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ACTA AGRICOLA ROMANICA -Tom 5, An 5, Nr.5.1 August 2023 <br> by Aurel-Florentin Badiu :: SSRN

THE FORAGE QUALITY FROM THE IMPROVED SUBALPINE GRASSLANDS, GRAZED WITH DAIRY COWS AND THE MILK'S CHEMICAL COMPOSITION

CALITATEA FURAJELOR OBȚINUTE PE PAJIȘTILE SUBALPINE AMELIORATE, PĂȘUNATE CU VACI DE LAPTE ȘI COMPOZIȚIA CHIMICĂ A LAPTELUI

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Abstract

Knowing the quality of fodder obtained on mountain meadows is important for ensuring a rational feeding of animals, as well as for choosing those improvement technologies, which allow satisfying the nutritional requirements in order to achieve the highest possible animal productions. The purpose of this work was to show the quality of the fodder obtained on 5 experimental plots, with different improvement measures applied, grazed with dairy cows, as well as the milk's chemical composition. The study was carried out in the Bucegi Mountains at 1800 m altitude over a period of three years (2017-2019). Forage quality assessment was performed by determining the main quality parameters: crude protein (CP), ash (ASH), crude fiber (CF), dry matter digestibility (DDM). The chemical composition of the fodder registered changes during the studied period, being influenced by the botanical composition, climatic conditions, as well as the improvement measures applied. A positive influence of the improvement methods on the quality of the fodder on the improved plots was found, especially for the second year of research, compared to the fodder obtained on the control plot. The chemical composition of cow's milk recorded different values between the pastured plots, but not statistically assured ($p > 0.05$), except for the content in SNF and protein, which registered significant differences, statistically assured ($p \leq 0.05$), between the three years studied.

Keywords**: **subalpine grasslands, floristic composition, forage quality, milk composition

Rezumat

Cunoașterea calității furajelor obținute pe pajiștile montane este importantă pentru asigurarea unei alimentații raționale a animalelor, precum și pentru alegerea acelor tehnologii de îmbunătățire, care să permită satisfacerea cerințelor nutriționale în vederea realizării unor producții animaliere cât mai ridicate. Scopul acestei lucrări a fost acela de a prezenta calitatea furajelor obținute pe 5 parcele experimentale, tratate diferit, pășunate cu vaci de lapte, precum și compoziția chimică a laptelui obținut. Studiul a fost realizat în Munții Bucegi la 1800 m altitudine și s-a desfășurat pe o perioadă de trei ani (2017-2019). Evaluarea calității furajelor a fost efectuată prin determinarea principalilor parametri de calitate: proteină brută (PB), cenușă (ASH), fibre brute (FB), digestibilitatea substanței uscate (DSU). Compoziția chimică a furajului a înregistrat schimbări în perioada studiată, fiind influențată de compoziția botanică, condițiile climatice, precum și de măsurile de îmbunătățire aplicate. S-a constatat o influență pozitivă a metodelor de îmbunătățire asupra calității furajului de pe parcelele ameliorate, cu precădere în anul doi de cercetare, față de furajul obținut pe parcela martor. Compoziția chimică a laptelui de vacă a înregistrat valori diferite între parcelele pășunate, nefiind asigurate statistic ($p > 0,05$), excepție făcând conținuturile în SNF și proteină, care au înregistrat diferențe semnificative, asigurate statistic ($p \leq 0,05$), între cei trei ani studiați

***Cuvinte cheie**: **pajiști subalpine, compoziția floristică, calitatea furajului, compoziția laptelu**i*

INTRODUCTION

The mountain grasslands provide the animals with a very heterogeneous vegetation, providing food for the main cattle species that people raised for milk production. The subalpine grasslands of Bucegi Mountains represent an important source of fodder for ruminants.

Grazing plays the most important role in the nutrition of dairy cows in many regions of the world (MARKOVI´C et al., 2017), this is one of the best uses of grassland as it contributes greatly to food and feed production, to botanical diversity and to the preservation of the entire agroecosystem (METERA et al., 2010).

The use of grazing in feeding of dairy cows has several advantages, such as increasing the opportunities to encourage the natural behaviour of cows and contributing to the image of the dairy sector, but it also has deficiencies, such as greater nitrate excretion and less balanced nutrition (HENNESSY et al., 2020; ELGERSMA et al., 2016). The monitoring of pasture quality through the evaluation of protein and fiber content over time is critical to define the nutritional value of pastures and design balanced diets for grazing animals (DEMANET et al., 2015).

Forage quality is affected by species composition and abundance (KHALSA et al., 2012) and soil resource availability (NIU et al., 2016), which are both in turn affected by climatic conditions. The milk composition and yield are affected by many factors (O'CALLAGHAN et al., 2018).

Milk yield and milk chemical composition can be a good indicator of animal response to the amount of pasture biomass, as well as composition and quality.

The purpose of this work was to show the quality of the fodder obtained on 5 experimental plots improved differently, grazed with dairy cows, as well as the chemical composition of the obtained dairy product.

MATERIAL AND METHOD

The researches were conducted for three years, in 2017-2019, in Blana Bucegi Mountains (coordinates: 45°21'24.3"N 25°27'33.6"E) on slightly sloping ground with western exposure, at 1800 m altitude. The climatic characteristics in this area is specific to the alpine floor, where the annual air temperature is 4.9^0C. The amount of precipitation recorded, in the months of June-September, during the vegetation period is approximately 400-600 l/m2, and the annual amount of precipitation is around 1200-1300 l/m2. The annual average wind speed is over 5-6 m/s.

The study used cows from the *Brown of Maramureş* (*Schwyz*) breed adapted to the harshest mountain conditions, with average production milk (12-14 l/head). For each plot taken in the study were distributed 3 cows, which grazed under the open sky, without shelter, these feeding only with grass from the plot, salt and water.

