF RAM SEMINAL MATERIAL PRESERVED BY FREEZING

STUDIUL PRELIMINAR AL PARAMETRILOR CALITATIVI AI MATERIALULUI SEMINAL DE BERBEC PREZERVAT PRIN CONGELARE

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Abstract

*The interest of sheep and goat breeders in increasing productivity and implicitly profitability has materialized through the modernization of the traditional exploitation system. To increase productive performance, breeders resort to natural mating with breeding males. The epizootic situation of recent years, respectively the sanitary-veterinary restrictions as a result of the evolution of infectious diseases (Bluetongue, PMM) have limited the circulation of males with implications for reproduction management. An alternative to solving this impediment is to resort to artificial insemination with semen from breeding males. In this sense, Palas Sheep and Goat Breeding Research and Development Institute, in partnership with National Association of Goat Breeders in Romania "CAPRIROM", carries out research activities in order to establish methodologies for preserving ram and goat seminal material in frozen form that would allow the creation of a sperm cryobank from valuable males. When examining frozen-thawed semen from **Palas Merino** rams, processed during the breeding season, a total motility ranging from 43.2 to 64.1% and a progressive motility ranging from 31.8 to 47.7% were recorded, while in the off-breeding season, values of 39.4-44.5% for total motility and 27.1-32.5% for progressive motility were obtained. The total and progressive motility values from **Palas Milk** rams had a very high variability.*

Keywords: ram semen, total/progressive motility

Rezumat

*Interesul crescătorilor de oi și capre pentru creșterea productivității și implicit a profitabilității s-a materializat prin modernizarea sistemului de exploatare tradițional, iar pentru creșterea performanțelor productive crescătorii apelează la monta naturală cu masculi amelioratori. Situația epizootologică a ultimilor ani, respectiv restricțiile sanitar-veterinare ca urmare a evoluției unor boli infecțioase (Bluetongue, PMM) au limitat circulația masculilor cu implicații în managementul reproducției. O alternativă de rezolvare a acestui impediment este apelarea la inseminarea artificială cu material seminal provenit de la masculi amelioratori. În acest sens, Institutul de Cercetare – Dezvoltare pentru Creșterea Ovinelor și Caprinelor Palas, în parteneriat cu Asociația Națională a Crescătorilor de Capre din România "CAPRIROM" desfășoară activități de cercetare în sensul stabilirii unor metodologii de prezervare a materialului seminal de berbec și țap sub formă congelată care să permită crearea unei criobănci de spermă de la masculi valoroși. La examinarea materialului seminal congelat-decongelat de la berbeci rasa **Merinos de Palas**, procesat în sezon de reproducție, s-a înregistrat o motilitatea totală care a variat între 43,2 și 64,1 % și o motilitate progresivă cuprinsă între 31,8 și 47,7, iar în contrasezon de reproducție s-au obținut valori de 39,4-44,5% pentru motilitatea totală și 27,1-32,5% pentru motilitatea progresivă. Valorile motilităților totală și progresivă de la berbecii din **Rasa de Lapte Palas** au avut o variabilitate foarte mare.*

Cuvinte cheie: material seminal berbec, motilitate totală / progresivă

INTRODUCTION

Sheep reproduction in temperate zones is seasonal, occurring in the autumn period, respectively with the decrease in daylight hours, for which reason they are also called "short-day" animals (Chemineau, P. et al., 2017, 1988). During the period with a daylight duration of over 10-12 hours, seasonal anestrus (lack of sexual activity, respectively of estrus and ovulation) is observed in ewes and a reduction in sexual activity is observed in rams. Photoperiod is a decisive factor in triggering sexual activity in sheep, which influences the general management of farms, by placing the calving campaign in the period December-March. Thus, the production of lamb meat and sheep milk is seasonal, not meeting the demands of the human population and bringing insufficient benefits to sheep farmers.

Off-season sheep breeding is increasingly practiced as producers adopt accelerated lamb rearing programs to better supply lamb markets and ensure a consistent supply of sheep milk throughout the year. Coupling this method with other biotechnical methods of sheep reproduction (induction and synchronization of estrus, early diagnosis of pregnancy, embryo transfer) amplifies the usefulness of artificial insemination. The intensive use of tested rams of high zootechnical value or the use of the gene pool offered by imported rams of various specialized breeds, leads to obtaining a superior quality offspring and intensifies the pace of genetic improvement of sheep flocks. By artificial insemination of a large number of sheep, approximately 600-1000 sheep / ram, the pace and degree of improvement is accelerated by using breeding rams of superior genetic value, tested according to their own performance or that of their offspring (Zamfirescu S., Sonea A., 2004).

Ways to intensify the reproductive activity of males in order to be able to mount during the breeding season include the application of non-hormonal treatments, namely maintaining them in photoperiod conditions (Nadolu D., Anghel A., 2007, José Abecia et al., 2020) or the application of melatonin implants (Șogorescu E et al., 2012).

By using artificial insemination, the main advantages for the breeder are the increase in the rate of genetic progress in terms of milk quantity, milk protein quantity and quality, obtaining a large number of daughters from a breeding ram from one's own farm or from another farm. By de seasonalizing reproduction and concentrating calving periods, the products demanded by the market (milk, meat) obtained with a reduced manpower requirement are ensured throughout the year. In addition, the danger of transmitting diseases through reproduction is reduced and the transport of animals is avoided, which is important, especially during the period of sanitary and veterinary restrictions, a situation of utmost topicality. By imposing quarantine measures and prohibiting the movement of animals, rational matings are prevented, and in situations when restrictions provide for the elimination of sick animals, the risk of losing valuable breeders is very high. By freezing the semen from valuable males in a cryobank, the genetic fund of each breed is protected.

MATERIALS AND METHODS

The present study was conducted at the Palas Sheep and Goat Breeding Research and Development Institute, Constanta, Reproductive Biotechnologies Laboratory, on semen collected from 2 **Palas Merino** rams and 4 **Palas Milk** rams, monitoring the variation of sperm parameters after freezing-thawing.

The results presented are part of a complex study currently underway, regarding the establishment of a freezing protocol and assessment of the suitability for freezing of ram semen under the conditions of using the C.A.S.A. (Computer - Assisted Sperm Analysis) system. The research is carried out through the collaboration of the institute with the National Association of Goat Breeders of Romania CAPRIROM, which purchased the complete semen freezing line, namely an automatic straw stamping system, an automatic straw loading system, an automatic station with a freezing program and connection

to a liquid nitrogen tank and the C.A.S.A. (Computer - Assisted Sperm Analysis) computerized sperm analysis system.

To determine sperm indices, 48 ejaculates were analysed, 8 ejaculates from each ram, 4 ejaculates collected during the off-season (last week of March 2024) and 4 ejaculates collected during the normal breeding season (last week of September 2024) (table 1).

Table 1. Semen collection scheme and frozen-thawed semen analysis / *Schema de recoltarea materialului seminal și analiza materialului seminal congelat-decongelat*

Specification	**Merino Palas** breed 1	**Merino Palas** breed 2	**Milk Palas** breed 1	**Milk Palas** breed 2	**Milk Palas** breed 3	**Milk Palas** breed 4
September 2024	4	4	4	4	4	4
March 2024	4	4	4	4	4	4
Total	8	8	8	8	8	8

The six rams, 2 of the **Palas Merino** breed (MP1 and MP2) and 4 of the **Palas Milk** breed, from which semen was collected benefited from the same maintenance and exploitation conditions. 2 ejaculates/day were collected from each male, at intervals of 2 days, and the sperm was processed in parallel, benefiting from the same environmental conditions. The only difference is 10 minutes between the semen collections from the two males, a difference that does not significantly influence the results of the research. It should be noted that from all males, collected during the off-season, semen was collected one week before the start of the study, in order to eliminate the sperm reserve with a high content of denatured spermatozoa due to non-use in mating and to stimulate spermatogenesis in the presence of females in hormonally induced estrus.

In the activity of the Reproduction Laboratory, the procedures provide for the cryobank of frozen semen which, when checking viability by the vital staining method, has values over 35%. Frozen-thawed semen with viability between 30 and 35% is kept as a buffer stock, as a reserve. It should be noted that the method of establishing viability has a degree of subjectivity depending on the experience, skill and conscientiousness of the operator performing the control. By using the CASA automatic system, these disadvantages are eliminated, the results being much more complex and precise. Thus, total motility indicates the percentage of live spermatozoa, regardless of the type of movement they perform (circular movements, backwards, zig-zag etc.), a parameter similar to the viability determined by vital staining. Therefore, following the verification of sperm parameters, the semen retained in the cryobank must register a total motility of over 35%.

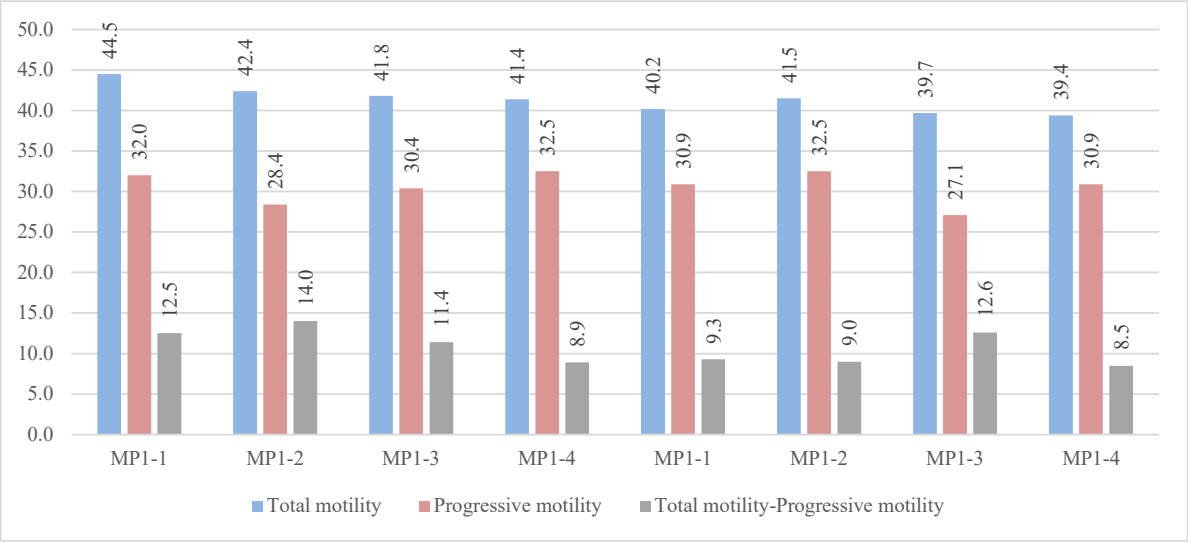
The research of the Laboratory of Reproductive Biotechnologies is aimed at finding a correlation between total motility and progressive motility and the factors that may influence this correlation. In establishing the minimum level of progressive motility of frozen semen, the verification of the fertilizing capacity of the semen following artificial insemination of sheep must also be taken into account.

Frozen preservation of semen was verified by analysing total motility and progressive motility within 24 hours of freezing for the same male in normal and off-season breeding.

RESULTS AND DISCUSSION

The results of the qualitative assessment of semen from **Palas Merino** rams harvested in the off-season and preserved in frozen form are presented in Graph 1.

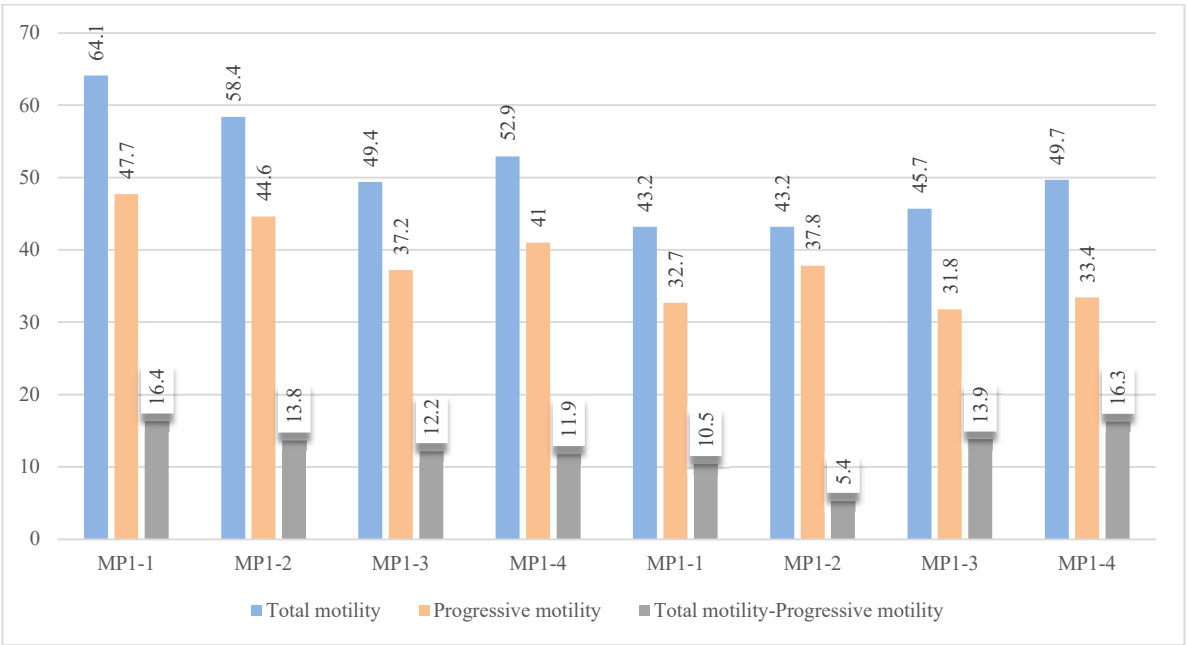
Total motility varies between 39.4 and 44.5%, with higher values recorded in the first ram (41.4 – 44.5%) and progressive motility is between 27.1 and 32.5%, with the differences between the two motilities being between 8.3 and 14%. What is notable about the semen from the first ram is the greater difference between the two motilities, between 8.9 and 14% compared to the ejaculates of the second ram, where the difference between total and progressive motility is between 8.5 and 12.6%.



MP1–first Merino de Palas ram, MP2- second Merino de Palas ram

Graph 1. Variation of total motility and progressive motility of frozen-thawed semen from Palas Merino rams, collected in the off-season / *Variația motilității totale și a motilității progresive a materialului seminal congelat-decongelat de la berbeci Merinos de Palas, recoltat în contrasezon de reproducție*

The results of the assessment of motility after freezing-thawing of semen from **Palas Merino** rams, harvested during the breeding season, are presented in graph 2.



MP1–first Merino de Palas ram, MP2- second Merino de Palas ram

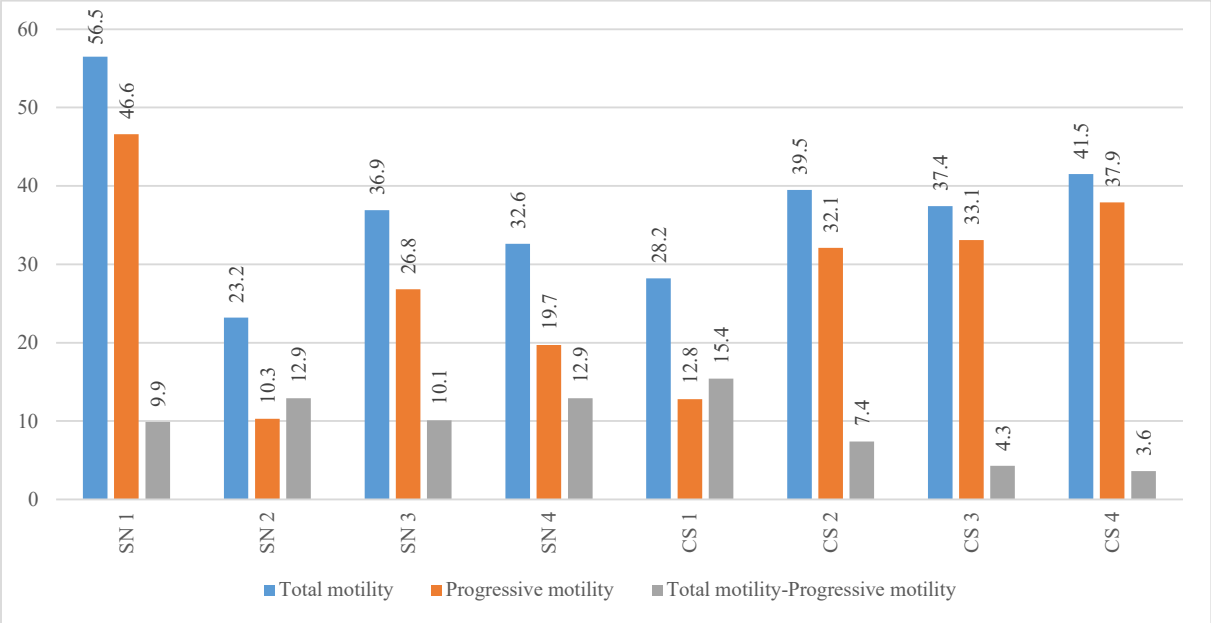
Graph 2. Variation of total motility and progressive motility of frozen-thawed semen from Merinos de Palas rams, harvested during the breeding season / *Variația motilității totale și a motilității progresive a materialului seminal congelat-decongelat de la berbeci Merinos de Palas, recoltat în sezon de reproducție*

The situation observed in the evaluation of semen collected in the off-breeding season and processed by freezing is also found in those collected in the breeding season. Thus, the ejaculates from the first **Palas Merino** ram, after freezing-thawing, recorded total motility values ranging between 49.4 and 64.1%, while in the second ram these were between 43.2 and 49.7%. The second ram stands out by obtaining close values of total (43.2-49.7%) and progressive (between 31.8 and 37.8%) motility, while the semen of the first ram has a greater variability between the sperm parameters determined in successive collections and with a greater difference between the motility values.

Ejaculates collected during the breeding season and cryopreserved recorded total values of over 35% and were stored in the semen cryobank. And progressive motility has values of over 30% in all 8 ejaculates collected, following that by insemination of the sheep the fertility rate will be increased. Therefore, the two **Palas Merino** males are suitable for semen collection for cryopreservation.

The other ejaculates analysed in this study came from 4 males of the **Milk Palas** breed. Graph 3 contains the total and progressive motility values of the ejaculates collected from the first male of the Milk Palas breed. It is observed that, in the normal breeding season harvests, only the first ejaculate collected in September 2024 and frozen had, after thawing, a high total and progressive motility of 56.5% and 46.6%, respectively.

The other ejaculates collected had, after freezing-thawing, total motilities ranging between 32.6% (harvest 4 of September) and 36.9% (harvest 3) and only in harvest 2 was a total motility below 30% (23.2%) obtained, which would lead to the removal of the frozen straws from the cryobank. Regarding progressive motility, except for the first collection with very good values and the 2nd collection in the case of removed ejaculate, it has values below 30%, respectively 26.8% (3rd collection) and 19.7% (4th collection).



SN – normal season, CS – off-season

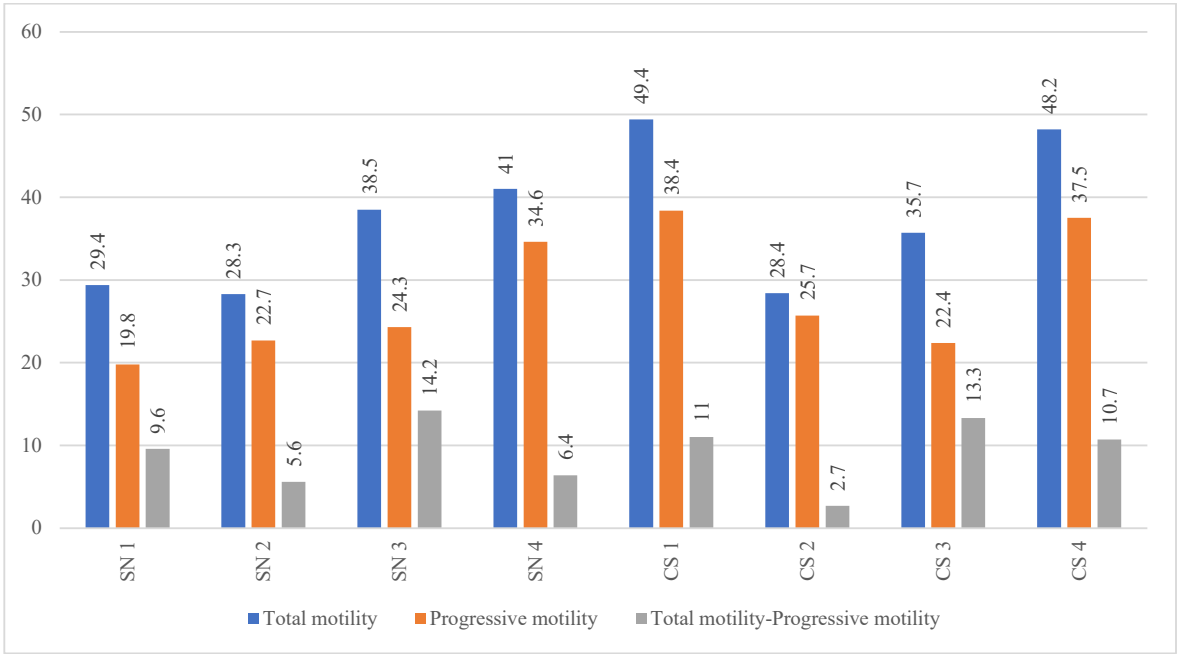
Graph 3. Variation of total motility and progressive motility of frozen-thawed semen collected from the Milk Palas 1 ram in the breeding season and off-season / *Variația motilității totale și a motilității progresive a materialului seminal congelat-decongelat recoltat de la berbecul de rasa Lapte Palas 1 în sezon și contrasezon de reproducție*

During the off-season breeding harvests (in March 2024), only the first ejaculate collected was removed from the cryobank, with a total motility of 28.2%. The other ejaculates with total motilities

ranged between 39.5% (harvest 2 in off-season breeding) and 41.5% (harvest 4 in March 2024). In the case of these 3 ejaculates retained in the cryobank, a progressive motility lower by 3.6% to 7.4% compared to the total motility is observed. These small differences in progressive motility compared to the total motility indicate a good suitability for freezing of the semen from the first analysed **Milk Palas** male, which will also be correlated with the fertility rate following artificial inseminations of sheep.