The 5 experimental plots A, B, C, D, and T were treated differently, as follows:

1. Plot (Group) A: Natural grassland (*Nardus stricta dominant species*) fertilized with chemical fertilizers, as follows: 200 kg/ha N + 100 kg/ha P_2O_5 +100 kg/ha K_2O in the year 2000; 150 kg/ha N + 75 kg/ha P_2O_5 +75 kg/ha K_2O in the year 2001; 100 kg/ha N + 50 kg/ha P_2O_5 + 50 kg/ha K_2O in the year 2002; 150 kg/ha N + 100 kg/ha P_2O_5 + 100 kg/ha K_2O in the year 2010; 100 kg/ha N in the year 2011; 50 kg/ha N in the year 2012; 150 kg/ha N + 100 kg/ha P_2O_5 + 100 kg/ha K_2O in the year 2014; 100 kg/ha N in the year 2015; 50 kg/ha N in the year 2016.

2. Plot (Group) B: Natural grassland (*Nardus stricta dominant species*) chemical fertilized in the period: 1996-1998 with a rate of 150 kg/ha N + 75 kg/ha P_2O_5 +75 kg/ha K_2O, then paddocked with dairy cows in the years 2004, 2010 and 2016. Before or immediately after paddocking, chemically fertilized by superphosphate at a dose of 100 kg/ha P_2O_5.

3. Plot (Group) C: Natural grassland (*Nardus stricta dominant species*), limed on 2/3 of Ah in 1995, chemically fertilized in the period 1996-1998 with a dose of 150 kg/ha N + 75 kg/ha P_2O_5 +75 kg/ha K2O, then paddocked with dairy cows in the years 2003, 2009 and 2015.

4. Plot (Group) D: Seeded and limed pasture in 1995 year, chemically fertilized with NPK between 1996-1998 years, identical to plots B and C, and paddocked with dairy cows in the years 2002, 2008 and 2014. For sowing it has been used a mixture of perennial grasses and forage legumes consisting

of: *Phleum pratense* Favorit variety (40%), *Festuca pratensis* Transilvan (25%), *Lolium perenne* Marta (5%), *Trifolium hybridum* - local population of Brasov (15 %), *Lotus corniculatus* Livada variety (15%).

5. Plot (Group) T: Natural grassland (*Nardus stricta dominant species*), 30 years rationally used, located within the experimental field of the Mountain Grassland Research Base from Bucegi.

The forage samples were grounded with a Grindomix GM 200 mill and prepared for chemical analysis. Crude protein content based on total N content was determined with Kjeldahl method (multiplying N content by the factor 6.25), with the Gerhardt system. The cude fiber (CF) content was analysed according to the manufacturer's procedure of the Gerhart Fibertherm fiber extraction device and in accordance to the Van Soest method (VAN SOEST and WINE, 1967; VAN SOEST, 1963). The total ash was determined by the gravimetric method after calcining the grass samples at 550^0C in the Nabertherm B150 furnace. The determination of the digestibility of the dry matter (DDM) of the fodder was carried out by the technique of Near Infrared Spectroscopy (NIRS).

The composition of cow's milk (fat, solids non-fat (SNF), density, pH, protein, lactose) was determined using the equipment Ekomilk Total, BULTEH 2000 model.

All analyses were performed using the MSTATC and STATISTICA software. Statistical differences in quality parameters were evaluated, and the significant differences were evaluated based on the Student's T test, by a one-way and bifactorial analysis of variance using the ANOVA procedure.

RESULTS AND DISCUSSION

In the floristic composition of the studied grasslands, a number of 14 grasses, 1 legume, 11 species from other botanical families and other species without fodder value were identified (Table 1). The participation of plant groups in the composition of the grassy carpet varied significantly over the three-year period (2017-2019). Figures 1, 2, 3 show the percentages regarding the participation of plant quality groups in the botanical composition of the experimental plots.

Table 1. Plant species present in the experimental field's grassy carpet // Speciile de plante prezente în covorul vegetal al câmpului experimental

Species	Forage quality index	Species	Forage quality index
Gramineae		**Leguminosae**	
Agrostis capillaris	7	*Trifolium repens*	8
Agrostis rupestris	5	**Other families**	
Anthoxantum odoratum	5	*Achillea stricta*	6
Dactylis glomerata	9	*Alchemilla vulgaris*	6
Deschampsia caespitosa	0	*Campanula abietina*	0
Deschampsia flexuosa	0	*Campanula napuligera*	0
Festuca nigrescens	7	*Geum montanum*	0
Festuca pratensis	9	*Hieracium aurantiacum*	0
Nardus stricta	0	*Ligusticum mutellina*	6
Phleum alpinum	6	*Polygonum bistorta*	5
Phleum pretense	9	*Potentilla aurea*	0
Poa annua	7	*Ranunculus montanus*	0
Poa media	5	*Taraxacum officinale*	7
Poa pratensis	8	Other species	0

The value of the fodder quality index can be between 0 and 9 for plant species, depending on the quality class (MARUȘCA, 2016). From the graphic representation (Figure 1), it is highlighted that in 2017, only in the floristic composition of plot D, species of excellent forage plants were identified, in a percentage of 3%. The highest share of very good fodder plant species was recorded in plot C (49%).