Motility after freezing-thawing of straws with ejaculate from the 2nd **Milk Palas** ram is presented in graph 4.

During the normal breeding season harvests, from September 2024, it is observed that the first 2 ejaculates have total motility below 30% and were removed from the cryobank. The other 2 ejaculates have total motility of 38.5% and 41%, values of over 35%, which leads to their preservation in the semen cryobank stock. As can be seen, the progressive motility of these ejaculates is good, with the largest decrease recorded by the ejaculate from the 3rd harvest, by 14.2%, the last harvest recording progressive sperm motility of over 30%.



SN – normal season, CS – off-season

Graph 4. Variation of total motility and progressive motility of frozen-thawed semen harvested from the Milk Palas 2 ram in the breeding season and off-season / *Variația motilității totale și a motilității progresive a materialului seminal congelat-decongelat recoltat de la berbecul de rasa Lapte Palas 2 în sezon și contrasezon de reproducție*

In the off-season collections, a large variation in total sperm motility is observed when checking after freezing-thawing, between 28.4% and 49.4%. The same variation is also recorded in progressive motility, which varies from 22.4% to 38.4%.

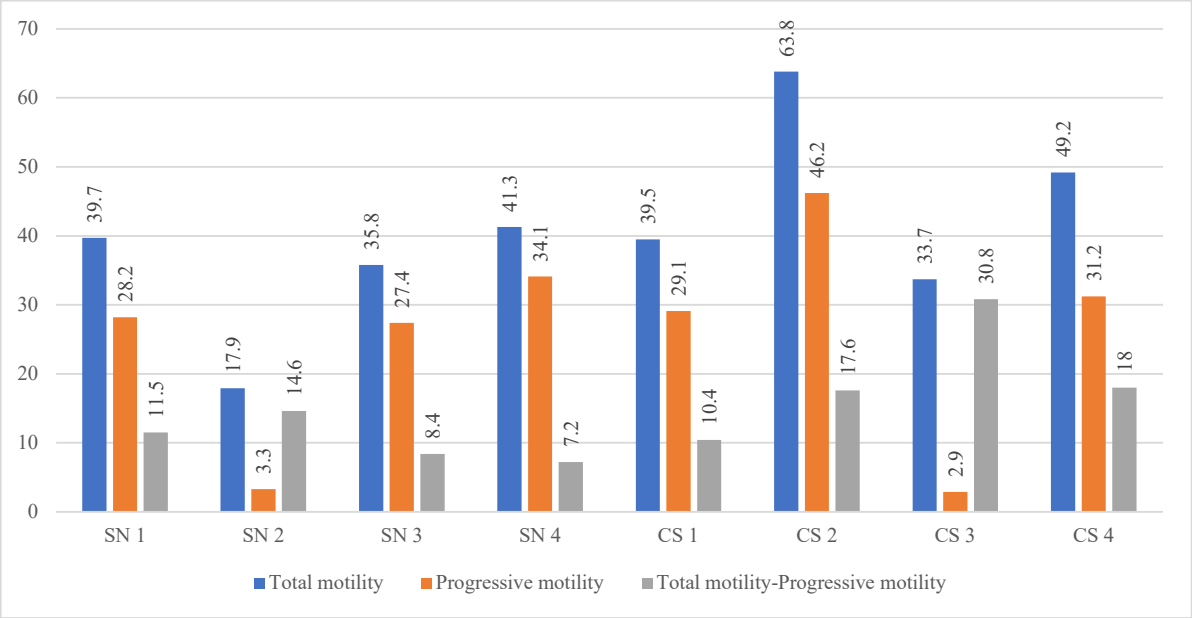
From the analysis of the evolution of motilities in the 8 ejaculates, we can conclude that the semen of the second male is suitable for freezing and storage in the sperm cryobank, specifying that in the off-season, the greater variation in results may be due to a lower rate of sperm production and therefore it must be monitored whether a lower frequency of collections is necessary with the interspersion of rest days in the collection to restore the sperm reserve.

Graph 5 shows the variation in total motility and progressive motility of frozen-thawed semen collected from the 3rd ram of the **Milk Palas** breed.

During the normal breeding season, a very high variability is observed in both total and progressive motility, and the differences between the 2 motilities are between 7.2% and 14.6%.

Of the 4 ejaculates collected in September, one ejaculate was removed and 3 were kept in the cryobank stock, and the fertility rate after artificial insemination was to be correlated with progressive motility to evaluate the lowest value of progressive motility at which acceptable fecundity indices are obtained.

In the breeding season (March 2024), 3 ejaculates had total motility of over 35% (39.5%- first harvest, 63.8%- 2nd harvest and 49.2%- 4th harvest). Of these, in one ejaculate, respectively in the 3rd harvest, a progressive motility of only 2.9% is observed. The decrease by 30.8% is probably due to a processing error, since on the monitor image it is observed that the spermatozoa have fast but static movements or with a reduced forward speed, which was not observed in the other ejaculates. This ejaculate was kept in the cryobank reserve, to be used for artificial insemination of goats to check the gestation rate and confirm whether it was just a processing error.



SN – normal season, CS – off-season

Graph 5. Variation of total motility and progressive motility of frozen-thawed semen collected from the Milk Palas 3 ram in the breeding season and off-season / *Variația motilității totale și a motilității progresive a materialului seminal congelat-decongelat recoltat de la berbecul de rasa Lapte Palas 3 în sezon și contrasezon de reproducție*

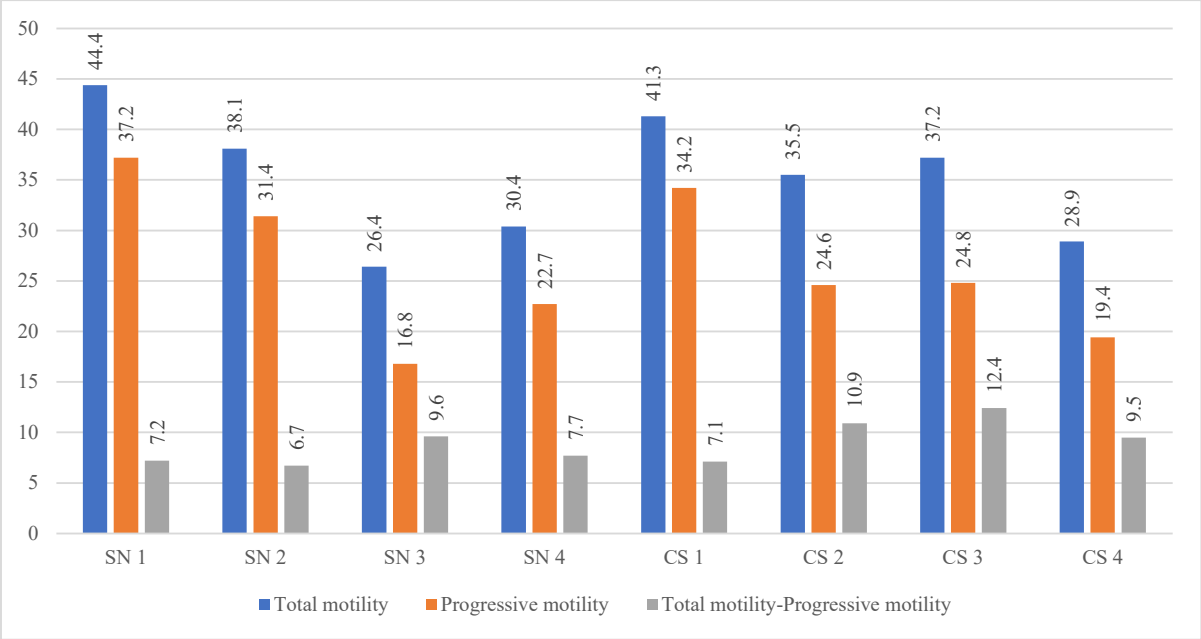
From the analysis of the quality of the semen collected in the two seasons, it can be concluded that the 3rd ram of the **Milk Palas** breed is suitable for freezing ejaculates, with better results being obtained in the off-season when the rams are not used for mating. It is recommended to check the ejaculates of this ram in the breeding season but when he does not also mate the ewes, thus having a control regime of ejaculations.

Graph 6 presents the evolution of the total motility and progressive motility of the frozen-thawed semen collected from the 4th ram of the **Milk Palas** breed.

In the ejaculates collected during the breeding season, the one corresponding to the 3rd collection had a motility after freezing-thawing of 26.4% and was removed from the semen cryobank and the 4th ejaculate, with a total motility of 30.4% was kept in the cryobank reserve stock. The other 2 ejaculates had a total motility of 44.4% (first collection during the breeding season) and 38.1% (2nd collection). In

these 2 ejaculates with total motility of over 35%, a progressive decrease in motility is observed with small percentages of 7.2% and 6.7%.

In the ejaculates collected in the off-season of reproduction, it is observed that only the 4th collection has a total motility of 28.9% and is removed from the sperm cryobank. The other 3 ejaculates have a total motility of over 35% and the progressive motility is between 24.6% and 34.2%.



SN – normal season, CS – off-season

Graph 6. Variation of the total motility and progressive motility of the frozen-thawed semen, collected from the Milk Palas 4 ram in the breeding season and off-season / ***Variația motilității totale și a motilității progresive a materialului seminal congelat-decongelat, recoltat de la berbecul de rasa Lapte Palas 4 în sezon și contrasezon de reproducție***

The analysis of the motility values obtained in the two reproduction seasons, with values of over 35% in total motility and values of 25% in progressive motility, recommends keeping this ram for collecting semen for freezing and is also a candidate for research on establishing the minimum level of progressive motility depending on the fertility rate of inseminated females.

Based on the analysis of the evolution of total and progressive motility of semen preserved in frozen and refrigerated form and by analysing the fertility rate obtained following artificial insemination, the minimum threshold for progressive motility will be established so that the sperm thus processed can be used or stored in the cryobank.

CONCLUSIONS

1. The quality and suitability for freezing of semen is influenced by the individual and the breeding season.
2. Semen collected from **Palas Merino** rams recorded higher values of total and progressive motility in the breeding season (total motility: 64.1%-43.2%, progressive motility: 47.7%-31.8%) compared to the non-breeding season (total motility: 44.5%-39.4%, progressive motility: 32.5%- 27.1%).

3. Semen from **Milk Palas** rams showed large differences between total and progressive motility and a high total motility does not guarantee a proportional progressive motility (example: total motility: 63.8% and progressive motility: 46.2%, total motility: 38.5% and progressive motility: 24.3%).
4. In the semen of **Milk Palas** rams, large differences in total motility are observed between successive collections (56.5% - 23.2%) from the breeding season compared to collections from the counter-breeding season (41.5% - 28.2%).
5. No direct correlation was established between total motility and progressive motility of frozen-thawed semen regardless of the breeding season in which the sperm was collected and processed. Research activity to establish the minimum value of progressive motility for the two forms of ram semen preservation is in progress.

ACKNOWLEDGEMENTS

The results presented in this paper are part of the research on establishing a technology for freezing ram and goat semen using state-of-the-art equipment. The purpose of the project, which was started with funding from the state budget - ASAS contract no. 7674 of 06.12.2023, is to establish a sperm cryobank from males of local or imported breeds raised in our country.

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COMPARATIVE RESEARCH ON MEAT PRODUCTION PERFORMANCE IN INTENSIVELY FATTENED LAMBS FROM NATIVE BREEDS

CERCETĂRI COMPARATIVE PRIVIND PERFORMANȚELE PENTRU PRODUCȚIA DE CARNE LA TINERETUL OVIN DIN RASELE AUTOHTONE ÎNGRĂȘAT INTENSIV

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Abstract

*In recent years, there have been significant changes in the breeding direction of native sheep breeds, with increasing concern for enhancing meat production. Thus, sheep breeders in our country have turned to crossbreeding with specialized meat breeds without thoroughly evaluating the current productive potential of the native sheep breeds.In this context, the present study aims to assess the main morpho-productive indices, as well as the performance of intensively fattened male lambs from native breeds (**Tsurcana, Tsigai, Palas Merino, Palas Prolific breed**, and **Palas Meat breed**) and crossbreeds (**Rouge de L'Ouest** × **Palas Prolific breed**, **Rouge de L'Ouest** × **Palas Meat breed**). The average birth weight of male lambs ranged between 3.82 kg/head and 5.29 kg/head, regardless of breed. At the end of the growth and fattening period, the lowest body weights were recorded in the **Tsigai** and **Tsurcana** breeds, at 30.55 kg/head and 31.67 kg/head, respectively. The highest body weights at the end of the growth and fattening period were observed in the crossbreeds **Rouge de L'Ouest** × **Palas Prolific breed** (45.16 kg/head) and **Rouge de L'Ouest** × **Palas Meat breed** (48.16 kg/head). The daily average gain of lambs during the fattening period was 320 g/day in the **Palas Meat breed**, while the crossbreeds achieved a daily average gain of 253.0 g/day (**Rouge de L'Ouest** × **Palas Meat breed**) and 245.71 g/day (**Rouge de L'Ouest** × **Prolific Palas breed**).*
*Among the experimental groups, the **Palas Merino** and **Palas Meat breed** lambs exhibited the lowest feed conversion ratio, both in terms of energy intake (4.37 – 4.78 N.U./kg gain) and crude protein intake (745.01 – 789.06 g D.C.P./kg gain).*
The obtained results highlight the good current potential of native breeds for meat production and shows the beneficial effect of crossbreeding with specialized meat breeds to improve the economic efficiency of sheep farming operations.

Key words: breeds, carcass, crossbreeds, fattening.

Rezumat

*În ultimii ani au existat schimbări privind direcția de creștere a raselor autohtone de ovine, crescând semnificativ preocuparea pentru sporirea producției de carne. Astfel, crescătorii de ovine din țara noastră au apelat la aplicarea de încrucișări cu rase specializate pentru producția de carne, fără a testa însă potențialul real actual al raselor autohtone de ovine. În acest sens, lucrarea de față are drept scop studierea principalilor indici morfo-productivi, precum și performanțele obținute la îngrășarea intensivă a tineretului ovin mascul din rasele autohtone (**Țurcană, Țigaie, Merinos de Palas, rasa Prolifică Palas, rasa de Carne Palas**) și a unor metiși (**Rouge de L'Ouest** x **rasa Prolifică Palas**, **Rouge de L'Ouest** x **rasa de Carne Palas**). Greutatea corporală medie a mieilor masculi la fătare a fost cuprinsă între 3,82 kg/cap și 5,29 kg/cap., indiferent de rasă. La finalul procesului de creștere și îngrășare, valoarea cea mai redusă a greutăților corporale a fost întâlnită la rasele **Țigaie** și **Țurcană**, acestea fiind de 30,55 kg/cap și respectiv 31,67 kg/cap. Cele mai mari greutăți corporale înregistrate la sfârșitul perioadei de creștere și îngrășare s-au întâlnit la metișii **Rouge de L'Ouest** x **rasa Prolifică Palas** și **Rouge de L'Ouest** x **rasa de Carne Palas**, acestea fiind de 45,16 kg/cap respectiv 48,16 kg/cap. Sporul mediu zilnic realizat de miei în perioada de îngrășare a fost de 320 g/cap la **rasa de Carne Palas**, în timp ce metișii au realizat un spor de 253,0 g/zi (**Rouge de L'Ouest** x **rasa de Carne Palas**) și respectiv 245,71 g/zi (**Rouge de L'Ouest** x **rasa Prolifică Palas**). În cadrul loturilor luate în studiu, lotul de miei din rasa **Merinos de Palas** și din **rasa de Carne Palas** au realizat cel mai redus consum specific atât la energie (4,37 – 4,78 UN/kg spor), cât și la proteină (745,01 – 789,06 g PBD/kg spor).*

Rezultatele obținute evidențiază potențialul actual bun pentru producția de carne al raselor autohtone și ilustrează efectul benefic al încrucișării cu rase specializate pentru producția de carne, în vederea rentabilizării exploatațiilor de ovine.

Cuvinte cheie: carne, îngrășare, metiși, rase.

INTRODUCTION

Native sheep breeds are generally breeds with high production mixed, presenting a high degree of variability of individuals, characterized through the existence of certain specimens with visible traits for meat or milk production.

The continuous scientific research in the field, relating to the improvement of native sheep breeds in the direction of meat production, seeking methods to increase the degree of capitalization of the potential productive by selection or by creating lines with consolidated genetics for meat production.

Many studies made in Romania (Ciolcă N. et al. 1972, Mireșan E. et al. 1989, Pascal C. 2007) have proven the fact that biological potential for meat production in sheep differs significantly, the differences existing between breeds due both specific precocity as well as fattening technology used.

The local breeds traits, **Tsurcana** and **Tsigai**, for meat production was studied by Pascal C. et al. (2012) for the semi-intensive fattening. In this way, by fattening for 175 days of lambs, the indigenous sheep breeds have achieved a daily average gain of 140.0 g for lamb's males of the **Tsigai breed**, 125.0 g in females of the **Tsurcana** breed, the variety black and 116.0 g in lambs males of the **Tsurcana breed**, the variety white.

After slaughter, the carcasses were evaluated, and the comparison of the results obtained with other scientific references led to the conclusion that both breeds have limited possibilities to produce high-quality carcasses, because the proportion of carcasses in the U and R categories of the EUROP grid was below 10%, those classified in the P category were over 20%, and the classification in the higher categories was null.

Another study conducted within Palas - Constanța Institute followed the evolution of body weight, feed conversion and slaughter results on batches of lambs from local breeds subjected to intensive fattening. In this way, the research conducted by Călătoiu A. et al. (1975, 1977, 1980) on fattening lambs from the **Merino, Spancă, Tsigai** and **Tsurcana breeds**, tested 3 energy levels (2500, 2750 and 3000 Kcal digestible energy/kg combined feed) and within each energy level three protein levels were ensured. The results obtained revealed that local breeds best utilized feed recipes that provided 2750 kcal E.D./combined feed, the differences between breeds being due to the protein level. In this way, Merino and Spancă lambs better utilized feed recipes with 15.95% protein, while **Tsigai** and **Tsurcana** lambs better utilized recipes with protein levels of 12.65% and 9.35%, respectively.

In recent years, concerns have grown in Romania for increasing the meat production of indigenous sheep breeds, frequently resorting to their crossbreeding with other specialized meat breeds. In this context, it appears appropriate to establish the current potential of indigenous breeds as well as of some crossbreeds obtained for meat production that can be capitalized on various markets.

MATERIAL AND METHOD

The research was carried out within the Palas Constanța Institute on lambs from the current year belonging to the following breeds and genotypes:

- **Palas Merino Breed**
- **Palas Meat Breed**
- **Palas Prolific Breed**
- **Tsurcana Breed**
- **Tsigai Breed** (rusty variety)

- **Rouge de L'Ouest x Palas Prolific Breed**
- **Rouge de L'Ouest x Palas Meat Breed**

The present work aims to study the main morphoproductive indices (body weight at calving, weaning), as well as the performances achieved during intensive fattening of young male sheep from the mentioned breeds and crossbreeds.

After weaning the lambs, the experimental groups were formed of 20 male lambs from each breed, after which they were introduced for fattening, the groups being homogeneous in terms of age and body weight.

The feeding of the batches was done with the same recipe, namely combined feed with an energy value of 2751 Kcal and a protein value of 16%, as well as with alfalfa hay granules, which had 2740 Kcal and 18.5% protein.

The administered feeds were weighed daily, and the unconsumed residues were harvested after a period of 3-5 days for each batch, thus determining the consumability of the administered rations.

Based on the average daily consumption of nutrients within each batch, the energy consumption (UN) and protein (PBD)/kg live weight gain was calculated.

The growth rate of lambs was thus monitored by individually weighing each batch at the beginning of the experimental period, and then monthly until the end of fattening and the achievement of body weights of 30-40 kg.

RESULTS AND DISCUSSIONS

Table 1 presents the evolution of body weight and the average daily gain achieved by **Palas Merino** lambs subjected to intensive fattening.

Table 1. Morphoproductive indices in Palas Merino lambs subjected to intensive fattening / *Indicii morfoproductivi la mieii din rasa Merinos de Palas supuși îngrășării intensive*

Breed	Body weight (kg)			Daily average gain (grams)
	Birth	Weaning	End of fattening period	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Palas Merino	3.96 ± 0.13	22.88 ± 0.55	40.63 ± 0.91	290.33 ± 0.64

The data presented show that **Palas Merino** lambs had an average body weight of 3.96 kg at calving and 22.88 kg at weaning. During the intensive fattening period, male lambs achieved an average daily gain of 290.33 g, with a live weight at the end of fattening of 40.63 kg/head. Table 2 presents the morphoproductive indices of male lambs of the **Palas Meat Breed**, subjected to intensive fattening.

Table 2 presents the morphoproductive indices of male lambs of the **Palas Meat Breed**, subjected to intensive fattening.

Table 2. Indices morphoproductive characteristics in lambs of the Palas Meat breed stall intensive / *Indicii morfoproductivi la mieii din rasa de Carne Palas îngrășați intensiv*

Breed	Body weight (kg)			Daily average gain (grams)
	Birth	Weaning	End of fattening period	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Palas Meat Breed	3.82 ± 0.13	22.75 ± 0.81	40.35 ± 0.82	320.0 ± 0.64

The lambs of the **Palas Meat Breed** weighed 3.28 kg and 22.75 kg at birth and weaning, respectively. During the fattening period, the young sheep achieved an average daily gain of 320.0 g/head, at the end of the period having a final body weight of 40.35 kg/head.

The morpho-productive indices achieved by male offspring of the **Palas Prolific Breed** subjected to intensive fattening are presented in Table 3.

Table 3. Morpho-productive indices in intensively fattened lambs of the Prolific Palas breed / *Indicii morfoproductivi la mieii masculi din rasa Prolifică Palas îngrășați intensiv*

Breed	Body weight (kg)			Daily average gain (grams)
	Birth	Weaning	End of fattening	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Prolific Palas Breed	3.83 ± 0.27	22.38 ± 0.91	38, 46 ± 1.74	191.56 ± 0.81

At birth and weaning, the lambs of the **Palas Prolific Breed** had a body weight of 3.83 kg and 22.38 kg, respectively. The body weight at the end of the fattening period was 38.46 kg, achieving a daily gain during the aforementioned period of 191.56 g/day/head.

In the crossbreeds **Rouge de L'Ouest** x **Prolific Palas Breed**, the morphoproductive indices presented in table 4 were obtained.

Table 4. The main morpho-productive indices in crossbred Rouge de L'Ouest x Prolific Palas breed subjected to intensive fattening / *Principalii indici morfoproductivi la metișii Rouge de L'Ouest x Rasa Prolifică Palas supuși îngrășării intensive*

Breed	Body weight (kg)			Daily average gain (grams)
	Birth	Weaning	End of fattening	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Rouge de L'Ouest crossbreed x Palas Prolific Breed	4.17 ± 0.10	22.52 ± 1.16	45.16 ± 0.83	245.71 ± 0.61

The table shows that the crossbreeds **Rouge de L'Ouest** x **Palas Prolific Breed** achieved an average daily gain of 245.71 g/head, with a final body weight of 45.16 kg. Also, at calving and weaning, the crossbred lambs had average body weights of 4.17 kg/head and 22.52 kg/head, respectively.

In the **Rouge de L'Ouest** x **Palas Meat Breed** crossbreds, the body weight of lambs at calving is 3.87 kg, as shown in the data presented in Table 5.

Table 5. Morpho-productive indices in intensively fattened Rouge de L'Ouest x Palas Meat Breed crossbreds / *Indicii morfoproductivi la metișii Rouge de L'Ouest x Rasa de Carne Palas îngrășați intensiv*

Breed/Genotipe	Body weight (kg)			Daily average gain (grams)
	Birth	Weaning	End of fattening	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Rouge de L'Ouest x Palas Meat Breed	3.87 ± 0.22	22.97 ± 1.14	48.16 ± 1.17	253.0 ± 0.54

Average body weight at weaning of **Rouge de L'Ouest** x **Palas Meat Breed** crossbreds was 22.97 kg, and after the fattening period. The final weight was 48.16 kg, the daily average gain by lambs' crossbreds being 253.0 g/cap.

In the **Tsurcana breed**, the average body weight of lambs at birth and at weaning was 5.29 kg and 24.98 kg, respectively, as shown in Table 6.

Table 6. Morphoproductive indices in Tsurcana lambs subjected to intensive fattening / *Indicii morfoproductivi la mieii din rasa Țurcană supuși îngrășării intensive*

Breed	Body weight (kg)			Daily average gain (grams)
	Birth	Weaning	End of fattening	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Tsurcana	5.29 ± 0.30	24.98 ± 1.02	31.67 ± 0.94	228.16 ± 0.01

During the growing and fattening period, the lambs achieved a daily average weight gain of 228.16 g/head, having at the end fattening an average body weight of 31.67 kg/head.
In the **Tsigai breed** (rusty variety), the average daily gain achieved by male lambs is presented in Table 7.

Table 7. Morphoproductive indices in Tsigai breed (rusty variety) lambs subjected to intensive fattening / *Indicii morfoproductivi la mieii din rasa Țigaie supuși îngrășării intensive*

Breed	Body weight (kg)			Daily average gain (grams)
	Birth	Weaning	End of fattening	
	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$	$X \pm S_x$
Tsigai (Rusty variety)	4.28 ± 0.14	16.12 ± 0.30	30.55 ± 0.63	206.45 ± 0.51

At birth and at weaning **Tsigai breed** lambs' rusty variety had an average body weight of 4.28 kg and 16.12 kg/head, respectively. The daily average gain achieved during the growth and fattening period was 206.45 g/head, the final body weight being 30.55 kg/cap.

Energy (N.U) and protein (D.P) consumption per kilogram of live body weight of the batches taken into consideration is presented in table 8.

Table 8. Specific energy and protein intake obtained in lambs undergoing the fattening process / *Consumul specific de energie și proteină obținut la mieii supuși procesului de îngrășare*

Breed/ Genotype	Specific energy consumption (N.U)	Specific protein intake (D.P) (g)
Palas Merino breed	4.37	745.01
Palas Meat breed	4.78	789.06
Prolific Palas breed	6.72	915.12
Crossbreds Rouge de L'Ouest x Prolific Palas Breed	7.79	1032.58
Crossbreds Rouge de L'Ouest x Palas Meat breed	7.72	990.05
Tsurcana breed	8.39	1059.34
Tsigai breed	5.27	899.98

Among the breeds taken into studies, the lowest specific consumption was found in the **Palas Merino** and **Palas Meat breeds**. Thus, the specific energy consumption in the above-mentioned breeds was between 4.37 N.U and 4.78 N.U, while the specific protein consumption had values of 745.01 – 789.06 g D.P, being lower in the **Palas Merino**. In the other groups, the specific nutrient consumption had values between 5.27 N.U and 899.98 g D.P/kg live weight gain and 8.39 UN and 1059.34 g PBD/kg gain.

CONCLUSIONS

Through comparative research on the performance of meat production in young sheep from native breeds (**Tsurcana, Tsigai, Palas Merino**) and some crossbreeds (**Rouge de L'Ouest** x **Palas Prolific Breed, Rouge de L'Ouest** x **Palas Merino Breed**) subjected to intensive fattening, the following conclusions can be drawn:

1. The body weight of lambs at calving was between 3.82 kg and 5.29 kg, regardless of breed. The late breeds (**Tsurcana** and **Tsigai**) had the highest weight of male lambs at calving, 4.28 kg for the **Tsigai** breed and 5.29 kg for the **Tsurcana** breed. In the other breeds and crossbreeds, the weight of male lambs at calving was between 3.82 kg/head and 4.17 kg/head.
2. At the end of the growing and fattening process, body weight was variable, depending on the breed or genotype. Thus, within the **Tsigai** and **Tsurcana** breeds, the average body weights were 30.55 kg and 31.67 kg/head, respectively.
3. The highest body weights recorded at the end of the growing and fattening period were found in the **Rouge de L'Ouest** x **Palas Prolific Breed** and the **Rouge de L'Ouest** x **Palas Meat Breed**, which were 45.16 kg/head and 48.16 kg/head, respectively. The other breeds achieved final body weights ranging between 38.46 kg/head (**Prolific Palas Breed**) and 40.63 kg/head (**Palas Merino**). The average daily gain achieved by lambs during the fattening period was 320 g in the **Palas Meat Breed**, while the crossbreeds achieved an increase of 253.0 g/day (**Rouge de L'Ouest** x **Palas Meat Breed**) and 245.71 g/day (**Rouge de L'Ouest** x **Palas Prolific Breed**), respectively.
4. The specific nutrient consumption was the lowest in the **Palas Meat Breed** and the **Palas Merino** breed, in which between 4.37-4.78 UN and 745.01-789.06 g PBD were consumed to achieve one kilogram of weight gain.
5. The results obtained highlight the current good potential for meat production of local breeds and illustrate the beneficial effect of crossing with specialized meat breeds in order to make sheep farms more profitable.

ACKNOWLEDGEMENTS

This research was carried out with the support of the Ministry of Agriculture and Rural Development and funded through the ADER Project 9.1.1/12.07.2023, an ongoing project. The results obtained highlights current good potential for meat production of indigenous breeds and illustrates the beneficial effect of crossbreeding with specialized breeds for meat in view cost effectiveness sheep farms.

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RESEARCH ON THE PRODUCTIVE PERFORMANCE RECORDED IN WHITE AND PINK LINE LAMBS OF THE KARAKUL OF BOTOSANI BREED

CERCETĂRI PRIVIND PERFORMANȚA PRODUCTIVĂ ÎNREGISTRATĂ LA MIEII DIN LINIILE ALB ȘI ROZ ÎN CADRUL RASEI KARAKUL DE BOTOȘANI

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Abstract

*During the period 2022-2024, on a representative flock of lambs belonging to the **Karakul of Botosani** breed, data on lambing weight and the score obtained in the breeding sheet were processed, representative data within the framework of the control of productive performance in the case of skin production. The biological material studied belongs to RDSSGB Popauti and is registered in the Genealogical Register of the **Karakul of Botosani** breed, being the subject of the multiplication and consolidation of individuals from the two lines in order to homologate them. Currently, the degree of homogeneity of individuals from the White and Pink lines is quite high in terms of curl quality and birth weight, with an average score of 490.8 points in White lambs for breeding 490.3 points for Pink lambs for breeding in 2024, aspects favorable to their homologation.*

Key words: pelts, sheep, Karakul of Botosani, breeding sheet

Rezumat

*În perioada 2022-2024, pe un efectiv reprezentativ de miei aparținând rasei **Karakul de Botoșani**, au fost prelucrate datele referitoare la greutatea la fătare și punctajul obținut în fișa de bonitare, date reprezentative în cadrul controlului privind performanța productivă în cazul producției de pielicele. Materialul biologic studiat aparține SCDCOC Popăuți și este înscris în Registrul Genealogic al rasei **Karakul de Botoșani**, făcând obiectul înmulțirii și consolidării indivizilor din cele două linii în vederea omologării acestora. În momentul actual, gradul de omogenitate al indivizilor din liniile Alb și Roz este destul de ridicat în ceea ce privește calitatea buclajului și greutatea la naștere, cu un punctaj mediu de 490,8 puncte la mieii de prăsilă din linia Alb și 490,3 puncte la mieii de prăsilă din linia Roz în anul 2024, aspecte favorabile omologării acestora.*

Cuvinte cheie: pielicele, ovine, Karakul de Botoșani, fișă de bonitare

INTRODUCTION

The activity of forming the **Karakul of Botosani** breed began a long time ago, namely in 1948, when the first specimens of **Karakul** males from the USSR, Austria and Germany were brought to RDSSGB Popăuți to be crossed with **Tsurcana** sheep. After an extraordinary work, in 1989 the Certificate of Approval was issued for the recognition of the **Karakul of Botosani** breed with the two varieties (black and greyish). Later, in 2010 the approval of the Brown Line within the breed was achieved and in 2018 the approval of the Gray Line within the **Karakul de Botosani** breed.

The **Karakul of Botosani** breed is a Romanian breed specialized in production of pelts, formed by combining the **Karakul** breed (males from the Bukhara basin – Turkmenistan, Kazakhstan, Germany,

Austria, Bessarabia) with females from the black and grayish **Tsurcana** breed, an indigenous breed with coarse wool well adapted to the specific conditions of northeastern Moldova. Of the 7 color lines within the breed, the first four are homologated till now, as follows: black, greyish, brown and grey (Alexandru M.F. et al., 2020, Nechifor I. 2019, Pascal C. et all 1994-2001, Albertyn, L.R. et all 1993, Alemneh T., et. al., 2019).

This breed was selected and subjected to the improvement process almost exclusively for the characters that influence the quality of the pelts. Regarding body maturity, expressed by weight at mating, many publications specify that in Merino breeds, reproduction can proceed without subsequent negative effects if the weight, at the time of mating, represents at least 60–70% of that of adults (Moise et al 2012, Sandu 1993, Stăncescu 2009, Pascal et al. 2005)

Individuals from the White and Pink lines are in the breeding and consolidation phase in view of their homologation, and production performances and compliance with the breed standard contribute significantly to achieving the objective, with homologation expected in 2026.

MATERIALS AND METHODS

The research was conducted on 382 individuals from the active population of the White and Pink lines of the **Karakul of Botosani** breed, representing lambs obtained in 2022, 2023 and 2024. The individuals are registered in the Official Control of production performances of the **Karakul of Botosani** breed and using the official control data, supplemented with direct personal observations, the following were analyzed: total score and birth weight.



Figure 1. Individuals from White and Pink lines of the Karakul of Botosani breed / *Miei din liniile Alb și Roz, Rasa Karakul de Botoșani*

Once systematized, the data were processed and interpreted using methods specific to such research - arithmetic mean, error of the mean. standard deviation, coefficient of variability, significance tests, using the GraphPad Prism and SPSS Amos statistical programs. Statistics were noted with Latin letters: arithmetic mean (± s), standard deviation (σ).

The complexity of the aspects pursued required the use of a diversified work methodology depending on the aspects pursued, using and respecting the investigation methodology recommended by the specialized literature (Drucker, A.G., et al, 2001).

RESULTS AND DISCUSSIONS

The **Karakul of Botosani** lamb's performance is given by the breeding sheet score, which for lambs from the White and Pink lines was centralized in tables no. 1 and no. 2. The study on the total

score highlighted an average score of 480.4 points for those from the Alb line and 486.5 points for those from the Roz line in 2022, with no statistical differences with the scores recorded in 2022 and 2023. This fact shows us that the populations of the two lines are close in terms of productive performance and the individuals from the two lines tend towards uniformity of the quality characteristics of the curl, thus creating opportune premises for their homologation as distinct lines within the breed. Of course, the quality of the curl can always be improved, taking into account the fact that the score recorded in the **Karakul of Botosani** breed can have a maximum of 725 points, but which is currently only reached in some individuals from the Brack and Greyish lines (lines approved precisely in 1989), which subsequently, through selection and improvement work, have currently reached this performance, but which is still manifested in a limited number of individuals within the two lines.

Table 1. Statistics for the characters studied in White and Pink lambs of the Karakul of Botosani breed in 2022-2024 / *Statisticii pentru caracterele studiate la mieii Alb și Roz din cadrul rasei Karakul de Botoșani în anii 2022-2024*

Scores (points)	White line			Pink line		
	2022	2023	2024	2022	2023	2024
N	36	72	55	73	73	73
Minimum	450	410	390	415	385	400
Maximum	545	575	555	600	550	555
± s	**480.4 ± 3.69 ns**	**489.2 ± 4.35 ns**	**481.7 ± 4.66 ns**	**486.5 ± 4.37 ns**	**482.5 ± 4.17 ns**	**485 ± 4.58 ns**
σ	22.14	28.22	34.58	37.33	35.59	39.17

ns - insignificant statistically differences

Table no. 2 summarizes the data on the average performance of curls quality of lambs destined for breeding, belonging to the White and Pink lines of the **Karakul of Botosani** breed. From the data summarized in table no. 2, we can see that the average score of the lambs retained for breeding was generally higher than the total average score, with the exception of 2023 for the Pink line, where an average score of 473.7 points was recorded for breeding lambs, due to our desire to multiply the breeding flock with a larger number of lambs than we initially planned. The performance of future generations of products depends on lamb's quality retained for breeding, and therefore lambs with superior curl quality are predominantly retained for breeding.

Table 2. Statistics for the characters studied in White and Pink lambs retained for breeding within the Karakul of Botosani breed in 2022-2024 / *Statisticii pentru caracterele studiate la mieii Alb și Roz reținuți pentru prăsilă din cadrul rasei Karakul de Botoșani în anii 2022-2024*

Lambs for breeding socres (points)	White line			Pink line		
	2022	2023	2024	2022	2023	2024
N	27	26	36	58	29	55
Minimum	450	460	420	420	425	415
Maximum	545	540	575	600	545	555
± s	**481.8 ± 4.52 ns**	**490.8 ± 4.38 ns**	**490.8 ± 5.17 ns**	**493.5 ± 4.73 ns**	**473.7 ± 6.2 ns**	**490.3 ± 5.02 ns**
σ	23.49	22.35	31	35.99	33.4	37.2

ns - insignificant statistically differences

The selection of lambs for breeding based on birth weight, in the **Karakul of Botosani** breed, will influence, in addition to the size of the skin surface, their subsequent development. Table no. 3 summarizes the data regarding the average birth weight of lambs retained for breeding from the two-color lines.

The retention of lambs for breeding is not only conditioned by the quality of the curl but also by the birth weight, as a higher lamb weight will generate a skin with a larger surface area.

For the years 2022 and 2023, according to the data summarized in table no. 3, there are no significant differences in the average birth weight of both the White and Pink line lambs, with a minimum of 4.037 kg in 2022 for the White line lambs and 4.44 kg in 2023 for the Pink line lambs. In 2024, due to the selection and improvement practiced throughout the period, an average weight of 5.1 kg was

obtained for the White line lambs and 5.29 kg for the Pink line lambs, with a maximum of 6.4 kg for the White line and 8.1 kg for the Pink line. By retaining for breeding products with a higher average birth weight, we ensure the improvement of future generations of lambs.

Table 3. Evaluation of the average weight of lambs retained for breeding from the White and Pink lines in 2022-2024 / *Evaluarea greutății medii a mieilor reținuți pentru prăsilă din linii Alb și Roz în anii 2022-2024*

Lambs for breeding birth weight (points)	White line			Pink line		
	2022	2023	2024	2022	2023	2024
N	27	26	36	58	29	55
Minimum	2.3	2.8	3.7	3.2	2.5	2.9
Maximum	5.6	5.4	6.4	6.5	7.2	8.1
± s	4.037 ± 0.17 ns	4.1 ± 0.17 ns	**5.1 ± 0.12** ****	4.641 ± 0.10 ns	4.44 ± 0.17 ns	**5.29 ± 0.14** ****
σ	0.897	0.92	0.71	0.78	0.92	1.02

ns - insignificant statistically differences
**** - very significant statistically differences (*P<0,0001)*

In order to highlight the degree of improvement of the two lines, regarding birth weight, information on the average weight of all products obtained in the years 2022-2024 within the White and Pink lines was centralized in table no. 4 and figure no. 2 shows the evolution of the average birth weight of lambs from the two lines during the studied period.

Table 4. Evaluation of the average weight of lambs from the White and Pink lines in 2022-2024 / *Evaluarea greutății medii a mieilor din linii Alb și Roz în anii 2022-2024*

Birth weight (kg)	White line			Pink line		
	2022	2023	2024	2022	2023	2024
N	36	72	55	73	73	73
Minimum	2.6	2.7	3	2	2	2.9
Maximum	5.6	6	6.4	6.5	7.2	8.1
± s	3.76 ± 0.16 ns	3.962 ± 0.12 *	**4.782 ± 0.11** ****	4.3 ± 0.12 ns	4.364 ± 0.11 ns	**5.085 ± 0.12** ****
σ	0.96	0.7997	0.8336	1.03	0.9334	1.015

ns - insignificant statistically differences
** - statistically significant differences (P <0.05);*
**** - very significant statistically differences (P<0,0001)*

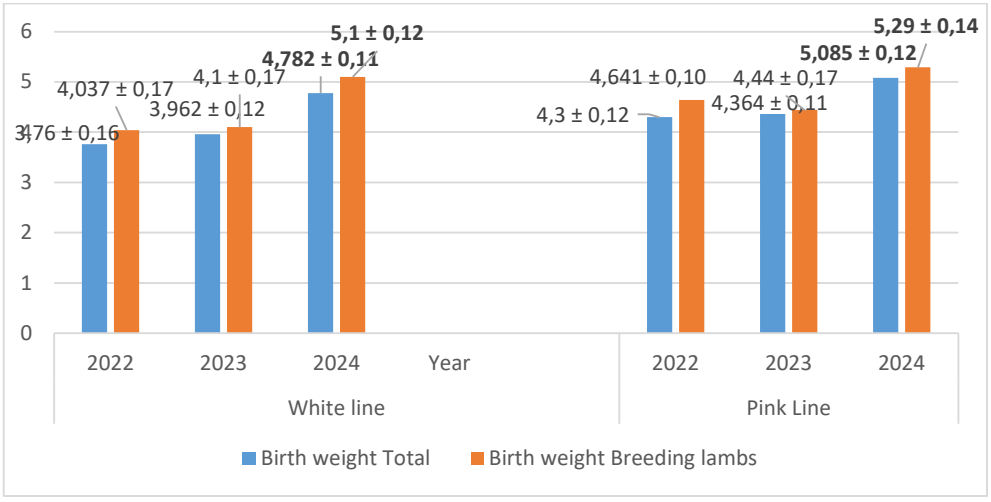


Figure 2. Average birth weight evolution of White and Pink lambs' lines during the analyzed period / *Evoluția greutății medii la naștere a mieilor din liniile Alb și Roz în perioada analizată*

According to the graph in fig. no. 2 and comparing the data centralized in tables no. 3 and 4, we can state that the individuals from the White and Pink lines obtained in 2024 had a higher average birth weight compared to those obtained in previous years, obtaining an average of 5.085 kg for the lambs retained for breeding from the Pink line and 5.1 kg for the lambs retained for breeding from the White line. The selection of breeding individuals based on body weight ensures the massiveness of the two lines and the increase in the development capacity of the next generations.