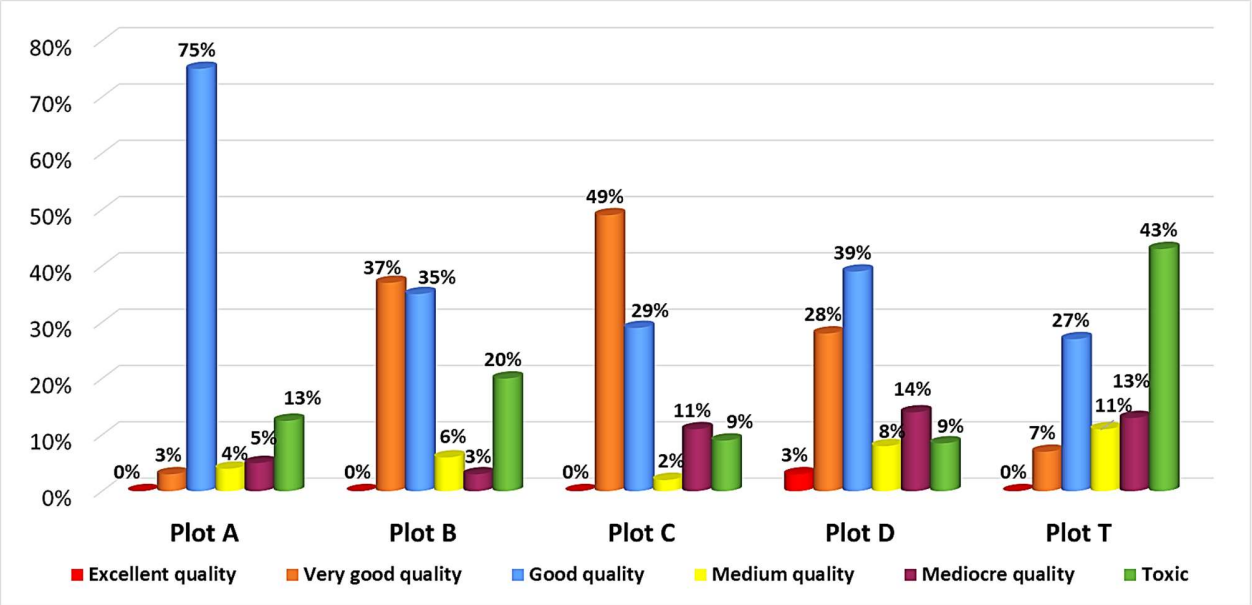


Figure 1. Participation of plant quality groups in the botanical composition of the 5 experimental plots (2017) // Participarea grupelor de calitate a plantelor în compoziția botanică a celor 5 parcele experimentale (2019)

In 2018, the participation of excellent and very good forage plant species was more increased on plots C and D, with percentages of 2% and 6%, respectively 50% and 46%. The highest percentage of participation of good fodder plant species (66%) was recorded in plot A, followed by plot B, with a percentage of 45% (Figure 2).

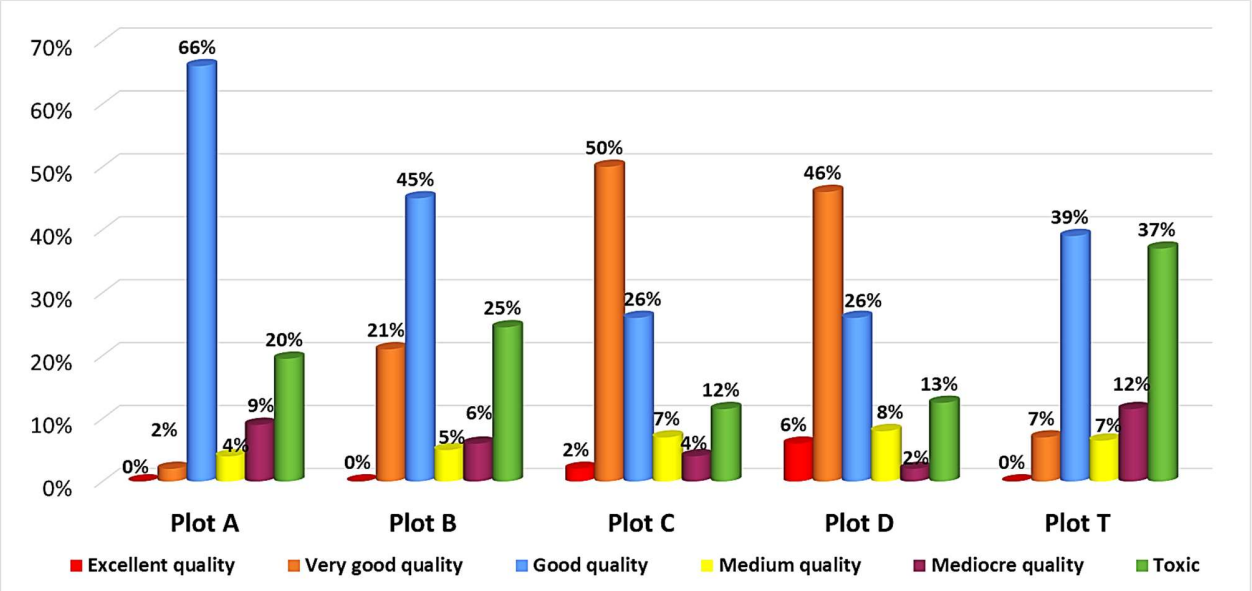


Figure 2. Participation of plant quality groups in the botanical composition of the 5 experimental plots (2018)// Participarea grupelor de calitate a plantelor în compoziția botanică a celor 5 parcele experimentale (2018)

In 2019, the participation of good and very good forage plant species in the grass carpet was increased, being between 57% and 71%, for all improved plots (A, B, C and D) compared to the control plot T, which had a participation of 50% (Figure 3).

Compared to the improved plots (A, B, C and D), in the floristic composition, the participation of toxic plant species was the highest in all three experimental years at the control plot T, with a percentage between 36-43%.