The individuals from the White and Pink lines are part of a research project aimed at their homologation, and based on the results obtained regarding birth weight and skin quality, we can state that we are on the right track regarding the opportunity of their homologation as soon as possible, respectively in 2026.

CONCLUSIONS

The following conclusions can be drawn from the study:

1. Individuals from 2024, both those from the White and Pink lines, had improved results in terms of birth weight, with an average weight of 4.78 kg for white lambs and 5.09 kg for pink lambs.
2. In each analyzed season, the average weight of individuals retained for breeding was higher than the average weight of the total lambs obtained in the season, and this aspect confirms the fact that a very important criterion in individuals selection for breeding is birth weight, the higher it is, the larger skin surface area the lambs will have.
3. The quality of the pelts is given by the score obtained in the breeding sheet and during the analyzed period, although there were no significant differences between seasons, the individuals retained for breeding had a higher score than the group average.
4. The fact that the lambs from the White and Pink lines do not record significant differences in the quality of the curl during the analyzed period confirms that the individuals from the two lines are homogeneous in terms of the quality of the curl, being a genetically consolidated population with the possibility of homologation.
5. Considering that the populations of the two lines are consolidated, we can focus our attention in the future on improving the production traits and improving the aspects related to the quality of the curl, because the maximum score limit is 725 points for the **Karakul of Botosani** breed.

ACKNOWLEDGEMENTS

White and Pink lines are an important category of **Karakul of Botosani** sheep, and this study helps us to complete the documentation for their homologation, documentation developed within the ADER 7.3.1 project.

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PRECISION LIVESTOCK FARMING AND REPRODUCTIVE BIOTECHNOLOGIES – MODERN SOLUTIONS FOR OPTIMIZING REPRODUCTIVE MANAGEMENT AND ENSURING NATIONAL FOOD SECURITY

ZOOTEHNIA DE PRECIZIE ȘI BIOTEHNOLOGIILE DE REPRODUCERE – SOLUȚII MODERNE PENTRU OPTIMIZAREA MANAGEMENTULUI REPRODUCTIV ȘI ASIGURAREA SECURITĂȚII ALIMENTARE NAȚIONALE

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Abstract

Precision livestock farming and reproductive biotechnologies, such as the use of sexed semen in dairy cows, play a crucial role in improving reproductive performance and increasing the economic efficiency of farms. A preliminary study was conducted in two dairy farms in northeastern Romania, which implemented the SenseHub® Monitoring system, based on sensors, to optimize reproductive management. The study included 40 nulliparous heifers undergoing their first artificial insemination, with procedures carried out by trained personnel based on data generated by the monitoring system. The results showed a statistically significant increase in conception rates in heifers inseminated using this system compared to conventional estrus detection methods. The implementation of such solutions—based on continuous monitoring, artificial intelligence, and real-time data analysis—allows for the precise identification of the optimal insemination time, reducing economic losses associated with inefficient reproductive cycles. Furthermore, optimizing herd genetic structure by increasing the proportion of female calves contributes to enhancing the productivity of future dairy cow generations and ensuring farm sustainability. Thus, applying modern monitoring and reproductive strategies in farms represents a viable solution for increasing the competitiveness of the livestock sector and ensuring national food security.

Keywords: **precision livestock farming, reproductive management, biotechnologies, sexed semen.**

Rezumat

Zootehnia de precizie și biotehnologiile de reproducere, precum utilizarea materialului seminal sexat la vacile de lapte, joacă un rol esențial în îmbunătățirea performanțelor de reproducție și creșterea eficienței economice a fermelor. Studiu preliminar a fost efectuat în două ferme de vaci de lapte din nord-estul României, care au implementat sistemul SenseHub® Monitoring, bazat pe senzori, pentru optimizarea managementului reproductiv. În studiu au fost incluse un număr de 40 de femele nulipare aflate la prima însămânțare artificială. Procedurile au fost realizate de personal specializat, pe baza informațiilor generate de sistemul de monitorizare. Rezultatele au indicat o creștere semnificativă statistic a ratei de concepție la femelele însămânțate, comparativ cu metodele convenționale de detecție a estrului. Implementarea unor astfel de soluții – bazate pe monitorizare continuă, inteligență artificială și analiza datelor în timp real – permite identificarea precisă a momentului optim pentru inseminare, reducând pierderile economice asociate cu ciclurile de reproducție ineficiente. În plus, optimizarea structurii genetice a turmelor prin creșterea procentului de viței contribuie la îmbunătățirea productivității viitoarelor generații de vaci de lapte și la sustenabilitatea fermelor. Astfel, aplicarea

strategiilor moderne de monitorizare și reproducere în ferme reprezintă o soluție viabilă pentru creșterea competitivității sectorului zootehnic și asigurarea securității alimentare naționale.

Cuvinte cheie: zootehnie de precizie, management reproductive, biotehnologii, material seminal sexat.

INTRODUCTION

Beef and dairy play a key role in food security and agricultural development globally and nationally. Increased competition for land and environmental water brings long-term structural sustainability issues for the global livestock sector (Bewley et al., 2017). In this context, enhancing efficiency within livestock production systems is paramount. Precision livestock farming (PLF) has emerged as a transformative approach, defined as the use of integrated technology to obtain relevant information from animals, thereby assisting in their management; it may also be referred to as precision farming, precision agriculture, or precision animal agriculture (Menendez et al., 2023; Monteiro et al., 2021; Tadele et al., 2025). This definition encompasses the broad applications of sensing technology in diverse production settings. Although pig and poultry production are commonly regarded as intensive systems, the dairy sector has been a particularly active adopter of PLF technologies, with a significant portion of PLF research originating from the bovine industry (Tzanidakis et al., 2023).

The core objective of PLF systems is the continuous, automated monitoring of animals or their environment, focusing on data collection, subsequent analysis, and the development of diagnostics pertinent to the overall production system, such as fertility status, lameness detection, or health alerts, ultimately providing actionable feedback to farm management (Morrone et al., 2022; Tzanidakis et al., 2023). The ultimate goal of PLF is to enhance the productivity and well-being of individual animals and the herd by continuously monitoring key parameters related to the producer, the production site, and the animals themselves (Simitzis et al., 2021). This involves tracking housing conditions, pen or herd parameters, and individual animal productivity metrics, often integrating data from existing farm management software (Bramley et al., 2022). While not always utilized for real-time on-farm decisions, individual performance data can be aggregated voluntarily or through paid services, allowing the industry to better understand production dynamics and respond to regulatory and consumer pressures. Continuous monitoring and subsequent data analysis enable the quantification of how various factors translate into bulk tank parameters and milk quality traits, facilitating targeted feedback to producers for enhanced performance while upholding animal well-being (Bramley et al., 2022).

The dairy industry is one of the most dynamic and innovative agribusiness sectors. Dairying significantly contributes to food security, economic development, livelihoods, and the alleviation of rural poverty. Modern dairy herds have been intensely selected and managed for high milk yield and production efficiency. High-yielding breeds, sophisticated management practices, advanced reproductive, nutritional, and health strategies, alongside stringent biosecurity measures, are increasingly common. However, these advancements often come with a trade-off: compromised fertility and reproductive efficiency, which represent major challenges in many herds worldwide (Bragança & Zangirolamo, 2018; Tolosa et al., 2021). Reproductive efficiency is a fundamental cornerstone for the economic viability and sustainability of dairy production systems, yet it is often suboptimal in high-producing herds. Improving fertility has been an ongoing challenge, with many herds experiencing declines in reproductive performance over time, often linked to environmental, management, nutritional factors, or complex interactions thereof.

Accurate and timely estrus detection is critical for successful artificial insemination (AI) and overall reproductive success (Rial et al., 2022; Sharifuzzaman et al., 2024). Traditional methods, primarily based on visual observation of estrus behaviors, are labor-intensive, time-consuming, and prone to error, especially in large herds or with animals exhibiting subtle or short estrus periods. Missed heats lead to extended calving intervals, reduced milk production per lifetime, and increased culling rates,

resulting in significant economic losses. PLF systems, equipped with sensors monitoring activity levels, rumination patterns, body temperature, or other physiological indicators, offer a promising solution for automated, continuous, and accurate estrus detection (Neculai-Valeanu et al., 2025).

Furthermore, reproductive biotechnologies, such as the use of sex-sorted semen (sexed semen), provide tools to accelerate genetic progress and optimize herd structure. Sexed semen allows producers to preferentially generate female offspring from genetically superior dams for replacements, while potentially using beef semen on lower-merit cows to produce more valuable crossbred calves. However, conception rates with sexed semen can sometimes be lower than with conventional semen, making precise insemination timing even more critical for success (Neculai-Valeanu AS & Ariton AM, 2021).

This study aims to evaluate the effectiveness of a commercially available PLF system (SenseHub®) for optimizing reproductive management, specifically focusing on improving conception rates following AI in nulliparous dairy heifers under typical farm conditions in northeastern Romania. We hypothesize that utilizing sensor-based estrus detection leads to more accurate identification of the optimal insemination window, resulting in higher conception rates compared to conventional detection methods. The findings are discussed in the context of enhancing farm efficiency, leveraging reproductive biotechnologies, and contributing to national food security objectives.

MATERIAL AND METHODS

Study Location and Animals This preliminary study was conducted on two dairy farms located in the northeastern region of Romania. Both farms utilized modern dairy management practices. A total of 40 nulliparous dairy heifers (predominantly **Holstein-Friesian** or local breeds crossed with **Holstein-Friesian**) approaching their first breeding cycle were enrolled in the study. The animals were housed and fed according to the standard operating procedures of the participating farms. The study period spanned approximately 6 months during 2024.

Precision Livestock Farming System Both participating farms had implemented the SenseHub® Monitoring system (Allflex Livestock Intelligence/MSD Animal Health). This system utilizes neck-worn sensors that continuously monitor animal activity (e.g., steps, head movements, rest) and rumination patterns. Data collected by the sensors are transmitted wirelessly to a central base station and processed by proprietary algorithms using artificial intelligence to detect deviations from individual baseline behavior. The system generates alerts indicating suspected estrus events, providing an estimated optimal insemination window for each alerted animal via farm management software and mobile applications (Figure 1).

Reproductive Management and AI Procedure. Estrus detection for the 40 study heifers was based primarely on the alerts generated by the SenseHub® system. Farm personnel responsible for reproduction were trained to interpret system alerts and inseminate heifers within the recommended optimal time window provided by the system. Artificial insemination was performed by certified AI technicians following standard protocols established on the farms, using conventional semen from proven bulls. All AI events, including date, time, and heifer ID, were recorded.

Conception Rate Determination. Pregnancy diagnosis was performed on all inseminated heifers approximately 45 days post-AI using transrectal ultrasonography, conducted by experienced veterinarians. The first-service conception rate (FSCR) was calculated as the number of heifers confirmed pregnant after the first AI divided by the total number of heifers inseminated for the first time (n=40), expressed as a percentage.

Comparison Data. The FSCR achieved using the SenseHub® system in the study group (n=40) was compared to the historical first-service conception rate data obtained from the records of the same two farms for nulliparous heifers of similar breed types inseminated during the preceding two years

(2022-2023). During that period, estrus detection relied primarily on conventional visual observation methods by farm staff (e.g., observing mounting behavior, restlessness, mucus discharge).

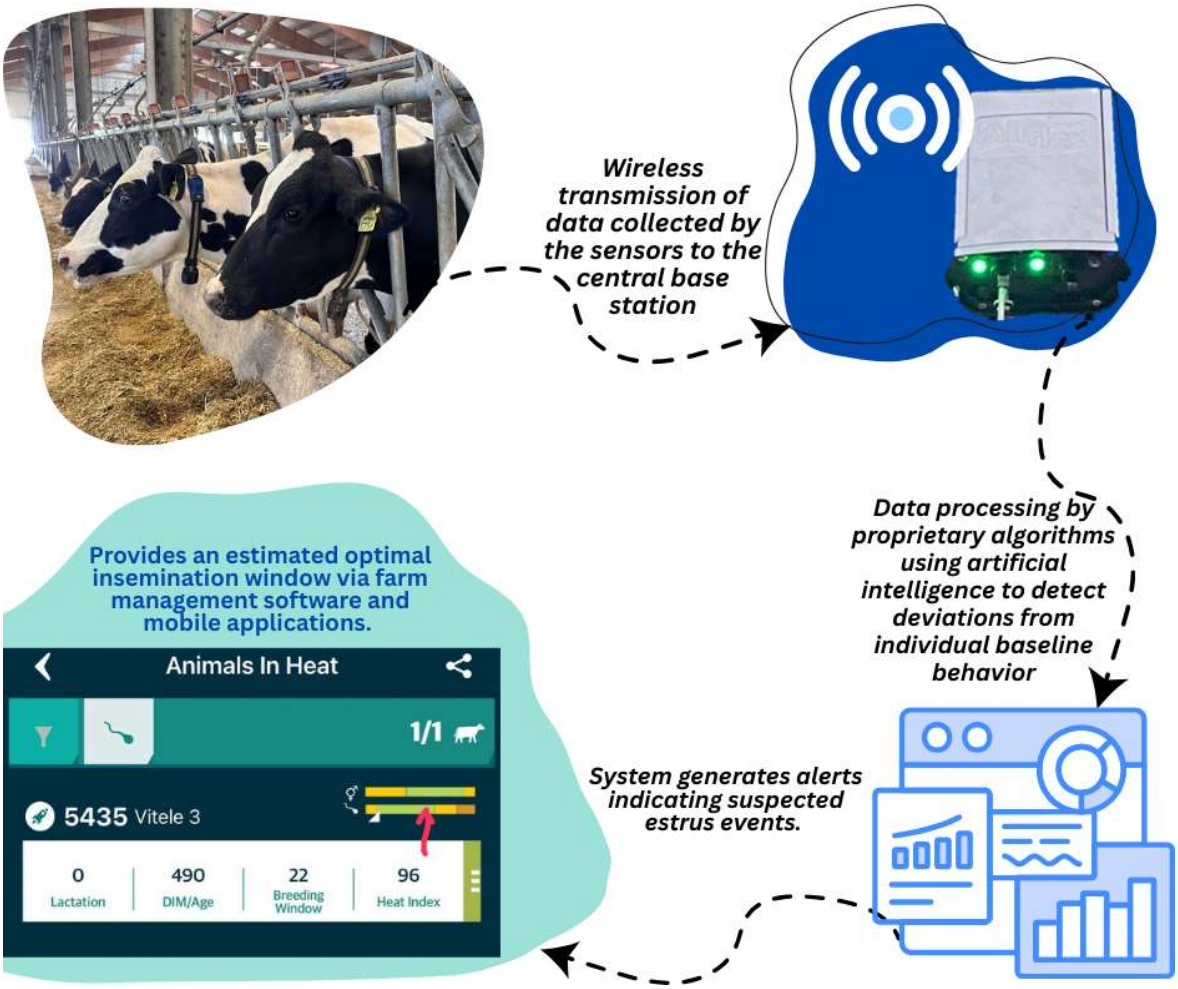


Figure 1. Schematic representation of the Allflex ecosystem / ***Prezentarea schematică a ecosistemului Allflex***

Statistical Analysis. The conception rate observed in the SenseHub® group was statistically compared to the expected conception rate based on the historical farm data using a Chi-square goodness-of-fit test. A p-value less than 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad software.

RESULTS AND DISCUSSIONS

Of the 40 nulliparous heifers monitored and inseminated based on SenseHub® system alerts, 26 were confirmed pregnant at the first service diagnosis. This resulted in a first-service conception rate (FSCR) of 65.0% for the SenseHub® group. Analysis of historical farm records from the preceding two years (2022-2023) for similar nulliparous heifers managed with conventional visual estrus detection indicated an average FSCR of 52.5% on these farms. The Chi-square goodness-of-fit test comparing the observed conceptions (26 out of 40) in the SenseHub® group against the expected conceptions based on the historical rate (expected 21 out of 40). This indicates that the conception rate achieved using the SenseHub® system was significantly higher ($p < 0.05$) than the historical baseline performance achieved

with conventional visual estrus detection methods on these farms. The SenseHub® system successfully identified estrus events and provided timely alerts, enabling farm personnel to perform AI procedures within the suggested optimal insemination window for the study animals. No major technical issues hindering the system's performance were reported during the study period.

The results of this preliminary study strongly support the hypothesis that utilizing PLF technology, specifically the SenseHub® monitoring system, significantly improves first-service conception rates in nulliparous dairy heifers compared to the historical performance achieved with conventional estrus detection methods on the same farms. The observed percentage point increase in FSCR (from 52.5% to 65.0%) demonstrates the system's practical efficacy in accurately detecting estrus and, crucially, identifying the optimal time for insemination. This enhanced precision likely overcomes the inherent limitations of visual observation, which can miss estrus events (especially silent or nocturnal heats) or misinterpret behavioral signs, leading to inseminations performed outside the optimal fertility window (Valenza et al., 2012; Stevenson, 2014; Endo, 2022). The significant improvement provides strong evidence for the system's positive impact.

The economic implications of improved conception rates are substantial. A higher FSCR translates directly into reduced days open, fewer inseminations required per pregnancy (lowering semen and labor costs), and ultimately, a shorter calving interval, moving heifers into production more quickly. These factors collectively enhance the overall economic efficiency and profitability of the dairy operation (Burgers et al., 2022, 2023; Krpálková et al., 2020) By minimizing reproductive inefficiency, PLF systems contribute to the farm's sustainability. The successful application of the SenseHub® system highlights the practical benefits of integrating continuous monitoring, data analysis, and artificial intelligence into routine farm management. Such systems provide objective, data-driven insights into individual animal status, allowing for more timely and precise interventions. This aligns with the broader goals of PLF, which aims to optimize production while potentially improving animal welfare through early detection of health or reproductive issues (Bianchi et al., 2022; Das et al., 2023; Trapanese et al., 2025).

Furthermore, the ability of PLF systems to pinpoint the optimal insemination time is particularly relevant when considering the use of reproductive biotechnologies like sexed semen. While sexed semen offers the advantage of controlling offspring sex for targeted herd development, it often exhibits slightly reduced fertility compared to conventional semen (Neculai-Valeanu & Ariton, 2021). Although conventional semen was used in this specific trial, the demonstrated improvement in timing accuracy suggests that PLF systems could be crucial for achieving acceptable conception rates when using sexed semen, thereby fully leveraging its potential for accelerating genetic gain and optimizing herd demographics. This contributes to enhancing the productivity of future generations and supports long-term farm planning.

In the context of Romania and national food security, improving the efficiency of the domestic dairy sector remains vital in 2025. Technologies that boost reproductive performance, like the PLF system evaluated here, can increase milk production efficiency, reduce reliance on imports, and enhance the competitiveness of local farms (Bianchi et al., 2022; Trapanese et al., 2025). By enabling more efficient reproduction and potentially facilitating better use of advanced genetics (including sexed semen in the future), these modern solutions contribute to a more resilient and secure national food supply chain.

This study, being preliminary, has certain limitations. The sample size (40 heifers) is relatively small, and the study was confined to two farms in northeastern Romania. The comparison relied on historical data rather than a concurrent, randomized control group; while common in farm studies, this approach cannot entirely rule out confounding factors related to time (e.g., subtle changes in nutrition or management between the historical period and the study period). Future research should ideally involve larger, multi-farm studies encompassing a randomized controlled trial design if feasible. Further investigation into the specific algorithms and sensor parameters most critical for estrus detection

accuracy would also be beneficial. Additionally, a detailed economic analysis quantifying the return on investment for implementing such PLF systems under Romanian conditions, specifically assessing the cost-benefit when used in conjunction with sexed semen strategies, is warranted.

CONCLUSIONS

This study demonstrated that the implementation of the SenseHub® PLF system significantly improved first-service conception rates (65.0% vs. 52.5%) in nulliparous dairy heifers on two Romanian farms compared to reliance on conventional visual estrus detection methods. The findings highlight the substantial potential of modern technological solutions, based on continuous monitoring and real-time data analysis, to optimize reproductive management in dairy cattle by precisely identifying the optimal time for artificial insemination. Improving reproductive efficiency directly impacts farm profitability and sustainability. When potentially combined with reproductive biotechnologies like sexed semen, PLF systems can further enhance genetic progress and herd management strategies. The adoption of such modern monitoring and reproductive approaches represents a viable pathway for increasing the competitiveness of the Romanian livestock sector and contributing positively to national food security.

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THE INFLUENCE OF DIFFERENTIATED FERTILIZATION OF NATURAL GRASSLANDS ON GREEN MASS PRODUCTION AND QUANTITATIVE AND QUALITATIVE MILK PRODUCTION IN SHEEP OF THE TSIGAI BREED

INFLUENȚA FERTILIZĂRII DIFERENȚIATE A PAJIȘTILOR NATURALE ASUPRA PRODUCȚIEI DE MASĂ VERDE ȘI A PRODUCȚIEI CANTITATIVE ȘI CALITATIVE DE LAPTE LA OILE DIN RASA ȚIGAIE

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Abstract

According to the data provided by the last Agricultural Census carried out in our country, permanent pastures and hayfields lost approx. 21% of their existing area in 1990 as a result of their abandonment and improper use. Efficient management of natural grasslands is essential for optimal animal nutrition, mitigation and adaptation to climate change, biodiversity conservation, landscape management and soil fertility. This study aimed to investigate the effect of differentiated fertilization of permanent pastures in the Moldavian Plateau area on green mass production and quantitative and qualitative milk production in sheep of the ***Tsigai*** *breed, the rust variety. The experiments were carried out within the RDSSGB Secuieni Bacău during 2023-2024. The best results in terms of pasture productivity and quality were obtained when using manure fertilization (15 tons/hectare) associated with over-seeding with valuable fodder plants (perennial grasses and legumes). In terms of quantitative and qualitative milk production, the best results were achieved by sheep fed with green mass from pasture fertilized with chemical and organic fertilizers ($N_{80}P_{50}K_{50}$/hectare + 7.5 tons of manure/hectare).*

Key words: *fertilization, green mass, natural meadows, milk production, sheep*

Rezumat

Conform datelor furnizate de ultimul Recensământ Agricol efectuat în țara noastră pășunile și fânețele permanente au pierdut cca. 21% din suprafața existentă în anul 1990, ca urmare a abandonării și a utilizării lor defectuoase. Gestionarea eficientă a pajiștilor naturale este esențială pentru hrănirea optimă a animalelor, atenuarea și adaptarea la schimbările climatice, conservarea biodiversității, gestionarea peisajului și fertilitatea solului. Acest studiu și-a propus să investigheze efectul fertilizării diferențiate a pășunilor permanente din zona Podișului Moldovei asupra producției de masă verde și a producției cantitative și calitative de lapte la oile din rasa ***Țigaie****, varietatea ruginie. Experiențele au fost efectuate în cadrul SDCOC Secuieni Bacău în perioada 2023-2024. Cele mai bune rezultate din punct de vedere al productivității și calității pășunilor s-au obținut în cazul utilizării fertilizării cu gunoi de grajd (15 tone/hectar), asociată cu supraînsămânțarea cu plante furajere valoroase (graminee și leguminoase perene). Sub raportul producției cantitative și calitative de lapte rezultatele cele mai bune le-au realizat oile hrănite cu masă verde provenită de pe pășunea fertilizată cu îngrășăminte chimice și organice ($N_{80}P_{50}K_{50}$/hectar + 7,5 tone de gunoi de grajd/hectar).*

Cuvinte cheie: *fertilizare, masă verde****,*** *pajiști naturale, producție de lapte, ovine*

INTRODUCTION

Permanent grasslands represent 34% of the agricultural area of the European Union, playing a key role in providing a wide range of ecosystem services vital to society. In recent decades, the area of these grasslands has decreased significantly (between 30 and 50%) in many Member States, and is still threatened by land-use change (Schils et al., 2022; Peeters, 2009).

In Eastern Europe, including Romania, political transformations since the late 1980s led to the massive abandonment of permanent grasslands, many of which were left unused and subsequently degraded (Torok et al., 2020). Thus, in the period 2010–2020, the area of pastures and hayfields in Romania decreased by 17.4% (INS, 2024).

The grassland is a multifunctional system with different utilities: fodder production, plant and animal biodiversity, stopping soil erosion, water storage, maintaining groundwater quality, ensuring landscape quality, carbon storage, supplying the soil with biologically fixed nitrogen etc. Permanent grasslands – natural or/and semi-natural – are an important part of the natural heritage, and their use through grazing represents the cheapest source of fodder.

Current research in the field of permanent grasslands focuses on sustainable management and conservation methods, focused on soil health, carbon sequestration and production efficiency. For the sustainable use of these ecosystems, an interdisciplinary approach that integrates climatic, edaphic and vegetation and animal management factors is essential.

The experiences within this work were carried out at RDSSGB Secuieni Bacău during 2023-2024. The aim of this study was to investigate the effect of differential fertilization of permanent pastures in the Moldavian Plateau area on green mass production and quantitative and qualitative milk production in **Tsigai** sheep, the rusty variety.

MATERIAL AND METHOD

The research began in 2023, with the completion of a study on the natural conditions in the Moldavian Plateau area, the identification of permanent grasslands suitable for conducting experiments and the organization of the experimental device (demarcation, fencing and establishment of fields). To achieve the proposed objectives, four experimental plots were set up, each with an area of 1.4 ha, with the following treatments applied: Plot 1 – control, without fertilization; Plot 2 – combined fertilization: chemical fertilizers ($N_{80}P_{50}K_{50}$/ha) + organic fertilizers (7.5 t manure/ha); Plot 3 – organic fertilization: 25 t manure/ha; Plot 4 – organic fertilization (15 t manure/ha) + overseeding.

Plots 3 and 4 were additionally fertilized with simple phosphate (P_2O_5 – 30 kg/ha), given the low phosphorus content of the manure. In experimental plots 2, 3 and 4, agro-ecological maintenance works were also carried out – stone removal, clearing of woody vegetation, weed control – to improve the quality of the available green mass.

To achieve the main purpose of the research, the following steps were taken: analysis of the quantitative and qualitative milk production performances of the **Tsigai** breed (rusty variety) sheep breed; evaluation of the productivity of the meadows and the quality of the forages in the experimental variants. The working methods adopted were those specific to research of this type.

Organization of animal lots. Four batches of sheep were organized, one control batch and three experimental batches. When establishing the batch size, the estimated green mass production to be obtained from the four permanent grassland areas was taken into account, as well as the following factors: breed, age, lactation stage, growing environment etc. Consequently, four batches of animals were established, in the amount of 10 sheep for the control batch and 15 sheep for each experimental batch, eliminating all variation factors.

The 2024 animal experiments were initially planned to be carried out over a period equivalent to the grazing period specific to the area (approx. 6 months). For objective reasons (delay in the acquisition of chemical fertilizers and implicitly the application of fertilization with this type of fertilizer, the difficult acquisition of the plant mixture used for over-seeding and the delay in carrying out this work), the experiments could only be carried out over a period of 4 months, respectively in the months of June-September. The experiments started with monitoring the quantitative and qualitative milk production performances of the sheep in the 4 batches, as well as evaluating the productivity of the meadows and the quality of the fodder in the experimental variants.

Monitoring the quantitative and qualitative milk production performances. The working methods used in the research were appropriate for the objectives of this kind. Thus, the evaluation of milk production performances was based on the application of successive productive controls, at 30-day intervals. At each control interval, the standard method was used, namely AT4, respecting the technical specifications recommended by the International Committee for Animal Recording (ICAR). At each control applied, the amount of milk milked from each female was determined by weighing using an approved instrument with an accuracy of ±5 g, and the estimation of the average total milk production obtained per lactation considered was carried out using the method described by Fleischmann (ICAR, 2018).

Research on milk quality was carried out only during the experiments (4 months), which coincided with the milking period of the sheep. For the analysis of milk quality, determinations were made at each control (5 milk samples/control/control batch and 7 milk samples/control/experimental batches) regarding the chemical composition of the milk in the main constituents, namely dry matter, and within the dry matter the content of fat, protein, lactose and mineral salts. The determination of the chemical composition of the milk samples was carried out in the own laboratory of RDSSGB Secuieni Bacău using the Funke Gerber milk analyzer type LactoStar.

Evaluation of green mass production. The evaluation of the green mass production obtained for each of the 4 permanent grassland plots was done according to the classical methodology (Bărbulescu et al., 1983), by harvesting at each mowing or grazing cycle (once a month), areas of 10 m^2 from 5 points (depending on the degree of uniformity of the vegetation and the area considered) located on the diagonal of the meadow. The production resulting from all the control points is weighed, then divided by the total harvested area and the green mass production per square meter (m^2) is obtained, denoted by G. By multiplying the green mass production per square meter in kilograms from each mowing (G1,2,3....) by the area of one hectare (10,000 m^2), the production per hectare per cycles (C1,2,3....) or mowing is obtained and is expressed in kg/ha. Subsequently, by adding all cycles (C1 + C2 + C3 + ...) or scythes, the total annual green mass production per hectare (C) is obtained, which can be expressed in kg/ha or tons/ha.

Calculation formulas:

a. $C_{1,2,3\ .....} = G_{1,2,3.....} \times 10000$

b. $C = C_1 + C_2 + C_3 + ...$

Statistical analysis. The data obtained from the determinations performed were processed according to the classical methodology of each type of determination, and subsequently they were interpreted by comparing them with the data from the specialized literature. The results are presented as mean values ± standard errors of the mean. Microsoft Office Excel 2016 was used to calculate all statistical parameters (mean, standard deviation, coefficient of variability and standard error of the mean values) and the Tukey test to determine the significance of the difference between the mean values. Differences were considered statistically significant at $P<0.05$ and indicated by specific superscripts.

RESULTS AND DISCUSSION

Quantitative and qualitative analysis of milk production

Daily, monthly and total milk yield. The **Tsigai** breed sheep, which constituted the experimental groups, achieved during the 4 months of control an average daily milk production relatively similar to the data available in the literature (Taftă et al., 1997). The results of the average daily milk production by groups and lactation months, recorded following the official control, are presented in Table 1.

The highest average daily production was recorded by the sheep in group 2 (388±17.18 ml), closely followed by the production achieved by the sheep in group 4, then by those in the control group, and in last place by the production achieved by the sheep in group 3. It should be noted that the differences between the groups are insignificant (P>0.05).

Table 1. Evolution of the average daily milk quantity during the period June-September (ml/day) / *Evoluţia cantităţii medii zilnice de lapte pe perioada iunie-septembrie (ml/zi)*

Specification	Control Lot	Lot 2	Lot 3	Lot 4
June	590±19.76	609±30.55	504±12.46	617±14.99
July	498±29.87	505±30.50	574±23.84	508±20.59
August	264±24.11	272±26.53	249±12.96	268±12.23
September	131±8.39	165±10.98	150±8.79	152±7.58
Mean	**371±16.49**	**388±17.18**	**369±9.10**	**386±8.16**
S	**52.11**	**66.50**	**45.48**	**40.80**
CV%	**14.05**	**17.15**	**12.31**	**10.56**

NS – non-significant differences (P>0.05); *significant differences (P<0.05); **distinctly significant differences (P<0.01); ***highly significant differences (P<0.001).

Following the ranking based on the average daily production achieved by the ewes in the experimental batches, the total milk production during the 4 months of the experiment followed the same path in terms of batch ranking (Table 2). The differences between batches in terms of total milk production are also insignificant (P>0.05).

Table 2. Evolution of monthly and total milk quantity during June-September (liters) / *Evoluţia cantităţii lunare şi totală de lapte pe perioada iunie-septembrie (litri)*

Specification	Control Lot	Lot 2	Lot 3	Lot 4
June	17.70±0.59	18.28±0.92	17.24±0.72	18.52±0.45
July	15.44±0.93	15.66±0.65	15.62±0.39	15.74±0.61
August	8.20±0.75	8.43±0.82	7.72±0.40	8.31±0.38
September	3.94±0.25	4.94±0.33	4.51±0.26	4.55±0.23
Mean	**45.27±2.03**	**47.30±2.10**	**45.16±1.12**	**47.13±1.00**
S	**6.41**	**8.13**	**5.59**	**4.99**
CV%	**14.15**	**17.18**	**12.38**	**10.59**

NS – non-significant differences (P>0.05); *significant differences (P<0.05); **distinctly significant differences (P<0.01); ***highly significan differences (P<0.001).

The highest total milk production was recorded by the sheep in batch 2 (47.30±2.10 liters), which benefited from green mass from the mixed fertilized pasture (pasture fertilized with chemical fertilizers $N_{80}P_{50}K_{50}$/hectare + organic fertilizers, 7.5 tons of manure/hectare), and the lowest level by the sheep in batch 3 (45.16±1.12 liters), which had as a source of volume feed the green mass obtained from the pasture fertilized only with organic fertilizers (25 tons of manure/hectare).

The results regarding the total milk production obtained during the period analyzed in our experiment are similar to those obtained by Tsiplakou et al. (2010), who found that sheep from the group

fed with conventionally obtained fodder recorded an average daily milk production 37.5% higher than that achieved by sheep from the group fed with organic manure-based fodder.

Contrary to the results obtained in the present experience, the results communicated within the ADER 7.1.1/2015-2018 project, carried out within RDSSGB Caransebeș, show that sheep that were fed green mass from the pasture fertilized with manure recorded a milk production with only 2.8% higher (78.95 liters versus 76.81 liters) compared to sheep that were fed green mass obtained from the pasture fertilized with chemical fertilizers. The data on the specific consumption for obtaining one liter of milk are presented in Table 3.

Table 3. Specific consumption per liter of milk / *Consumul specific pe litru de lapte*

Specification	Control Lot	Lot 2	Lot 3	Lot 4
Specific energy consumption (UNL/liter of milk)	4.031	4.029	4.222	4.168
Differences (%) compared to the control group	-	-0.05	4.74	3.40
Specific protein consumption (g PDIN/liter milk)	410.606	427.619	448.173	429.264
Differences (%) compared to the control group	-	4.14	9.15	4.54
Specific concentrate consumption (kg/liter milk)	0.567	0.685	0.718	0.688
Differences (%) compared to the control group	-	20.81	26.63	21.34

From the data presented in table 3, it is found that the best energy consumption was recorded in group 2, which is lower by 0.05% compared to the control group, while the consumption in groups 3 and 4 is higher by 4.74% and 3.4% respectively. From the analysis of protein consumption, it is found that it is higher by 4.14-9.15% in experimental groups 2, 3 and 4 compared to the control group. Regarding the consumption of concentrates, it is found that in experimental groups 2, 3 and 4 the consumption is also higher by 20.81-26.83% compared to the control group.

In conclusion, it appears that the most efficient batch in terms of specific energy consumption is batch 2, which received green mass from the mixed fertilized pasture as feed (pasture fertilized with chemical fertilizers $N_{80}P_{50}K_{50}$/hectare + organic fertilizers, 7.5 tons of manure/hectare), and in terms of protein and concentrate consumption it is the control batch that received green mass from the unfertilized pasture as feed.

Qualitative analysis of milk production. Milk quality is understood as its ability to satisfy consumer requirements. This is expressed through 6 basic and 3 additional components. The basic components of quality are: sensory, nutritional, hygienic-sanitary, psycho-social, technological and commercial. The additional components are: quality constancy, quality perception and quality-price ratio. Data on milk quality in experimental sheep flocks are presented in Table 4.

Table 4. Milk quality results (%) (n=5 in the control group and n=7 in groups 2, 3 and 4) / *Rezultate privind calitatea laptelui (%) (n=5 la lot martor și n=7 la loturile 2, 3 și 4)*

Specification	Control Lot		Lot 2		Lot 3		Lot 4	
	$\bar{X} \pm s_{\bar{x}}$	CV%	$\bar{X} \pm s_{\bar{x}}$	CV%	$\bar{X} \pm s_{\bar{x}}$	CV%	$\bar{X} \pm s_{\bar{x}}$	CV%
Fat	7.95±0.11	2.98	8.17±0.11	3.44	8.08±0.06	2.06	8.15±0.03	2.31
Protein	5.90±0.04	1.35	5.81±0.05	2.34	5.49±0.04	2.01	5.82±0.02	2.10
Lactose	4.48±0.04	1.89	4.61±0.05	3.14	4.58±0.06	3.28	4.56±0.02	3.73
Minerals	0.90±0.02	4.57	0.95±0.02	4.57	0.94±0.02	5.03	0.93±0.01	5.99
Dry Mattter	19.24±0.08	0.90	20.15±0.13	1.71	19.39±0.11	1.53	19.45±0.04	1.57

The analysis of the data in Table 4 shows the following:

- the highest average fat percentage is recorded in group 2 (8.17±0.11%), and the lowest percentage in the control group (7.95±0.11%);

- the highest average protein percentage is recorded in the control group (5.90±0.04%), and the lowest percentage in group 3 (5.49±0.04%);

- the highest average dry matter percentage is recorded in group 2 (19.24±0.08%), and the lowest one in the control group (19.24±0.08%);

These values are within the normal limits of raw whole sheep milk, specific for the **Țigaie** breed (Taftă, 2008). Following the analysis of the significance of the differences in dry matter content, it is found that it is very significantly higher (P<0.001) in group 2 compared to the control group, very significantly higher (P<0.001) in group 2 compared to group 3 and distinctly significantly higher (P<0.01) in group 2 compared to group 4 (Table 5).

Table 5. Difference in dry matter content (%) and statistical significance between batches / *Diferența privind conținutul în substanță uscată (%) și semnificația statistică între loturi*

Tukey Test	Control Lot	Lot 2	Lot 3	Lot 4
Control Lot	0	0.91***	0.15^{NS}	0.21 ^{NS}
Lot 2	-	0	0.76***	0.70**
Lot 3	-	-	0	0.06 ^{NS}
Lot 4	-	-	-	0

NS – non-significant differences (P>0.05); *significant differences (P<0.05); **distinctly significant differences (P<0.01); ***highly significant differences (P<0.001).

The results regarding milk quality obtained during the period analyzed in our experience are similar to those obtained by Tsiplakou et al. (2010), who found that the sheep in the group fed with conventionally obtained feed recorded a dry matter content 8.1% higher than that achieved by the sheep in the group fed with organically obtained feed based on manure, the difference being significant (P<0.05). Similar results to those obtained in the present experience are also those communicated in the ADER 7.1.1/2015-2018 project, carried out within RDSSGB Caransebeș, where it is found that the sheep that were fed with green mass from the chemically fertilized pasture had a dry matter content higher by approx. 2% compared to the sheep that were fed with green mass from the pasture fertilized with manure.

Analysis of green mass production and consumption

The green mass requirement for animals was ensured by administering daily feed rations to the manger. The daily feed ration was weighed in the morning before administration, being administered in two doses/day, respectively in the morning and in the evening. At the same time, uneaten leftovers were weighed every morning in order to calculate feed consumption. Data on the amount of feed administered and the amount of uneaten leftovers were entered daily in the experiment record book, separately for each type of feed and for each batch of animals.

The rations administered to the animals during the experiment are presented synthetically in Tables 6 and 7. During the August-September period, due to the drought, green mass production was considerably reduced and we had to reduce the amount of green mass in the animals' rations by almost half, and the need for nutrients was covered by administering alfalfa hay.

From the analysis of the two tables it can be seen that the ration used for the control group is different from the rations of the experimental groups in terms of the amount of green mass administered, while the amount of concentrates and alfalfa hay is identical for all four groups, the rations differing only in the amount of green mass and the origin of the green fodder (each group of animals received mowed green mass and administered to the manger from the plot specific to the group).

The height of the plants at harvest was approx. 15-20 cm. The indicative daily delimitation of the pasture area to be mowed was made as follows: 75-100 m^2 for 1 UVM (1 large cattle unit = 6.6 sheep)

for the first two grazing cycles (mowing); 150-300 m^2 for 1 UVM for the following grazing cycles (mowing).