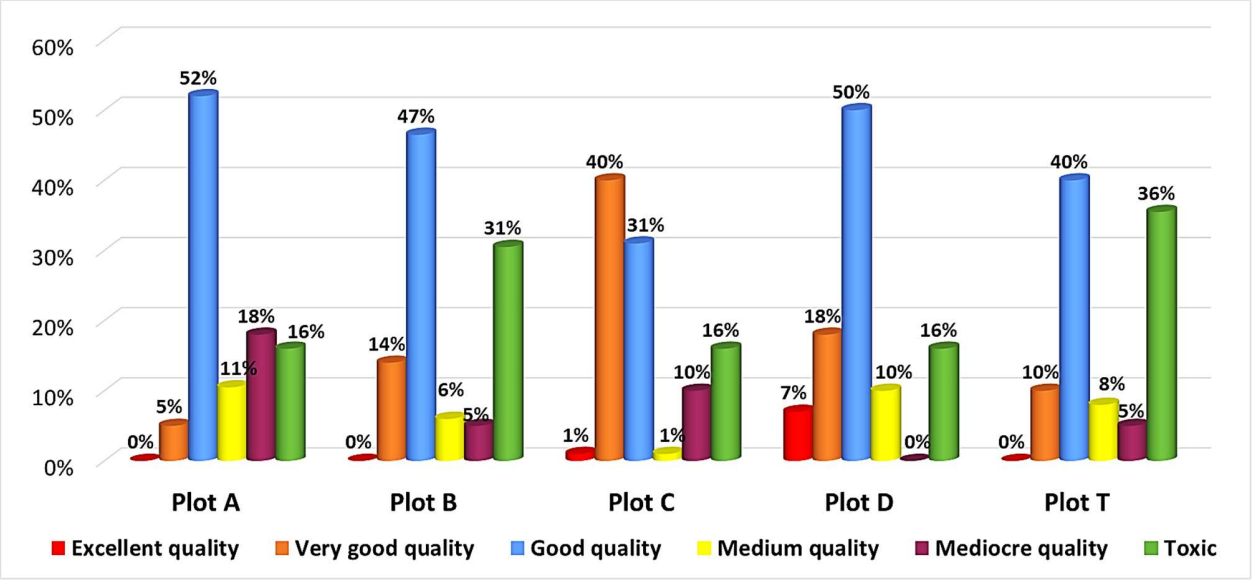


Figure 3. Participation of plant quality groups in the botanical composition of the 5 experimental plots (2019)// Participarea grupelor de calitate a plantelor în compoziția botanică a celor 5 parcele experimentale (2019)

The chemical composition of the fodder registered changes during the studied period, which are showed in the Table 2. Regarding the crude protein content of forages taken from the experimental plots A, B, C and D, compared to the control plot T, the crude protein content of the forages in 2017 was significantly higher ($p \leq 0, 05$) for plots A and D. The favourable temperature conditions and the more abundant rainfall recorded in the months of May-July of 2018 favoured the accumulation of protein, especially on the improved plots.

The absolute values of the crude protein (CP) content of the forage obtained on the improved experimental plots were significantly higher ($p \leq 0.05$) compared to the control plot T, unimproved, as a result of the residual effect of paddocking and the application of chemical fertilizers.

In 2019, overall protein contents decreased compared to the previous year, for all five plots studied. The protein content, from the fodder taken from all the improved plots, did not show significant differences ($p > 0.05$) compared to the control plot.

According to the Student's test, in terms of crude fiber (CF) content in the first experimental year, no statistically significant differences ($p > 0.05$) were revealed, compared to the control plot, for any of the plots improved by different methods. The content in fiber, together with that in protein compounds, contributes significantly to the qualitative value of the fodder intended for animals (LINSKENS and JACKSON, 1989), being that component that helps to regulate intestinal transit, to stimulate the feeling of satiety (GEORGESCU et al. 2007).

In 2018, a decrease in fiber content is observed in the feed from the improved plots, with highly significant ($p \leq 0.001$) and distinctly significant ($p \leq 0.01$) lower differences compared to the control plot T. A content of crude fiber, between 20-30%, characterizes a good quality forage, optimal for the forage ration of ruminants (NICHITA, 1984).

Forage samples, taken in the third year of the study, 2019, had a slightly higher crude fiber content for all improved plots, with the exception of control plot T.

Regarding the total content in mineral elements (crude ash), in the first experimental year, the fodder harvested from the improved plots did not show significant differences ($p > 0.05$) compared to the control plot. In the second year of the study, the total ash content was higher, compared to the year 2017, for all the experimental plots, these registering significantly greater differences, statistically assured ($p \leq 0.05$) compared to the control. In 2019, regarding the total ash content in the feed, it did not register significant differences ($p > 0.05$), for the improved experimental plots, compared to the control plot T, these not being statistically ensured.

The digestibility coefficient of the dry matter (DDM), in the first year of the study, 2017, in the fodder harvested from the improved experimental plots A, B, C and D, did not register significant differences ($p \leq 0.05$), compared to the fodder on the plot control T, unenhanced.

In the second year of the study, the values of the digestibility coefficients of dry matter (DDM), recorded on the improved experimental plots, showed very significantly ($p \leq 0.001$) higher differences compared to the unimproved plot. The values were between 60.50% - 68.13%.

In the last year of the study, the only experimental plot, which registered a distinctly significant difference ($p \leq 0.01$), was plot D, 13.60% higher than the control plot T. The other experimental plots did not register significant differences ($p \leq 0.05$), in terms of dry matter digestibility (DDM), compared to the control plot T.

Table 2. The chemical composition of the fodder in the three years (2017-2019)// Compoziția chimică a furajelor în cei trei ani (2017-2019)

Parameter	Year	PLOT					DL		
		A	B	C	D	T	5%	1%	0.1%
CP (%)	2017	13,20*	11,90ns	12,33ns	13,07*	9,87wt	3,19	4,64	6,97
	2018	16,40***	15,30**	15,20**	16,20***	11,57wt	1,82	2,65	3,98
	2019	13,47ns	11,23ns	11,13ns	13,97ns	10,67 wt	4,1	5,96	8,96
CF (%)	2017	32,83ns	36,17ns	31,90ns	28,70ns	31,50wt	7,06	10,27	15,43
	2018	28,3700	26,10000	28,0300	24,53000	32,60wt	2,48	3,61	5,43
	2019	29,00ns	28,47ns	28,87ns	25,130	31,10wt	4,81	6,99	10,51
ASH (%)	2017	7,53ns	6,97ns	7,77ns	8,23ns	7,57wt	1,62	2,36	3,54
	2018	10,10**	9,37*	10,23***	10,17**	8,17wt	0,95	1,38	2,07
	2019	8,73ns	7,77ns	7,73ns	8,30ns	7,43wt	2,09	3,04	4,56
DDM (%)	2017	56,50ns	52,80ns	55,80ns	63,23ns	57,47wt	13,27	19,31	29,01
	2018	62,37***	66,50***	60,50***	68,13***	48,90wt	3,34	4,86	7,31
	2019	61,87ns	62,90ns	63,53ns	69,70**	56,10wt	8,71	12,68	19,04

Significance level: ns $p > 0,05$; * $p \leq 0,05$; ** $p \leq 0,01$; *** $p \leq 0,001$

In Table 3 are presents a summary of the average of the main physico-chemical parameters of the milk collected from the cows that grazed on the five experimental plots A, B, C, D and T, which were analysed during the study period 2017-2019. During the three experimental years, the average percentage

of crude fat in the milk samples was in the range of 3.651% - 3.706%, the effect of the grazed plot being statistically uncertain ($p > 0.05$).