Table 6. Feed rations used in animal experiments (sheep) in 2024 (kg/head/day) during June-July / *Raţii furajere utilizate în experienţele cu animale (oi) din anul 2024 (kg/cap/zi) în perioada iunie-iulie*

Crt. No.	Specification	Meadow (green mowed grass)	Corn grain	Wheat grain
1.	Control lot (green mass source: unfertilized pasture)	7.0	0.15	0.15
2.	Lot 2 (green mass source: chemically fertilized pasture + manure)	6.7	0.15	0.15
3.	Lot 3 (green mass source: pasture fertilized with manure)	6.7	0.15	0.15
4.	Lot 4 (green mass source: pasture fertilized with manure + overseeding)	6.7	0.15	0.15

Table 7. Feed rations used in animal experiments (sheep) in 2024 (kg/head/day) during August-September / *Raţii furajere utilizate în experienţele cu animale (oi) din anul 2024 (kg/cap/zi) în perioada august-septembrie*

Crt. No.	Specification	Meadow (green mowed grass)	Alfalfa hay	Corn grain	Wheat grain
1.	Control lot (green mass source: unfertilized pasture)	3.3	1.0	0.15	0.10
2.	Lot 2 (green mass source: chemically fertilized pasture + manure)	3.0	1.0	0.15	0.10
3.	Lot 3 (green mass source: pasture fertilized with manure)	3.0	1.0	0.15	0.10
4.	Lot 4 (green mass source: pasture fertilized with manure + overseeding)	3.0	1.0	0.15	0.10

The biomass quantity was calculated according to the classical methodology (repeated mowing method) (Bărbulescu et al., 1984). The biomass obtained per hectare after monitoring the 4 mowing cycles, carried out during the months of June, July, August and September, is presented in Table 8.
From the analysis of the data recorded in Table 8, it is found that the green mass production was different depending on the type of plot. Thus, during the analyzed period (June-September), the highest green mass production was recorded in lot 4 (8840 kg/ha), which is approx. 65.85% higher than the production of the control lot, followed by lot 2 (8050 kg/ha), then by lot 3 (6560 kg/ha) and by the control lot in 4th place (5330 kg/ha).

The results regarding green mass production expressed in dry matter are presented in Table 9, taking into account the 5:1 ratio between green mass and dry matter (Marușca et al. 2014). Regarding the monthly amount of green mass consumed, the results obtained are presented in Table 10.

Table 8. Green mass production during June-September (kg/ha) / *Producţia de masă verde pe perioada iunie-septembrie (kg/ha)*

Specification	Cycle 1 (June)	Cycle 2 (July)	Cycle 3 (August)	Cycle 4 (September)	Production (June-September)	Comparative production (%)
Control meadow	1900	1510	1110	810	5330	100
Meadow Lot 2	2720	2500	1610	1220	8050	151.03
Meadow Lot 3	2310	1950	1300	1000	6560	123.07
Meadow Lot 4	3010	2710	1820	1300	8840	165.85

Table 9. Influence of fertilization type on dry matter (DM) production of pasture plots within the experiment / *Influenţa tipului de fertilizare asupra producţiei de substanţă uscată (SU) a loturilor de păşune din cadrul experienţei*

Specification	Dry matter (t/ha)	Absolute differences (t)	Percentage differences (%)
Control lot (green mass source: unfertilized pasture)	1.066	-	100
Lot 2 (green mass source: chemically fertilized pasture + manure)	1.610	0.544	151.03
Lot 3 (green mass source: pasture fertilized with manure)	1.312	0.246	123.07
Lot 4 (green mass source: pasture fertilized with manure + overseeding)	1.768	0.702	165.85

Table 10. Monthly quantity of green mass consumed during the analyzed period /
Cantitatea consumată lunar de masă verde pe perioada analizată

Specification	Lots	Amount of green mass consumed (kg/head/day)
June	Control Lot	6.76
	Lot 2	6.57
	Lot 3	6.57
	Lot 4	6.57
July	Control Lot	6.77
	Lot 2	6.58
	Lot 3	6.58
	Lot 4	6.58
August	Control Lot	3.09
	Lot 2	2.80
	Lot 3	2.80
	Lot 4	2.80
September	Control Lot	3.19
	Lot 2	2.90
	Lot 3	2.90
	Lot 4	2.90

The monthly amount of green mass consumed was relatively close in all 4 groups, namely in June it was 6.76 kg/head/day in the animals in the control group and 6.57 kg/head/day in the animals in the experimental groups, in July it was 6.77 kg/head/day in the animals in the control group and 6.58 kg/head/day in the animals in the experimental groups, in August it was 3.09 kg/head/day in the animals in the control group and 2.80 kg/head/day in the animals in the experimental groups, and in September it was 3.19 kg/head/day in the animals in the control group and 2.90 kg/head/day in the animals in the experimental groups.

In conclusion, the best influence on green mass production per hectare had a fertilization variant applied to lot 4 – pasture fertilized with organic fertilizers (15 tons of manure/hectare) + overseeding. The results obtained in our experience are similar to those obtained in the ADER 7.1.1/2015-2018 project carried out within RDSSGB Caransebeș, as well as with other research in the specialized literature (Marușca, 2012).

CONCLUSIONS

Following the activities carried out to achieve the objective of this stage, the following conclusions can be drawn:

1. The highest average daily milk production was recorded by the sheep in batch 2 (388±17.18 ml), closely followed by the production achieved by the sheep in batch 4, then by those in the control batch, and in last place by the production achieved by the sheep in batch 3, the differences between batches being insignificant ($P>0.05$).
2. The highest total milk production was recorded by the sheep in batch 2 (47.30±2.10 liters), which benefited from green mass from the mixed fertilized pasture (pasture fertilized with chemical fertilizers $N_{80}P_{50}K_{50}$/hectare + organic fertilizers, 7.5 tons of manure/hectare), and the lowest by the sheep in batch 3 (45.16±1.12 liters), which had as a source of bulk feed the green mass obtained from the pasture fertilized only with organic fertilizers (25 tons of manure/hectare).
3. The most efficient batch in terms of specific energy consumption is batch 2, which received green mass from the mixed fertilized pasture as volume feed (pasture fertilized with chemical fertilizers $N_{80}P_{50}K_{50}$/hectare + organic fertilizers, 7.5 tons of manure/hectare), and in terms of protein and concentrate consumption it is the control batch that received green mass from the unfertilized pasture as volume feed.

4. Following the analysis of the significance of the differences in milk quality, it was found that the dry matter content is very significantly higher (P<0.001) in batch 2 compared to the control batch, very significantly higher (P<0.001) in batch 2 compared to batch 3 and distinctly significantly higher (P<0.01) in batch 2 compared to batch 4.
5. The highest green mass production over the 4 mowing cycles (June-September) in the pasture plots within the experiment was recorded in batch 4 (8840 kg/ha), which is approx. 65.85% higher than the production of the control batch, followed by batch 2 (8050 kg/ha), then by batch 3 (6560 kg/ha) and by the control batch in last place (5330 kg/ha).
6. The best influence on green mass production per hectare was that by a fertilization variant applied to lot 4 – pasture fertilized with organic fertilizers (15 tons of manure/hectare) + overseeding with a mixture of perennial grasses and legumes.
7. The monthly amount of green mass consumed was relatively close in all 4 groups, respectively in June being 6.76 kg/head/day in the animals in the control group and 6.57 kg/head/day in the animals in the experimental groups, in July being 6.77 kg/head/day in the animals in the control group and 6.58 kg/head/day in the animals in the experimental groups, in August being 3.09 kg/head/day in the animals in the control group and 2.80 kg/head/day in the animals in the experimental groups, and in September being 3.19 kg/head/day in the animals in the control group and 2.90 kg/head/day in the animals in the experimental groups.
8. The research carried out highlighted the fact that the technology of free-range animal maintenance in collective stalls with an outdoor paddock, used in the experiment, led to the good performance of all feeding, watering, milking operations, as well as the possibility of making detailed observations on the behavior of the sheep, the way of utilizing the feed, the assessment of their health status etc.

ACKNOWLEDGMENTS

This work was supported through the Sectoral Plan for Research and Development of the Ministry of Agriculture and Rural Development, for the years 2023-2026 "Agriculture and Rural Development - ADER 2026", Cod Project ADER: 15.2.3.

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MODERN MANAGEMENT STRATEGIES TO REDUCE LABOR DEPENDENCY ON DAIRY FARMS

STRATEGII MODERNE DE MANAGEMENT PENTRU REDUCEREA DEPENDENȚEI DE FORȚA DE MUNCĂ ÎN FERMELE DE VACI DE LAPTE

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Abstract

Labor shortage is one of the biggest challenges for dairy farms in Romania, having a direct impact on their productivity and sustainability. In this context, digitalization and automation become essential solutions to optimize processes and reduce dependence on manual labor. This paper analyzes the organizational and human resources management in six dairy cow farms with different farming systems (intensive, semi-intensive and extensive) in Northeast Romania. The study examines the organization of the daily work schedule on the farm, the efficiency of specialized teams compared to generalized service, and the impact of management strategies on productivity. It also discusses digitization and automation solutions applied in managerial decision-making, highlighting the advantages of using modern technologies to increase operational efficiency. The results of the study demonstrate that the implementation of these technologies contributes to optimizing production processes, reducing costs and improving the safety and quality of dairy products, as well as food security.

Keywords: dairy farms, food safety, automation, precision livestock farming, artificial intelligence.

Rezumat

Lipsa forței de muncă reprezintă una dintre cele mai mari provocări pentru fermele de lapte din România, având un impact direct asupra productivității și sustenabilității acestora. În acest context, digitalizarea și automatizarea devin soluții esențiale pentru optimizarea proceselor și reducerea dependenței de muncă manuală. Această lucrare analizează managementul organizațional și al resurselor umane în șase ferme de vaci de lapte, cu diferite sisteme de exploatare (intensiv, semi-intensiv și extensiv) din nord-estul României. Studiul examinează organizarea programului zilnic de lucru în fermă, eficiența echipelor specializate comparativ cu deservirea generalizată, precum și impactul strategiilor de management asupra productivității. De asemenea, sunt discutate soluțiile de digitalizare și automatizare aplicate în luarea deciziilor manageriale, evidențiindu-se avantajele utilizării tehnologiilor moderne pentru creșterea eficienței operaționale. Rezultatele studiului demonstrează că implementarea acestor tehnologii contribuie la optimizarea proceselor de producție, reducerea costurilor și îmbunătățirea siguranței și calității produselor lactate, precum și a securității alimentare.

Cuvinte-cheie: ferme de vaci de lapte, securitate alimentară, automatizare, zootehnie de precizie, inteligență artificială.

INTRODUCTION

In recent years, the livestock sector in Romania, especially dairy farms, has been facing a major structural challenge: the acute shortage of specialized labor. This problem affects both large farms with intensive production systems and small and medium-sized farms, particularly in rural areas where the migration of the active population has led to a significant decline in available human resources. The

Romanian dairy sector stands at a critical juncture, facing profound challenges that threaten its productivity, competitiveness, and long-term sustainability. Foremost among these is a deepening dependency on and shortage of agricultural labor.

This issue is significantly amplified by the sector's structural characteristics, notably its high degree of fragmentation and the overwhelming prevalence of small, often subsistence-level farms. These farms typically operate with limited resources and face difficulties in achieving economies of scale, making them particularly susceptible to labor market pressures. Farms grapple not only with diminishing labor availability, driven by demographic shifts such as an aging rural population and significant outward migration (especially of younger, potentially more skilled individuals), but also with a qualitative gap in the skills required for modern agricultural practices.

In this context, the economic efficiency, food security, and sustainability of farms are increasingly influenced by their capacity to adapt to new socio-economic and technological conditions. This complex labor situation necessitates an urgent exploration of contemporary solutions capable of mitigating dependency on manual work while enhancing operational efficiency and overall farm performance.

Against this scenario, modern management strategies that include digitalization, process automation, and the use of artificial intelligence are becoming not just innovative options, but imperative necessities for maintaining competitiveness. Technologies ranging from robotic milking systems (AMS) to sophisticated Precision Livestock Farming (PLF) tools offer the potential to automate tasks, optimize resource use, and provide valuable data for improved decision-making.

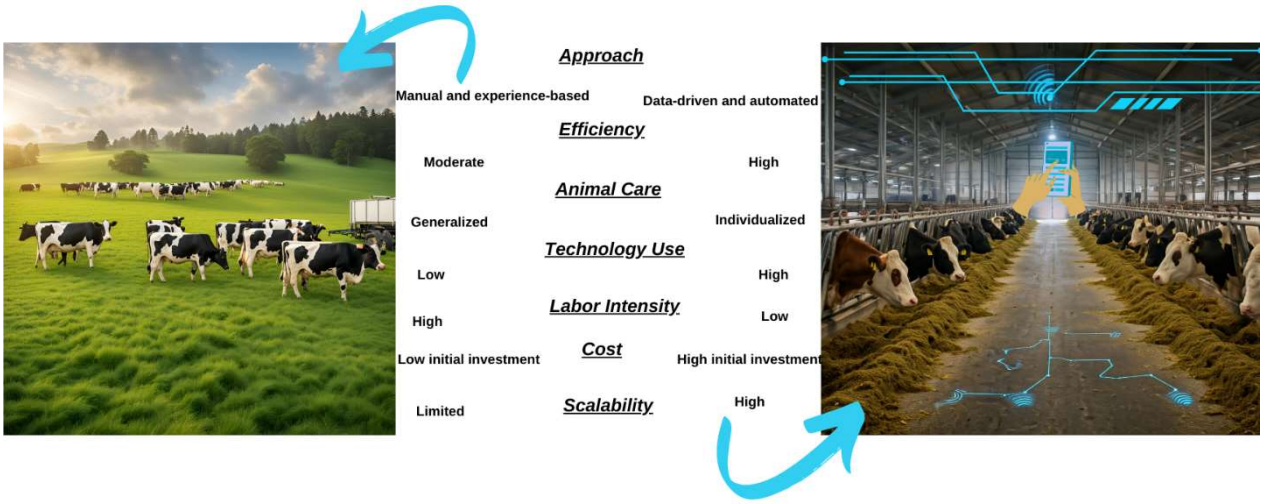


Figure 1. Livestock Management / ***Managementul efectivelor de animale***

Precision livestock farming technologies offer effective solutions for optimizing daily activities, reducing operational costs, and increasing product quality, while at the same time reducing dependence on constant human intervention (Giordano, 2024).

The present paper aims to analyze the impact of modern technologies on the organization of work on dairy cow farms, with a focus on organizational and human resource management strategies in the context of reducing the need for physical labor and to assess how digitalization and automation can contribute to reducing labour dependency on dairy farms, to identify the most effective management practices adapted to the Romanian context. The case study is carried out on a selected set of six farms in north-eastern Romania, representing different farming models (intensive, semi-intensive, and extensive).

The research questions clarify how digital and automated technologies affect dairy farm organization and performance. The first question identifies key differences in work organization between automated and traditional farms, addressing task structure, standardization, labor requirements, and

responsibility delegation. The second explores digitalization's role in managerial decisions, emphasizing the benefits of real-time data, automated monitoring, and computerized management systems for faster, informed choices. Finally, the third question examines how modern tech impacts productivity and product quality, focusing on digitalization's economic and qualitative effects and potential competitive advantages from these innovations.

Through a comparative analysis of these farms, the paper provides concrete insights into the potential for transforming Romanian farms into a more sustainable, efficient, and resilient agricultural system.

MATERIAL AND METHOD

This paper was based on an applied study conducted on six dairy farms in the north-eastern region of Romania, strategically selected to cover a variety of farming systems - intensive, semi-intensive and extensive. The main objective of this research was to assess the impact of modern management strategies and digital technologies on the organization of daily farm activities, human resource efficiency and productivity in the context of reduced labour dependency.

Table 1. General characterization of the analyzed farms / *Caracterizarea generală a fermelor supuse analizei*

Farm	General location	Size (heads)	Farming system	Technologies used
F1	Iasi county (plain area)	>2.000	Intensive	Multiple milking parlours, mechanized feeding, automated manure management, specialized teams
F2	Neamt county (hilly area)	~1.000	Intensive	DeLaval SenseHub™, DeLaval feeding robot, automatic sprinklers, audio system, Afimilk, microclimate sensing
F3	Iasi county (peri-urban area)	~890	Semiintensive	Mechanized feeding, standard milking parlours, mechanized manure treatment, team organization, strict schedule
F4	Iasi county (Siret river valley)	~1.000	Semiintensive	Mechanized feeding and cleaning systems, natural and forced ventilation, own feed base
F5	Rural region - Iasi (low hills area)	<90	Extensive	Traditional forage, minimal technological equipment, predominantly manual labor
F6	Sirețel commune, Iasi county	<30	Extensive	Basic equipment, lack of automation, mainly manual work

The six farms included in the study were grouped as follows: two intensive farms (F1 and F2), two semi-intensive farms (F3 and F4) and two extensive farms (F5 and F6). They were selected taking into account several factors: location, size, diversity of technologies used, openness to innovation and accessibility for research.

Data collection was mainly through field visits and direct observation, supplemented by semi-structured interviews with farm managers and staff involved in day-to-day activities. Technological sheets and internal farm documents were also used to validate data on work organization, livestock structure and investments in modern equipment.

The aspects analysed included: the structure of human resources (number of employees, qualifications, team organization), the daily work schedule, the technologies used in milking, feeding, animal health and welfare monitoring, and the degree of automation of activities. The level of digitalization of each farm, the presence of real-time monitoring systems (e.g. SenseHub, Afimilk, mobile environmental monitoring applications), as well as management strategies for future investments in technologies were also taken into account.

Comparisons between farms were made according to the type of farming system, linking data on technological inputs with operational outcomes such as labor productivity, resource consumption, dairy product quality, and the ability of the farm to operate efficiently in the absence of an abundant labor force. The analysis aimed to highlight good practices, but also barriers encountered in the transition to digitized and automated farms.

RESULTS AND DISCUSSIONS

Agriculture continues to be a cornerstone of Romanian employment, accounting for a substantial 20.9% of the total workforce in 2020, a figure dramatically higher than the EU average of 4.2%. This highlights the sector's socio-economic importance but also underscores its potential vulnerability to labor market fluctuations and suggests potentially lower labor productivity compared to EU counterparts. Despite this high employment share, the agricultural labor force in Romania is experiencing a rapid decline, potentially shrinking 50% faster than in other sectors like tourism. This contraction points to significant challenges in retaining workers within the agricultural domain.

A critical dimension of this challenge is the demographic profile of the agricultural workforce. It is predominantly elderly, with approximately 59.2% of workers aged between 40 and 64, and a further 9% aged 65 or over. Young people constitute only a small fraction of the workforce, and the proportion of farm managers under 40 is notably low at 10.4%. While aging is an EU-wide agricultural concern, with only 11.9% of EU farm managers under 40, the figures for Romania suggest a particularly acute demographic imbalance. This aging workforce not only poses a critical succession problem for family farms, which dominate the Romanian landscape, but also potentially limits the sector's adaptability to new technologies and practices. The difficulty in performing labor-intensive tasks, such as conserving forages for winter milk production, has been directly linked to the advanced age of many traditional dairy farmers.

In a global context where agriculture is facing multiple challenges, from climate change and diminishing natural resources to labor shortages and economic volatility, the livestock sector is under increasing pressure to adopt advanced technological solutions. In the literature (Yılmaz, 2023), several modern directions are outlined that can help reduce the dependence on manual labor on dairy farms, among which the following stand out: digitalization of processes, automation of repetitive tasks, and the use of artificial intelligence in decision-making.

According to recent studies (Sangode, 2024; Espinoza-Sandoval et al., 2024), labor shortages are one of the most important barriers to the sustainable development of livestock farms. The phenomenon is aggravated by the migration of young people from rural areas and the unwillingness of the working population to work in physically labor-intensive and irregular working hours environments.

In Romania, research shows that this deficit is more acute in rural northeastern regions, where farms are often family-run and have limited access to training and capital for technologization (Popescu et al., 2022). The structure of the Romanian dairy sector, characterized by extreme fragmentation, makes it acutely vulnerable. Over 90% of farms are essentially households with one or two cows, primarily for self-consumption, and family holdings constitute around 87-92% of all farms. These small units rely heavily on family labor, which, as established, is aging and diminishing. This structure inherently limits efficiency, prevents effective farm management, and weakens farmers' bargaining power in the market, often forcing them to accept low prices from processors. Many small dairy farms struggle with low profitability, outdated facilities, lack of investment, and difficulties accessing markets and finance, contributing to a decline in cow numbers and overall milk production.

The implementation of digital technologies - such as sensors for animal health monitoring, milking robots, automatic feeding systems - has become a practical solution to reduce the need for human labor in many European countries. Studies such as the one by Eastwood et al., (2015) showed that farms equipped with milking robots reduced the time needed for daily animal care by up to 30-40%, while increasing milk productivity and quality.

In Romania, the adoption of these solutions is still timid but growing. According to a report by Ministry of Agriculture and Rural Development - Romania (2023), more than 120 farms have integrated "smart farm" technologies, most of them in the central and north-eastern areas.

Classical models of work organization, based on a generalist workforce and manual routines, are gradually being replaced by specialized teams, in which each employee has precise responsibilities and is assisted by technological equipment. The literature (Kimmerer et al., 2024) emphasizes the importance of continuous training of staff and managers who understand the digital agricultural ecosystem.

A key point highlighted by the researchers is that technology does not completely replace human work, but transforms it, requiring new skills (digital, data interpretation, technical maintenance etc.).

The use of artificial intelligence (AI) in agriculture is on the rise, especially for monitoring animal behavior, predicting productivity and optimizing feed. Algorithms can analyze real-time data from a variety of sources (sensors, video cameras, production records) to support quick and accurate decisions by farmers (Tangorra et al., 2024). A relevant example is the CowManager system, which provides alerts on cow health and fertility. Case studies (Doidge et al., 2024, Priyan, 2024) show a reduction in veterinary treatment costs and an improvement in economic returns through early interventions.