Regarding the average value of the concentration of SNF (solids non-fat), from the milk samples, the plot effect was not statistically assured ($p > 0.05$), and statistically assured values for SNF were obtained within the study years ($p \leq 0.05$).

The highest average value of crude milk protein (3.429%) was obtained in the last year of the study (2019), statistically assured ($p \leq 0.001$). Changes in milk fat and protein content are affected by changes in the floristic and chemical composition of biomass (DUBLJEVI´C et al., 2020).

The average of the three years studied (2017-2019), regarding the lactose content of the milk taken from the five batches of cows A, B, C, D and T, varied between 4.863% and 4.954%.
For the pH value in the milk samples, significant values ($p \leq 0.05$) were recorded, statistically ensured for the groups of cows, from the five plots.

Table 3. The average values of the physico-chemical parameters of milk (2017-2019)// Valorile medii ale parametrilor fizico-chimici ai laptelui (2017-2019)

Parameter	**Lot/Plot** (P)					**Year** (Y)			Effect and signification		
	A	**B**	**C**	**D**	**T**	**2017**	**2018**	**2019**	P[2]	Y[3]	PxY[4]
Fat (%)	3,655	3,622	3,681	3,706	3,651	3,609	3,652	3,727	ns	ns	ns
SNF[1] (%)	8,976	9,026	8,915	8,891	8,866	8,902	8,866	9,037	ns	*	ns
Density g/cm^3	1,030	1,030	1,030	1,0302	1,029	1,030	1,031	1,032	ns	ns	ns
Protein (%)	3,399	3,400	3,393	3,409	3,345	3,393	3,346	3,429	ns	***	ns
Lactose (%)	4,895	4,903	4,954	4,915	4,863	4,938	4,867	4,913	ns	ns	ns
pH	6,44	6,46	6,50	6,49	6,44	6,47	6,49	6,44	*	ns	*

[1] SNF = Solids Non Fat
[2] P = Plot
[3] Y =Year
[4] PxY = Plot x Year interaction

Level of significance: ns $p > 0,05$;* $p \leq 0,05$; ** $p \leq 0,01$; *** $p \leq 0,001$

CONCLUSIONS

1. The changes in the composition of the grassy carpet regarding the participation percentage of plant species were due to the improvement methods applied and the climatic conditions of the respective years.
2. The values of the crude protein content of the feed obtained on the improved plots were in 2018 significantly higher ($p \leq 0.05$), compared to the protein content of the feed obtained from the control plot.
3. The crude fiber content of the feed from the improved plots decreased in 2018 with highly significant ($p \leq 0.001$) and distinctly significant ($p \leq 0.01$) lower differences compared to the control plot.
4. In the second study, the values of the digestibility coefficients of the dry matter (DSU), recorded on the improved experimental plots, recorded very significantly ($p \leq 0.001$) higher differences compared to the unimproved plot.
5. The chemical composition of the fodder registered changes during the studied period, being influenced by the botanical composition, climatic conditions, as well as the improvement measures applied.

6. A positive influence of the improvement methods on the quality of the fodder from the improved plots was found, especially for the second year of research, compared to the fodder obtained from the control plot.

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INNOVATIVE TEHNOLOGIE OF GRAIN SORGHUM PROCESSING

TEHNOLOGIE INOVATOARE DE PRELUCRAREA SORGULUI DE BOABE

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Abstract

As sorghum is a plant originating from southern regions, its temperature requirements are high. The amount of heat expressed by the thermal constant for sorghum is between 2,000 and 5,000 °C, depending on the precocity of the hybrids. It is known that there is a negative correlation between production and precocity. Through the spread of hybrids with a longer vegetation period and high yield potential, there is a risk that it will not reach a humidity that does not require pre-drying. Now that energy is very expensive it forces us to find economical cover solutions. For several years we have tested almost the entire range of sorghum hybrids from several continents in our unit and we came to the conclusion that since we are located in an area with a limited heat regime (multiannual average of the sums of active temperatures of 1270 ° C based on 10 ° C between the last spring frost and the first autumn frost) we can only spread in the area extra-early hybrids, which reach the storage humidity of 12-14%.The introduction of hybrids from the semi-early and semi-late groups with yields of 9-10 t / ha, which in the warmest year reach a humidity of 27-30% until the first frost, requires the identification of conservation technologies not in the form of grain - which is also very expensive, but instead as paste. During 2021 we tested this process as our own solution and developed a technology for preservation at higher humidity and we also want to test it on groups of cattle in our own unit.

Keywords: Grain Sorghum, grain croshing/crimping, silobags, preservation

Rezumat

Sorgul fiind o plantă origninară din regiunile sudice, pretențiile față de temperatură sunt mari.

Cantitatea de căldură exprimată prin constanta termică pentru sorg este cuprinsă între 2000 și 5000 °C în funcție de precocitatea hibrizilor. Se știe că între producție și precocitate este o corelație negativă. Prin extinderea hibrizilor cu perioadă mai lungă de vegetație și cu potențial mare de producție, există riscul ca acesta să nu ajungă la o umiditate încât să nu necesite o uscare prealabilă, ori acum energia fiind foarte scumpă ne obligă la găsirea unor soluții acoperitoare economic. Pe parcursul mai multor ani am testat în unitate aproape toată gama de hibrizi de sorg de pe mai multe continente și am ajuns la concluzia că fiind situați într-o zonă cu regim termic limitat (media multianuală a ∑ temperaturilor active de 1270 °C cu baza de10 °C între ultima brumă de primăvară și prima brumă de toamnă) nu putem extinde în zonă decât hibrizi extratimpurii, care ajung la umiditatea de păstrare (12-14%). Introducerea de hibrizi din grupele semi-timpurii și semi-tardivi cu producții de 9-10 t/ha, care în cel mai cald an ajung la umiditatea de 27-30% până la prima brumă, impune găsirea de tehnologii de conservare nu sub formă de boabe care este și foarte costisitoare, ci sub formă de pastă. În anul 2021 am testat acest procedeu ca fiind o soluție proprie și am elaborat o tehnologie de conservare la umiditate mai ridicată urmând o testare pe grupe de bovine în unitate.

Cuvinte cheie: Sorg de boabe, zdrobirea/ sertizarea boabelor , silobag, conservare

INTRODUCTION

The climatic conditions of the last years were very unsuitable for most fodder crops, due to a drastic water and thermal stress that occurred during the critical periods of vegetation. In recent years, the drought established during the summer in many European countries was also felt in all regions of Romania, causing the scorching of pastures and compromising a high proportion of cereal, oilseed and fodder agricultural crops located in areas characterized by a high degree of aridity such as they are in Dobrogea, eastern Bărăgan, southern Moldavia, the southern extremity of the Romanian Plain, but also local areas of the Transylvanian Plain.

Of all the branches of the economy, agriculture suffered the most from the phenomenon of aridification, a fact that determined the explosive increase in the prices of agri-food products and the decrease in the purchasing power and the standard of living of the population. Taking into account the increasing aridification phenomenon, the vulnerability of current crops manifested by the sharp reduction

of yields due to the absence of water at key moments of development, it is necessary to diversify the structure of field crops and fodder crops, promote drought-tolerant species and adopt technologies and soil processing systems that have the effect of preserving the water reserve (www.icpa.ro).

In this context, the crop that went well over the climate shock, without showing significant yield drops, was sorghum, a species that shows a special resistance to the stress caused by dryness, not demanding on the type of soil. The high ecological plasticity, the high photosynthetic yield places sorghum, along with corn, among the most productive cultivated cereals (www.caussade.ro).

Sorghum is an ecological, energetic, economic crop. Ecological: 1 Ha of sorghum absorbs 50 t of CO2, 3 times more than 1 Ha of forest, 5 times more than 1 Ha of field crop. Energy: 1 Ha of sorghum = 4-5 t biodiesel, 12 t briquettes. Economic: For 1 kg of SU consumes 271 l of water, 3 times less than 1 ha of alfalfa, 1.5 less than 1 ha of corn (Bilteanu, 1979).

Sorghum is a short-day plant, needing short days and long nights to go through the vegetative phase. Any shortening or lengthening of the photoperiod causes a change in the duration of the vegetation period. During the entire vegetation period, the sum of the temperature degrees required for sorghum is 2500-3500°C (average positive temperatures) (Bilteanu, 1989). The required thermal constant is closely related to the variety and there is a wide range of hybrids, some flowering in about 45 days after sowing and the very late ones that bloom only after 120 days and do not reach maturity in the pedo-climatic conditions of our country (Muresan et al., 1965). The variation in temperature also has a great influence on the rate of development of sorghum. Thermal stress, especially in the reproductive phenophase, significantly affects plant height and productivity elements, such as the number of seeds in the panicle and the size of the grain (Bekele E.,1998).

Sorghum is a thermophilic species, with very high temperature requirements. The minimum temperature for seed germination is 10-12°C, and the favorable temperature during plant growth is 25-27°C. At temperatures lower than 5-10°C, sorghum stops growing. Among the annual fodder plants cultivated in our country, sorghum has the highest resistance to drought, due to the highly developed root system and the reduction of growth in case of insufficient water. This also explains the low transpiration coefficient of sorghum (140-170) (Sin Gheorghe, 2005).

Due to the adaptation to arid climatic conditions, the average production in drought conditions can even exceed the average corn yield by 30%, and in optimal rainfall conditions it can be compared with similar yields, a fact by which sorghum contributes to the safety of production (www.euralis.ro).

Although very promising, the expansion of sorghum production was not spectacular, despite the fact that the breeders set as their objectives the increase of grain production in sorghum, based on the increase in the number of grains in the panicle, the increase in the number of panicles per plant and MTG, the creation of hybrids of sorghum resistant to drought, to aphid attack, to tolerance of soil alkalinity and salinity as well as to varieties with a low tannin content (Besancon T., 2003).

We grow sorghum because it utilizes soil water very well due to its deep root system and has low evapotranspiration level, and in case of heat, it pollinates and does not suffer as much as corn, being able to produce profitable yields. It capitalizes on poor lands and minimal fertilization conditions (Berenji J., Dahlberg J., 2004). Sorghum is not sensitive to the preceding plant, it also produces under monoculture conditions. Crops that leave the land clean of weeds are recommended as a precursor plant, because in the first 2-3 weeks after emergence, due to the slow growth rate in this phase, there is a danger of strong weeding. In this sense, the best are fallow crops (maize, sunflower) and fodder plants (Starodub Victor, 2008).

Table 1. The chemical composition and nutritional value of sorghum in various forms// Compoziţia chimică şi valoarea nutritivă a sorgului sub diferite forme

The type of feed	Water	% Dry Substance					
		Proteins	Fats	Non nitrogenous extracts	Cellulose	Ashes	N.U. /100 kg of fodder
Silage sorghum	72.1	10.1	2.5	50.2	29.8	7.5	23.4
Last harvest of silage sorghum	77.0	14.4	2.6	44.4	27.4	11.3	19.4

The type of feed	Water	% Dry Substance					
		Proteins	Fats	Non nitrogenous extracts	Cellulose	Ashes	N.U. /100 kg of fodder
Silage fodder	71.5	8.8	3.8	48.2	29.8	9.8	22.0
Hay of the lost harvest	15.0	12.6	3.7	52.0	22.2	9.5	57.4
Hay	15.0	11.3	2.4	45.1	31.7	9.7	49.2
Grains	16.0	12.9	3.2	77.9	3.5	2.6	118.8

Sursa/Source: Starodub, 2008

Used in animal feed in its green state, hay or silage, sorghum is imposed by yields comparable in quantity to corn, with close fodder value (90-100% of the value of corn) - see table 1.