The primary goals driving the development and adoption of these technologies include:

✓ Reducing Labor Dependency: Automating repetitive and time-consuming tasks like milking and feeding directly addresses labor shortages and reduces labor costs.

✓ Increasing Efficiency and Productivity: Optimizing processes like milking frequency, feed conversion, and health management can lead to significant gains in milk yield and overall operational efficiency.

✓ Improving Animal Health and Welfare: Continuous monitoring allows for early detection of health issues (e.g., mastitis, lameness, metabolic disorders), enabling prompt intervention and potentially reducing morbidity and mortality. Automation can also offer animals greater freedom and reduced stress.

✓ Optimizing Resource Use: Precision feeding minimizes feed wastage, and optimized health management can reduce veterinary expenses and medication use. Efficient operations may also lower energy and water consumption.

✓ Enhancing Decision-Making: Data gathered by sensors and automated systems, when analyzed effectively, provides farmers with actionable insights for improving breeding strategies, nutritional plans, health protocols, and overall farm management.

✓ Improving Sustainability: By enhancing efficiency, optimizing resource use, and potentially reducing the environmental footprint per unit of milk produced, these technologies can contribute to the economic and environmental sustainability of dairy farming.

The main categories of technologies comprising the PLF/PDF toolkit are Automated Milking Systems (AMS), Automated Feeding Systems, various Herd Monitoring Sensors (wearable and non-wearable), and integrated Farm Management Software (FMS) powered by data analytics, often incorporating Artificial Intelligence (AI) and Machine Learning (ML) (Oliveira et al., 2024).

Organization of work

The differences between the six farms analysed (F1-F6) clearly highlight the direct relationship between the level of technologization and the degree of specialization of the staff.

In intensive farms (F1 and F2), the work is mainly carried out by specialized teams (e.g. milking, feeding, calf care, cleaning, technical maintenance), with a high number of employees with higher education and regularly trained staff. For example, in F1, more than 15% of the employees have university education (veterinarians, engineers), while in F2 rigorous sanitation, milking and management procedures are implemented.

In contrast, the semi-intensive farms (F3 and F4) show a mixed organization: there is a tendency towards specialization, but in practice many employees cover multiple tasks, depending on needs. The lack of specialized staff is compensated by the use of basic technologies (partial mechanization, strict working hours).

On extensive farms (F5 and F6), the work is carried out almost exclusively by non-specialized staff, with an informal organization of tasks. Milking, feeding and sanitation are carried out manually and tasks are not clearly divided into functional teams.

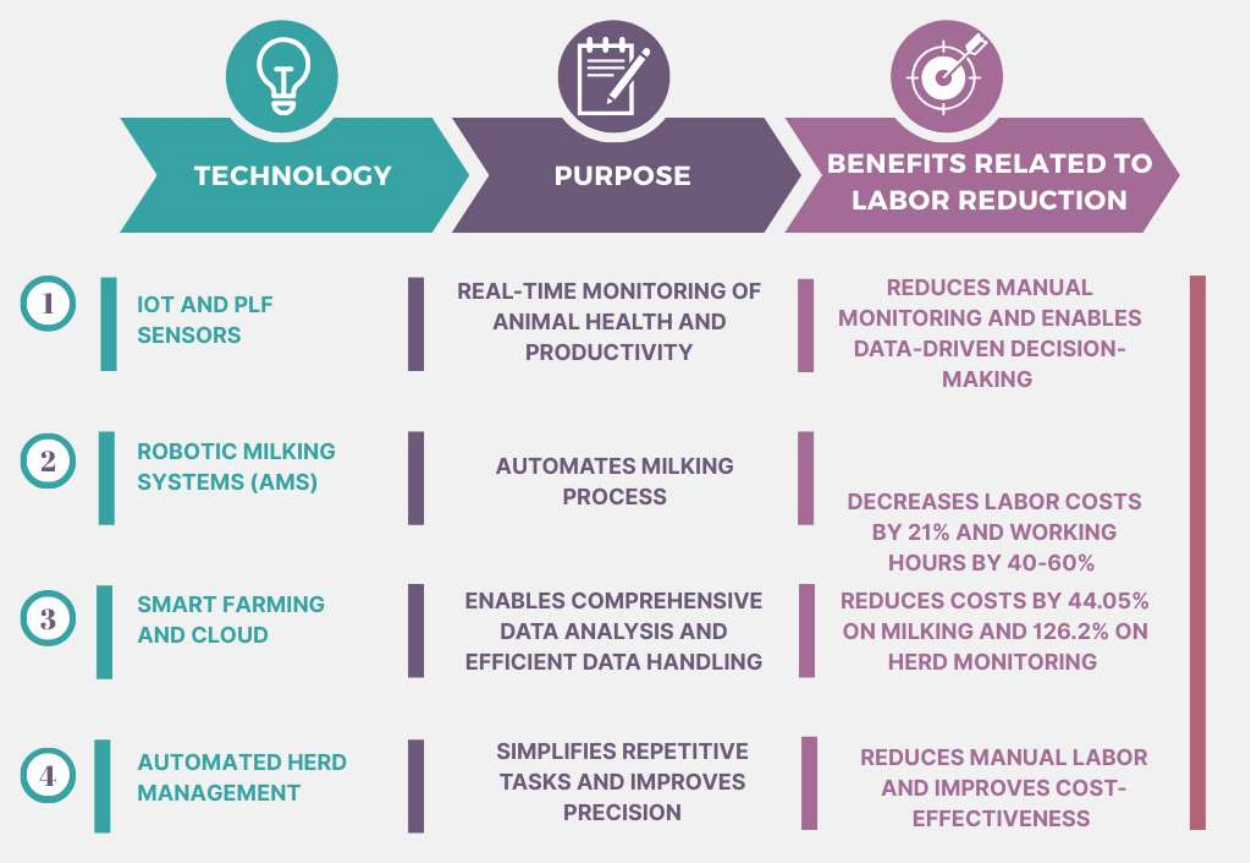


Figure 2. Comparative Analysis of Key Technologies / ***Analiza compratаivă a tehnologiilor cheie***

Addressing labor dependency involves not only exploring technological solutions but also optimizing the management of the existing human capital. Effective labor management practices are crucial for enhancing productivity, ensuring consistency, improving employee retention, and maximizing the return on labor investments, which constitute a significant portion of farm expenses. As farms grow and increasingly rely on non-family employees, the need for structured and professional human resource management becomes paramount. Poor management practices can undermine the potential benefits of even advanced technologies.

Labor efficiency

Labor efficiency varies directly proportional to the level of technologization. On F1 and F2 farms, average labor time/man/day for key activities is significantly lower due to automation. Mechanized feeding and push robots (F2) reduce the time required by 30-40% compared to traditional methods.

Automation demonstrably reduces labor input, especially for milking. Surveys and studies report significant reductions in daily milking time, such as a 62% decrease (from 5.2 to 2.0 hours/day) or savings of 2.5-3.4 hours/day. Overall labor savings associated with automated milking systems are often cited in the range of 20% to 50%, with some estimates suggesting potential savings up to 70%. Automation can also lead to a reduction in the number of employees required; one Canadian survey found a decrease from 2.5 to 2.0 employees (a 20% reduction) after automated milking system adoption. In a striking

example, one US farm added 1,200 cows after installing 30 robots without increasing its labor force. However, the nature of labor changes; time is shifted from manual milking to tasks like fetching reluctant cows (a median of 3-4% of the herd per day needed fetching in one survey) and, more significantly, to managing the technology, interpreting data, and troubleshooting. Some studies found that while labor hours decreased, the reduction was not always statistically significant, particularly on larger or non-family farms, suggesting that labor might be reallocated rather than purely eliminated.

Reducing labor dependency and promoting a sustainable future for Romanian dairy farming requires a dual strategy. Smaller farms should focus on improving management skills, access to finance, and infrastructure (like connectivity and irrigation) while adopting low-cost digital tools through enhanced advisory services and cooperatives. For larger, commercial farms, targeted support in advanced automation and PLF technologies, along with data management skills, can yield significant efficiency gains. A uniform, high-tech approach across the diverse sector is unlikely to succeed and may worsen existing inequalities.

The impact of modern technologies

The implementation of digital and automated technologies has brought measurable benefits on both intensive and, in part, semi-intensive farms. The use of monitoring sensors, such as SenseHub™ on the F2 farm, has enabled early detection of health problems and the optimal time for insemination, helping to reduce productivity losses by up to 25% and increase gestation ratesIn parallel, management software, such as Afimilk used in F2 and F1, has supported fast and accurate decision making in terms of treatment application, animal selection and herd reform. The introduction of automatic feeding systems and push robots in F2 also led to an increase of more than 15% in feed consumption per head, which was directly reflected in increased daily milk production.

Farm Management Software serves as the central hub, integrating data streams from milking systems, sensors, automated feeders, and potentially manual inputs. Advanced software utilizes algorithms, often incorporating AI and machine learning, to analyze this vast amount of data, identify patterns, generate alerts for cows needing attention (e.g., for heat, health issues, overdue for milking), and provide comprehensive reports. Examples include Lely Horizon, DeLaval DelPro™, AfiMilk, GEA's platform, and DTM feed management software. These systems empower farmers with data-driven insights for making more informed decisions regarding breeding, feeding strategies, health treatments, culling, and overall herd management. Cloud-based platforms offer the additional benefit of remote access and monitoring via smartphones or computers.

Farmers have seen a number of clear benefits from the adoption of digital and automated technologies, including a reduction in labor requirements by up to 30-40%, a decrease in disease incidence due to better hygiene and constant monitoring, increased predictability in production, and stabilization of work teams by reducing physical effort. However, implementing these solutions is not without its limitations (Kaur et al., 2023). Among the main challenges are the high upfront costs associated with the purchase of equipment and software, the need for continuous training of staff to effectively use the new technologies, the increased dependence on specialized technical support and IT maintenance, and the reluctance to change, especially on traditional farms, where adoption of new technological solutions is often slower.

Pletsch et al. (2024) highlights that technologically advanced farms tend to adopt simple but clear hierarchical structures, in which tasks are delegated to specialized teams, which increases efficiency and allows scaling of the activity without proportionally increasing the number of employees.

The automated farms (F1, F2 - intensive system) are characterized by a clear organization, structured in specialized teams with well defined tasks: milking team, feeding team, maintenance team, veterinary team, etc. The schedule is standardized and correlated with the biological needs of the animals (e.g. compliance with milking, feeding, resting times).

In contrast, in traditional farms (F5, F6 – extensive system), work is organized informally, generalist, with most activities being carried out by a small number of people, often without specialization. Tasks overlap, are distributed based on availability, and activity planning is flexible but less efficient.

The key differences between modern and traditional farms are evident both in terms of organization and daily operations. In large farms, the activity is characterized by increased efficiency, continuity in processes, standardization of operations and a clear delegation of tasks, aspects that contribute to predictable and controlled production management. In contrast, traditional farms often rely on improvisation and multitasking, and the success of activities depends largely on the physical effort and individual experience of staff, which makes processes less homogeneous and more vulnerable to fluctuations in human resources and working conditions.

According to Burda et al. (2021), dairy farms implementing modern technologies (e.g. milking robots, automated management systems) require a profound reorganization of work: new roles emerge (AI operators, maintenance technicians), and farmers become "data managers" rather than physical executors.

Digitalization plays a key role in managerial decision-making, especially in high-tech farms, completely transforming the way data and interventions are managed. By using integrated monitoring systems, such as SenseHub™ or Afimilk, alongside herd management applications and software dedicated to sanitary and veterinary records, managers can access detailed information on production, animal health, reproduction and performance in real time. Thus, digitalization directly contributes to the substantiation of rapid and well-argued decisions, optimizing interventions and reducing the risks associated with uncertainty or delayed reactions. In this context, the research question "What role does digitalization play in the decision-making process at the managerial level?" becomes essential to understand the impact of new technologies on modern livestock management.

This rapid data flow allows for:

• prompt and data-driven decisions, not intuition
• preventive (not reactive) interventions in case of illness or production decline
• optimization of resource planning (feeding, employee rotation, weaning, reform)

Steeneveld et al. (2012) show that farms equipped with sensors and management software make faster decisions based on real data (data-driven decisions), rather than subjective observations. Thus, decisions regarding treatments, reproduction or selection are more accurate, with reduced risk and better economic results.

In traditional farms, where digitalization is lacking, decisions are often made empirically, based on the farmer's direct experience and visual observations. This can lead to delayed interventions and loss of efficiency. Gargiulo et al. (2018) mention that digitalization transforms the role of the agricultural manager into a data analyst, emphasizing the importance of continuous training in related fields: agricultural IT, biological data interpretation, economic analysis.

The use of modern technologies significantly influences the productivity, costs and quality of dairy products, having a direct impact on the overall performance of farms. In terms of productivity, automated farms manage to achieve 30–40% higher average yields per cow, due to the optimization of feeding, milking processes and ensuring increased comfort for animals, which reduces stress and stimulates lactation (Liu et al., 2023). Implementing modern technologies in the dairy sector requires a high initial investment, but it significantly reduces long-term operational costs, particularly labor, by up to 25–35%. Automation minimizes downtime and technological losses, enhancing economic efficiency. The quality of dairy products benefits from better hygiene in milking, early mastitis detection, and consistent maintenance of quality parameters like fat content and protein levels in milk. This raises the question of how technological innovation impacts productivity, costs, and product quality, emphasizing the importance of understanding its role in sustainable performance in livestock.

Dayoub et al. (2024) emphasize that the implementation of technologies such as milking robots, automated feeding systems and AI significantly contributes to increasing productivity per animal, reducing waste and increasing the quality of dairy products, through better control over hygiene and nutrition.

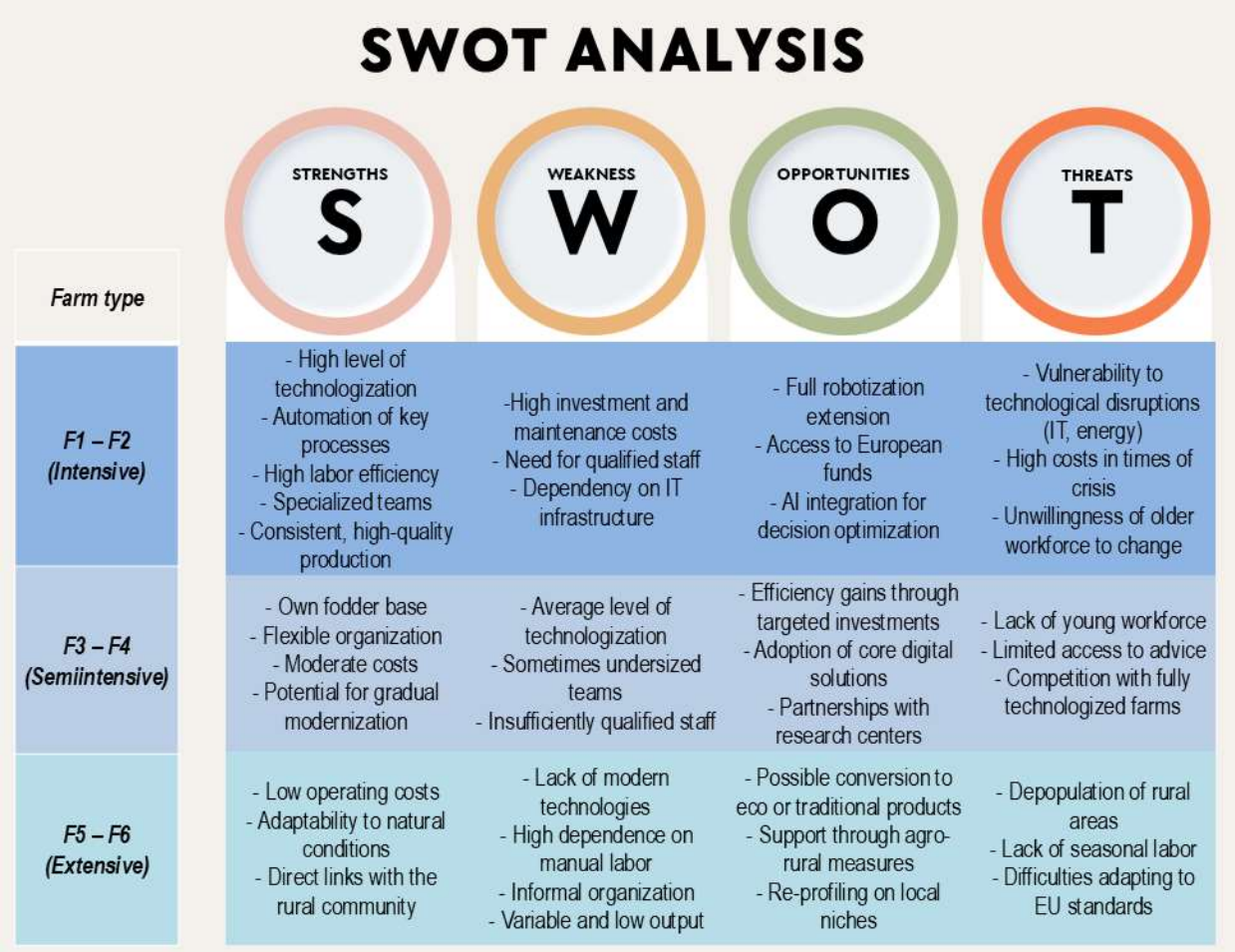


Figure 3. Comparative SWOT analysis of the three types of farms / ***Analiza comparativă SWOT a celor trei tipuri de ferme***

Concrete examples from practice support the impact of modern technologies on farm performance. In the F2 farm, the introduction of the feeding robot led to an increase of over 15% in voluntary feed consumption per animal, which was directly reflected in a higher milk production per cow. On the other hand, in the F1 farm, the automation of the milking process contributed to maintaining a constant level of milk quality, through a more rigorous control of hygiene and process parameters, which led to a reduction in the incidence of mastitis and, implicitly, to obtaining milk with higher quality standards. These examples highlight how modern technologies support both economic efficiency and improved dairy product quality.

Barkema et al. (2015) show that farms with advanced digitalization have been able to reduce total production costs by up to 20%, even though they initially involved large investments. These savings come from optimizing resource consumption (water, energy, feed) and reducing disease incidence through continuous monitoring.

CONCLUSIONS

The Romanian dairy sector faces a profound labor crisis characterized by an aging workforce, significant emigration, critical skills gaps, and exacerbated by extreme farm fragmentation. This situation poses a direct threat to the sector's productivity, viability, and long-term sustainability. Modernization, through the adoption of improved management strategies and advanced technologies like automation and Precision Livestock Farming (PLF), offers a potential pathway to mitigate labor dependency, enhance efficiency, and improve animal welfare.

Our study demonstrates that technologies such as automated milking systems, automated feeding, and sensor-based monitoring can deliver quantifiable benefits, including significant reductions in labor requirements, potential increases in milk yield, and opportunities for improved animal health management. Modern human resourses management practices, including structured training and standard operating procedures, are equally important for optimizing workforce performance and ensuring operational consistency.

However, the impact and suitability of these solutions are highly context-dependent. High-cost automation is often only economically viable for larger, typically more intensive operations, presenting a significant challenge for the predominantly small-scale Romanian dairy farms. Furthermore, the successful implementation of technology is heavily reliant on the quality of farm management and the ability to effectively utilize the data generated. Profitability is not guaranteed and requires careful planning and adaptation.

In Romania, the adoption of these modern solutions remains very low. Progress is severely hampered by a complex web of interconnected barriers, including prohibitive investment costs, limited access to finance, critical infrastructure deficits (especially rural connectivity and irrigation), pervasive skills gaps and low digital literacy, structural impediments related to small farm size and weak farmer organization, and potentially inadequate or poorly implemented policy support. This systemic lock-in makes modernization exceptionally difficult for the majority of the sector.

To forge a sustainable path forward, a dual strategy is essential. Improving basic management practices, skills, access to finance, and infrastructure is crucial for Romanian dairy farms. Targeted support should also aid smaller farms in adopting advanced technologies. Strengthening the Agricultural Knowledge and Innovation System (AKIS) is vital for training and support, while farmer cooperation can enhance scale and bargaining power.

Ultimately, addressing labor dependency and ensuring the future resilience of Romanian dairy farming demands more than just technological fixes. It requires strategic investment in human capital, infrastructure, and institutional capacity, alongside policies that recognize the diversity of the sector and support a transition towards greater efficiency and sustainability for all types of farms. Failure to address these systemic challenges risks further decline and consolidation, potentially undermining rural economies and food security objectives. The successful integration of management and technology, tailored to context and supported by a robust ecosystem, holds the key to navigating the challenges and unlocking the potential of the Romanian dairy sector.