The ARVALIS institute, in collaboration with the state agency FranceAgrimer, carries out analysis on grain sorghum every year. The results indicate that in terms of chemical composition, grain sorghum is similar to other grains such as wheat or maize. The starch content of sorghum (source of energy) represents 74% of the dry matter; exceeding wheat and equaling corn. The protein content of sorghum (which is, on average, 11%) can vary between 10 and 12%, in the best cases. Due to its chemical composition, energy value and high protein content, as well as its low sensitivity to mycotoxins, sorghum presents important advantages in animal feeding, integrating perfectly into the rations of both monogastrics and cattle (www.sorghum-id.com).

MATERIAL AND METHODS

The Research and Development Station for Cattle Breeding is located on the territory of Sangeorgiu de Mures, which is located 3 km north of the city of Targu Mures, having the geographical coordinates: the meridian 24°33' east longitude and the parallel 46°33' north latitude. It has tested different varieties of sorghum for grain and silage for several years.

The culture was placed on a brown forest soil, weakly podzolic (representative of over 80% of the surface of Targu Mures), with a humus content of 2.2, pH of 5,8, P_2O_5 suply is 17.4 mg/100 g soil, K_2O 21,7 mg/100 g soil and an N index of 1.4, water table above 12 m.

The culture technology consisted of the following stages: the land was plowed in the fall to 25 cm, and in the spring when it was possible to enter the field, there was a discussion of uniformity and to favor the development of perennial weeds (Agropyron repens, Convolvulus arvensis, Cirsium arvense, Symphytum officinale). Since sorghum is sown after May 10, we waited for good perennial weed development, and applied a non-selective herbicide Glyphogan. 4-5 days before sowing we carried out work with the combiner for the formation of the germinative bed and the incorporation of fertilizers. The culture being located on an acid soil, we applied 200 kg of Nitrolime. When the soil reached 12-13 °C with a growth tendency, sowing was carried out (starting from May 10-15). Sowing was done with the SPC6 seeder using a density of 250,000 pl/ha.

Immediately after sowing, a pre-emergence herbicide was carried out with Dual Gold 1.5 l/ha, to control annual monocotyledons, and to combat perennial dicotyledons on vegetation at 5-6 leaves, a combination of 0.7 l/ha Tomigan and 0.8 l was used /ha Amino.

During the vegetation period no other interventions were needed. Since the last years these have been quite atypical from a climatic point of view, the hybrids tested in our area did not lose moisture enough to be stored as such, requiring prior drying, which is very expensive.

We chose to preserve the grains in the form of a paste at a humidity of over 25%. The transformation of the grains into paste was achieved with a special mill with rollers driven from the power take-off of the tractor at 540 rpm.

The resulting paste was blown by pressure into a special silobag equipped with double foil walls. During grinding, the paste was treated with a special inoculant for better preservation. It was stored for 3 months, after which it was introduced into the feed, replacing corn in the feed ration of young cattle.

RESULTS AND DISCUSSIONS

A limiting factor in the expansion of sorghum in our area is the amount of active temperatures accumulated to reach maturity. In order to identify hybrids with thermal requirements that can be realized in the area, we undertook a series of experiments with sorghum hybrids with different periods of maturity. In table no. 2, 3 and 4 shows yields and moisture at harvest.

Hybrids from 3 maturity groups were tested: early, mid-early and mid-late.

Table 2. Results regarding the yield of some grain sorghum hybrids experimented at SCDCB Tg. Mures in 2018// Rezultate privind producția a unor hibrizi de sorg boabe experimentat la S.C.D.C.B. Tg. Mureș în anul 2018

Nr.	Hybrid	Maturity group	Humidity	Yield kg/ha	Avg. yield kg/ha
1	Quebec	Early	16.0	4700	4790
2	Arfiro	Early	16.9	4900	
3	Queyras	Early	16.7	4400	
4	Arlys	Early	16.9	5880	
5	Armida	Mid-early	17.7	7400	7550
6	Es Alize	Mid-early	17.5	7300	
7	Arkanciel	Mid-early	17.0	7940	
8	Solaris	Mid-late	19.6	7700	7725
9	Mistral	Mid-late	19.8	7750	

The obtained yields were 4970 kg/ha at the early hybrids, 7750 kg/ha at the mid-early hybrids and 7725 kg/ha at the mid-late hybrids.

It should be noted that the humidity at harvest was between 16%, 17.5% and 19.8% depending on the precocity of the hybrids, but no hybrid reached 14% even though the thermal input of 2018 was over 1200 °C active.

In 2019, we sowed and monitored 10 hybrids, also from Lidea of the three maturity group.

Table 3.Rezults regarding the yield and chemical composition of some grain sorghum hybrids experimented at S.C.D.C.B. Tg. Mureș in 2019// Rezultate privind producția și compoziția chimică a unor hibrizi de sorg boabe experimentat la S.C.D.C.B. Tg. Mureș în anul 2019

Nr.	Hibryd	Maturity group	Humidity	Yield kg/ha	Avg. yield kg/ha	Protein	Fats	Starch
1	Aquilon	Early	17.8	5400	6500	9.6	2.2	64.5
2	Arfiro	Early	17.5	5900		10.0	2.8	64.6
3	Arlys	Early	17.5	6700		8.8	2.3	64.9
4	Alize	Early	18.0	8000		9.3	2.6	64.1
5	Arakan	Mid-early	17.0	8100	8675	9.2	2.8	65.3
6	Arak	Mid-early	17.5	8500		10.5	2.0	63.7
7	Armida	Mid-early	17.8	8300		9.6	1.8	64.4
8	Arkansiel	Mid-early	17.9	9800		10.3	2.6	63.0
9	Solaris	Mid-late	18.4	9700	9600	9.1	2.1	65.3
10	Mistral	Mid-late	19.6	9500		9.4	1.9	64.1
Maize			21.0	8943		8.9	3.4	65.3

The obtained yields were between 5400 kg/ha and 9800 kg/ha depending on the precocity of the hybrids. The protein content was between 8.8% and 10.5%, and the starch content was between 63.7% and 65.3%. Moisture at harvest was between 17% and 19.6%.