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RESEARCH ON THE EVOLUTION OF THE MAIN GENETIC FACTORS IN THE NEW BIOLOGICAL CREATIONS FROM R.D.I.S.G.B. PALAS CONSTANTA

CERCETĂRI PRIVIND EVOLUȚIA PRINCIPALILOR FACTORI GENETICI LA NOILE CREAȚII BIOLOGICE DE LA I.C.D.C.O.C. PALAS CONSTANȚA

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Abstract

This paper presents the genetic history of the three new breeds created by the scientific research at the Research and Development Institute for Sheep and Goat Breeding Palas Constanta, namely: ***Palas Milk Breed, Palas Meat Breed*** *and* ***Palas Prolific Breed****. For each breed are presented: index of reproductive isolation, genetic similarity with the founding breeds, the evolution of the interval between generations, inbreeding and subdivision of the breed and the genetic size of each breed (N_e). The study showed that the sheep breeds created at R.D.I.S.G.B. Palas are genetically stable and do not present any danger of degeneration.*

Keywords: reproductive isolation, genetic similarity, generation interval, inbreeding, genetic size.

Rezumat

Lucrarea prezintă istoria genetică la cele trei rase nou create de cercetarea științifică de la Institutul de Cercetare – Dezvoltare pentru Creșterea Ovinelor și Caprinelor Palas Constanța și anume: ***Rasa de Lapte Palas, Rasa de Carne Palas*** *și* ***Rasa Prolifică Palas****. Pentru fiecare rasă sunt prezentate: indicele de izolare reproductivă, asemănarea genetică cu rasele fondatoare, evoluția intervalului între generații, a consangvinizării și subdivizării rasei și mărimea genetică a fiecărei rase (N_e). Urmare a studiului efectuat a rezultat faptul că rasele de ovine create la ICDCOC Palas sunt stabile genetic și nu prezintă pericol de degenerare*.

Cuvinte cheie: izolare reproductivă, asemănare genetică, consangvinizare, mărimea genetică.

INTRODUCTION

The integration of the sheep farming sector in the EU has involved the rapid completion of mandatory steps regarding the improvement of meat and milk production and the quality of the products obtained, a context in which the problem of the exploitation of specialized breeds has become particularly relevant.

Using the local genetic pool and the genetic pool of some imported breeds, research has been carried out at the Palas Research and Development Institute for Sheep and Goat Breeding in Constanta (R.D.I.S.G.B. Palas), in order to create new populations of sheep specialized for meat, milk and high prolificacy.

The concept of breed development based on cross-breeding, which was practiced many decades ago, has today become very successful in the field of improvement of desirable traits of sheep breeds.

After selection, cross-breeding is the second factor that exploits the genetic variation that exists both between and within populations.

The main breeds used for crossbreeding to create the three new breeds are the following: **Friesian**, **Awassi** (for milk), **Ile de France** (for meat) and **Romanov**, **Border Leicester** and **Finish Landrace** (for prolificacy).

Imported specialized sheep breeds have been used in cross-breeding programs in many countries in Europe, resulting in hybrid populations with traits close to the intended objective.

The data on the performance of the breeds used for the creation of prolific populations by crossing and their morphoproductive parameters were communicated by Maijala K., et al. (1977), Nitter (1977), Ricordeau G. (1978). Radu R. et al. (2011) presents data on the improvement of milk production in Romanian sheep breeds following crossbreeding with specialized breeds. Vicovan G. et al. (2009) presents in a paper, aspects regarding the improvement of meat production in Romanian sheep breeds by crossing with specialized breeds. The **Palas Merino** breed, a mixed wool-meat breed, considered the most valuable autochthonous sheep breed created by scientific research in the field, was the basis for the creation of three specialized sheep breeds by crossing with imported specialized breeds, namely: the **Palas Milk Breed**, the **Palas Meat Breed** and the **Palas High Prolificity Breed**, breeds that have been homologated in the last 15 years in Romania.

These breeds have become part of the national genetic heritage, they are bred at R.D.I.S.G.B. Palas Constanta and from them breeding animals with high breeding value are delivered annually to improve meat and milk production in sheep breeding units in the country.

In terms of flocks, they are relatively few in number, and it is necessary to find a breeding approach that leads to the preservation of their genetic structure under the conditions of maintaining inbreeding below 1% per generation.

This paper presents important aspects of the creation of the three breeds and their genetic history.

MATERIAL AND METHOD

The research was carried out on three breeds of sheep, specialised for milk, meat and high fertility, certified as new breeds and bred at the Institute. Each of the three breeds is studied in terms of morpho-productive and reproductive indices, and each year they offer breeding animals of high breeding value to breeders interested in improving their flocks.

The work carried out presents, for each breed, the mode of formation and the results obtained after lambing. The reproduction of the flocks of the three breeds is organised in such a way that inbreeding does not increase by more than 1% per generation, so the flocks of each breed are divided into ram families (10-12), with rotational mating between unrelated or slightly related ram families.

After weaning, a number of lambs are intensively fattened each year until they reach a body weight of 40 kg, and the males with the highest weight gains, over 320 g/day in the **Palas Meat Breed** and over 230 g in the **Palas Prolific Breed**, provide data for testing the progeny of the sires and are used to decide whether to keep the sires for breeding.

The **Palas Milk Breed** is used to breed rams whose mothers, grandmothers by dam and grandmothers by sire, have produced large quantities of milk, 20-30% more than the average of the population, and have had long lactations, more than 270 days.

Palas Milk Breed

The main economic characteristic of the **Palas Merino** breed is its milk production, which is higher than that of local breeds.

The breed was created in 1975 by crossing the **Palas Merino** with the **East Frisian** breed, then with the **Awassi** (from Israel), reproductive isolation for 5 generations and genetic consolidation.

The *coefficient of reproductive isolation (R.I.C.)* is the basic criterion for considering a population of sheep as a breed and is determined according to the formula:

$$R.I.C.=\frac{AA - (AI+II)}{AA + AI+ II}$$

where: AA - the number of individuals admitted for reproduction with both parents from their own flock;

AI - the number of individuals with one immigrant parent acquired through purchase or acquisition outside their own flock;

II - the number of individuals with both parent's immigrant.

The *breeding isolation coefficient* of the **Palas Milk Breed** is shown in Table 1.

Table 1. The reproductive isolation coefficient in the Palas Milk Breed / *Coeficientul de izolare reproductivă la Rasa de Lapte Palas*

Specification	Years				
Reproductive Isolation Coefficient	1988	1993	1998	2003	2022
	+0.137	+0.52	+1	+1	+1

Until 1993, the population had a lower degree of reproductive isolation (1988) and a relative degree of reproductive isolation (1993), and since 1998 a total reproductive isolation was reached, the RIC being +1. Under these conditions, according to Drăgănescu C, 1970, the analysed population with CIR values close to +1 can be considered a breed.

It was homologated as a new indigenous sheep breed in 2010.

At present, the **Palas Milk Breed** is genetically 55% similar to the Friesian, 35% to the Palas Merino and 10% to the **Awassi**, as shown in Table 2, which shows the *degree of similarity* to the founding breeds in the period 1988-2022.

Table 2. The genetic similarity of the Palas Milk Breed with the founder breeds / *Asemănarea genetică a Rasei de Lapte Palas cu rasele fondatoare*

Breed	R_{xy} (%) of sheep born between 1988-2022 R_{xy} % al oilor născute în intervalul anilor 1988-2022				
	1988	1993	1998	2003	2022
Frisian	56	59	57	55	55
Palas Merino	34	30	34	35	35
Awassi	10	11	9	10	10

The genetic similarity of the **Palas Milk Breed** to the founding breeds has remained relatively constant since 1988 and throughout the evolution of the breed. The data on the evolution of the *interval between generations* in this breed (Table 3) show that in the period 1988-2003 the interval on father-son and father-daughter lines was 3.16 and 3.32 years, respectively, lower values compared to the interval on mother-son and mother-daughter lines, which had values of 4.03 and 4.43 years, respectively. For the entire period analysed and for all filiations, the average intergenerational interval was 3.84 years.

Table 3. Evolution of the interval between generations of Palas Milk Breed (1988-2003) / *Evoluția intervalului între generații la Rasa de Lapte Palas (1988-2003)*

Year	Yearly generation interval				
	Father-son	Father-daughter	Mother-son	Mother- daughter	Average
1988	4.38	4.04	3.40	4.67	4.04
1993	3.05	3.10	4.12	4.05	3.32
1998	2.50	2.32	4.0	3.88	3.13
2003	2.90	2.25	4.58	4.82	3.57
Average 1988-2003	3.16	3.32	4.03	4.43	3.84

The inbreeding and subdivision of the **Palas Milk Breed** (Table 4) shows that in 2003 the breed had low *inbreeding* -3.52%, similar to the **Palas Merino Breed** from which it derives.

Table 4. Evolution of inbreeding of the Palas Milk Breed in the period 1988-2003 / *Evoluția consangvinizării Rasei de Lapte Palas în perioada anilor 1988-2003*

Specification	Year			
	1988	1993	1998	2003
Total inbreeding – F (%)	0,51	3,69	8,50	3,52
Average inbreeding per generation - ΔF (%)	0,17	0,97	1,75	0,73

The average inbreeding per generation increased from 0.17% in 1988 to 1.75% in 1998 and then decreased to 0.73% in 2003.

The total milk production of the **Palas Milk Breed** is shown in Table 5.

From the table it can be seen that the total milk yield of the **Palas Milk Breed** was 214.57 litres, which was significantly ($p<0.001$) higher ($p<0.001$) than the maternal **Palas Merino** breed by 95 litres and 179.5%, respectively, and the lactation duration of the dairy breed was 69 days longer than that of the **Palas Merino** breed.

Table 5. Total milk production in Palas Milk Breed ewes compared Palas Merino ewes / *Producția totală de lapte la oile din Rasa de Lapte Palas comparativ oile din rasa Merinos de Palas*

Breed	Total milk yield (litres/head)			Total milk yield litres/head)		Lactation duration (days)			Lactation duration (days)	
	n	X±sx	V(%)	Minim	Maxim	n	X±sx	V(%)	Minim	Maxim
Palas Milk Breed	25	214,57± 8,7813	20,46	155,8	325,7	25	264±2,0704	3,91	248	299
Palas Merino	25	119,53± 5,5340	23,23	83,5	181,1	25	195±2,3452	6,01	155	2

The *genetic size (Ne)* of the breed is given by the formula:

$$Ne = \frac{4Nm \times Nf}{Nm + Nf},$$

where:

Ne – effective (genetic) size;

Nm – the number of reproductively active males;

Nf – the number of females.

The breed has a genetic size of Ne = 60.3 individuals, which gives it a high level of security in the evolution of future generations.

The flock of this breed bred at the Research and Development Institute for Sheep and Goat Breeding Palas Constanta is currently 700 ewes, of which 350 are ewes, 48 are rams and 302 are young males and females.

Palas Meat Breed

A cross between the **Palas Merino** and **Ile de France** breeds, created through rigorous selection.

It was recognized as a native breed in 2012.

After 1989, the population was reproductively isolated, crossbreeding was stopped and the population itself was bred and selected for increased meat production.

Reproductive isolation of the **Palas Meat Breed** was determined using the same formula as presented for the **Palas Milk Breed** (Table 6).

Table 6. The reproductive isolation coefficient in the Palas Meat Breed / *Izolarea reproductivă la Rasa de Carne-Palas*

Specification	Year								
	1973	1976	1981	1989	1994	1999	2003	2009	2022
Reproductive Isolation Coefficient	-1	+ 0.54	+ 0.81	+1	+ 0.94	+ 0.98	+ 1	+ 1	+ 1

During the period 1973-1981 the breed had a relative degree of reproductive isolation. Since 1989 and until now reproductive isolation is total.

The *genetic similarity* of the **Palas Meat Breed** to the founding breeds is shown in Table 7.

Table 7. The genetic similarity of the Palas Meat Breed with the founder breeds / *Asemănarea genetică a Rasei de Carne-Palas cu rasele fondatoare*

Breed	R_{xy} (%) of sheep born between 1989-2009 R_{xy} (%) al oilor născute în intervalul anilor 1989-2009				
	1989	1994	1999	2003	2009
Ile de France	55.67	58.45	53.29	56.67	56.78
Palas Merino	41.25	40.15	39.71	34.10	36.28
Other breeds*	3.03	1.39	6.98	9.19	6.90

* Other breeds (**Romanov, East Friesian, Suffolk**)

The similarity of the **Palas Meat Breed** with the **Palas Merino** was 41.25% in 1989 and decreased progressively in all the analysed periods, reaching about 36% (36.28%) in 2009.

After 2009, the **Palas Meat Breed** did not change its genetic structure, remaining reproductively isolated and genetically stable.

The values of the *interval between the generations* in the period from 1989 to 2009 in the **Palas Meat Breed** are shown in Table 8.

Analysing the evolution of the interval between generations in the period 1989- 2009, it was observed that the average interval for father-son and father-daughter filiations was 3.24 years, respectively 3.12 years, lower than for mother-son and mother-daughter filiations, where it resulted 4.15 years and 4.38 years. For the total period analysed and for the total progeny, the average intergenerational interval for the **Palas Meat Breed** was 3.65 years.

Table 8. The interval between generations of the Palas Meat Breed in the period 1989-2009 / *Intervalul între generații la Rasa de Carne Palas în perioada anilor 1989-2009*

Year	The range in years				
	Father-son	Father-daughter	Mother-son	Mother- daughter	Average
1989	3.31	3.19	4.51	4.30	3.77
1994	4.10	4.01	3.75	3.46	3.83
1999	2.60	2.49	4.65	4.83	3.33
2003	2.85	2.81	4.40	4.70	3.53
2009	3.32	3.12	3.44	4.63	3.78
Average 1989-2009	3.24	3.12	4.15	4.38	3.65

*Inbreeding of the **Palas Meat Breed***

The relatedness of a population (breed) itself means the average relatedness of all its individuals to each other and is due to reproductive isolation, limited size and selection. The individuals of a population (breed) are related to each other at all times, meaning that they are descended from a series of common ancestors that existed previously (in ancestry).

The evolution of total inbreeding and average inbreeding per generation of the **Palas Meat Breed** is presented in table 9.

Table 9. The evolution of inbreeding of the Palas Meat Breed in the period 1989-2009 / *Evoluția consangvinizării Rasei de Carne-Palas în perioada anilor 1989-2009*

Specification	The years analysed				
	1989	1994	1999	2003	2009
Pedigree number	163	33	41	37	62
Total inbreeding – F (%)	5.06	17.42	9.15	14.19	10.00
Average inbreeding per generation - ΔF (%)	1.06	3.30	1.48	2.38	1.04

Total inbreeding increased from 5.06% in 1989 to 17.42% in 1994 and then decreased to 14.19% in 2003 and 10% in 2009.

The average inbreeding per generation was 1.06% in 1989 and after increasing to 3.30% in 1994, it decreased to 1.04% in 2009.

The body weight of ewes and rams of the **Palas Meat Breed** is shown in Table 10.

The table shows that the difference in weight in favor of the **Palas Meat Breed** was 4.26 kg for ewes (7.54%) and 5.63 kg for rams (6.40%). The differences were statistically very significant for ewes ($p<0.001$) and significant for rams ($p<0.05$).

Table 10. The body weight of ewes and rams of the Palas Meat Breed / *Greutatea corporală a oilor și berbecilor din Rasa de Carne Palas*

Breed	Category	Body weight (kg)	
		X±sx	V(%)
Palas Meat Breed	rams	95,23±1,1732	6,28
	ewes	60,75±0,3232	7,41
Palas Merino	rams	89,50±2,8893	13,70
	ewes	56,49±0,5849	13,46

The *genetic size* of the **Palas Beef Breed** *(Ne)* was calculated using the formula presented above for the **Palas Milk Breed**.

Calculation of the genetic size of the breed with 338 females gives a value of 93.11 individuals.

The **Palas Meat Breed** has a herd of 300 ewes, 20 rams, 20 young male and 44 young female.

Palas Prolific Breed

The **Palas Prolific Breed** was created in the period 1973-1988 through a complex program of crossbreeding between the **Palas Merino** breed and major prolific breeds. Many combinations of **Palas Merino** with **Romanov, Friesian, Border Leicester** and **Finnish Landrace** breeds were tested and in 1989 the desired type was obtained.

It was homologated in 2020.

The population has been reproductively isolated since 1990 and has been self-breeding for 7 generations. During this time, selection has been aimed at maintaining a fertility of over 150%, a high lactation capacity and an increase in inbreeding of less than 1%.

The value of the *Reproductive Isolation Coefficient* (RIC) is presented in Table 11.

Table 11. Reproductive isolation in the Prolific Palas Breed / *Izolarea reproductivă la Linia cu Prolificitate Ridicată Palas*

Specification	Year 1973	Year 1978	Year 1988	1989 up to the present
Reproductive Isolation Coefficient (RIC)	- 1.0	- 1.0	- 1.0	+ 1.0

In the period 1973-1988, the value of the Reproductive Isolation Coefficient (RIC) was -1, which led to the fact that the population was opened and used for the reproduction of rams of different breeds until the "desired type" was obtained.

Since 1989 and until now, for more than 7 generations, the breed's (line's) reproductive isolation is total, with a RIC value of +1.

The *genetic similarity* with the founding breeds is presented in Table 12.

To establish the genetic similarity with each of the founding breeds, the following formula was used:

$$\text{Similarity of breed X} = \frac{\text{Number of occurences in the pedigree of X breed}}{\text{Number of pedigree analyzed x 4}}$$

Table 12. Genetic similarity of the Prolific Palas Breed to the parent breeds / *Asemănarea genetică a Rasei Prolifice Palas cu rasele parentale*

Breed	Breed genetic similarity of individuals born in 2015-2017 to the founder breeds (%)		
	Rams	Ewes	Total population
Romanov	38.46	39.22	39.07
Palas Merino	27.08	28.44	28.13
East Frisian	18.69	14.73	15.63
Border Leicester	12.31	8.51	9.36
Ile de France	2.92	7.23	6.25
Finnish Landrace	0.85	1.77	1.56

It can be noted that **Palas Prolific Breed** breed is most genetically similar to the **Romanov** breed (39.07%), then to the **Palas Merino Breed** (28.13%), to the **Friesian** breed (15.63%), to the Border **Leicester** breed (9.36%), to the **Ile de France** breed (6.25%) and to the **Finnish Landrace** (1.56%).

Evolution of the *interval between generations* in the **Prolific Palas Breed** is presented in Table 13.

Table 13. Evolution of the interval between generations / *Evoluția intervalului între generații*

Specification	Father - son	Father - daughter	Mother - son	Mother - daughter	Average
Intergenerational interval (in years)	3.49	3.94	5.18	5.12	4.49

The intergenerational intervals for father-son and father-daughter consanguinity were 3.49 and 3.94 years, respectively, lower than those for mother-son and mother-daughter lineages, which were 5.18 and 5.12 years, respectively. The average intergenerational interval for total lineages was 4.49 years. The size of this interval indicates that in this breed, as generations evolved, young sheep were retained for breeding from older ewes that had to have at least 5 progenies at first lambing.

The *inbreeding* of the **Palas Prolific Breed** is presented in Table 14.

Table 14. Inbreeding of the Palas Prolific Breed / *Consangvinizarea Rasei Prolifice Palas*

Specification	Mean values
Total inbreeding (%)- F	4.39
Inbreeding per generation (%)	0.57

Total inbreeding was 4.39% and inbreeding over generations has the value of 0.57%.

The prolificacy value for the **Palas Prolific Breed** is shown in Table 15.

The data in the table show that the prolificacy of primiparous ewes of the **Palas Prolific Breed** was 146.43% and that of multiparous ewes was 153.80%, with ewes weaning 1.40 lambs per ewe lambed.

Table 15. Prolificacy in the Palas Prolific Breed compared with the Palas Merino / *Valoarea prolificității la Rasa Prolifică Palas comparativ cu rasa Merinos de Palas*

Breed / Category		Prolificacy (%)	Weaned lambs %	Weaned lambs On ewe (head)
Prolific Palas Breed	Primiparous ewes	146.43	91.46	1.34
	Multiparous ewes	153.80	92.81	1.43
	Total ewes	151.13	92.34	1.40
Palas Merino	Primiparous ewes	100.0	95.16	0.95
	Multiparous ewes	115.0	96.0	1.10
	Total ewes	111.0	95.83	1.00

Palas Merino ewes from a breeding flock of the Association for Sheep Breeding in Dobrogea achieved a lower fertility of 46.43 percentage points in primiparous ewes, 36.13 percentage points in multiparous ewes and 49. 13 points on the total population of Merino sheep.

The *genetic size* of the **Palas Prolific Breed** (Ne) determined was 87,7192 individuals.

Currently the total flock of sheep existing at R.D.I.S.G.B. Palas- Constanța is 700 heads, of which 350 mother ewes, 40 rams and 310 young sheep.

CONCLUSIONS

1. As a result of the study it was found that the sheep breeds created at R.D.I.S.G.B. Palas - Constanta are genetically stable and can be further used for the production of rams for milk, meat and lambs per 100 ewes lambed.
2. These breeds produce 100-120 rams per year.
3. The use of **Palas Milk Breed** rams is estimated to increase the milk production of their daughters by 50-53%.
4. The use of **Palas Meat Breed** rams for breeding purposes increases the body weight of the progeny by 17.54% in females and 6.4% in males.
5. The use of **Palas Prolific Breed** rams increases the prolificacy of daughters by 69%.

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