In 2020 we studied 7 hybrids, this year achieving the highest yields, reaching 13200 kg/ha at the hybrids with late maturity group. Protein % was between 8% and 10.5% and starch % between 62.2% and 64.1%.

Table 4. Rezults regarding the yield and chemical composition of some grain sorghum hybrids experimented at S.C.D.C.B. Tg. Mureș in 2020// Rezultate privind producția și compoziția chimică a unor hibrizi de sorg boabe experimentat la S.C.D.C.B. Tg. Mureș în anul 2020

Nr.	Hybrid	Maturity group	Humidity	Yield kg/ha	Avg. Yield. Kg/ha	Protein	Fats	Starch
1	Arfiro	Early	18.0	7300	9566	10.5	3.0	63.7
2	Es Typhon	Early	18.9	10600		10.5	2.0	63.6
3	Es Alize	Early	18.6	10800		9.6	2.6	63.1
4	Arakan	Mid-early	21.5	10600	10350	8.7	2.8	64.1
5	Arkansiel	Mid-early	20.7	10100		9.2	3.2	62.5
6	Aquilon	Mid-late	23.0	13200	12450	8.0	2.4	63.9
7	Es Foehn	Mid-late	23.7	11700		8.4	2.9	62.2
Maize			19.4	12500		7.7	3.3	65.0

Grain humidity at the harvest was between 18% and 23.7% at the hybrids with the mid-late maturity group.

The examined grain sorghum hybrids have never reached storage moisture (14%) in the past few years, raising the question of whether grain sorghum can be promoted in the central area of Romania. In this situation, we conducted analyses over the last 13 years regarding the amount of active temperatures with a base of 12°C, presented at table 5.

Table 5. Analysis over the last 13 years of the ∑ activ temperatures with a base of 12°C// Analiza pe ultimii 13 ani a ∑.t.a. cu baza de 12°C

	April	May	June	July	August	September	October	Total
2010	11.3	126.1	209.4	268.5	282.0	81.5	0	978.8
2011	15.5	118.9	201.6	254.9	249.5	156.3	5.9	1002.6
2012	39.2	130.5	258.8	379.0	292.7	178.3	52.3	1330.8
2013	53.0	154.5	223.8	278.,1	315.9	66.9	16.4	1108.6
2014	26.0	106.4	180.0	276.0	255.4	168.3	35.1	10472
2015	21.7	121.0	202.0	302.2	294.3	164.3	11.3	1116.8
2016	57.7	94.4	264.7	294.3	260.6	157.4	8.3	1137.4
2017	25.3	135.1	250.3	282.9	325.2	135.7	6.7	1161.2
2018	116.6	210.4	241.9	270.8	338.1	146.1	29.4	1353.3
2019	37.1	117.8	302.0	272.,7	327.2	160.1	21.2	1238.1
2020	10.4	78.1	230.2	279.7	309.1	189.3	50.,0	1146.8
2021	8.5	95.,0	238.4	320.3	262.8	162.6	2.2	1089.8
2022	9.8	114.6	289.2	337.6	325.1	94.8	11.2	1212.3
Avg.	33.23	125.6	237.8	293.6	295.2	143.2	19.2	1147.9

The active temperatures of the months of April and October and those of the first decade (1-10) of the month are still unused by sorghum in most years. Sorghum does not germinate normally unless the april temperature is consistently above 12°C, and October temperatures when atmospheric humidity is high do not help sorghum ripen.

Therefore the useful temperatures for sorghum in our area only add up to 1060°C.
1147-33.23+19.2+34 (first decade of May and October) = 1060, or at this amount of heat even the earliest hybrids do not reach maturity The climatic conditions in our area are not favorable for the realization of high grain yields with adequate storage moisture (14-15%). In this situation we are forced to resort to other solutions to preserve the grains without spending significant energy. In this sense, we have implemented the grain preservation technology with a moisture content of 25% by turning the grains into a flour and storing them in a silobag.

Silobag, more precisely, silage bagging is an innovative technical solution for farmers, which favors the efficiency of harvest storage processes. This new system significantly reduces both quantitative and qualitative losses.

During the winter time, the sorghum flour was preserved very well, with no undesirable phenomena. After 3 months of preservation, the silobag was opened and an analysis of the chemical composition was made by an authorized company in comparison to corn flour. We reproduce the comparative results in table 6.

Table 6. Comparative results of chemical analyzes of sorghum flour //Rezultatele comparative a analizelor chimice al făinii de sorg

	Shorgum flour (paste)	**Maize flour**
Dry matter	77,21	83,18
Humidity	22,79	16,82
Starch	75.94	64.43
Total protein	10,30	8,9
Digestible protein	8.44	7.36
Crude fat	1,81	1,70
Crude fibre	5,34	4,89
Ash	1,75	1,54
ADF *= non-digestible cellulose	12,01	8,25
NDF *= dry substance intake of the rumen	31,85	28,54

The amount of dry matter was lower in sorghum flour because preservation was done at 25% moisture and in corn at 17%.

An economic analysis of preserving grains in the form of paste highlights that the cost price is lower than the option of drying with a thermal agent. Drying costs 23 lei + VAT/%/t (24% y 14% = 273 lei +VAT), and preservation in the form of paste costs 70.4 lei + VAT/t (Together with mill rental, silo bag's price, the price of the inoculants and workmanship).

The paste was administered to young cattle. From the quality analysis we can conclude that sorghum paste can be an alternative to maize, but further experiments need to be carried out both with young cattle and milk cows.

CONCLUSIONS

1. The high costs of feed, its production in the needed quantities and ensuring the economic efficiency of a cattle farm in the context of global warming generate worldwide problems for which scientific research is required to find innovative solutions for optimizing feed rations and increasing the digestibility of basic feed without affecting the production, quality and health of the animals.
2. As a crop, sorghum makes very good use of soil water making use of poorer lands. In addition, pollination is not as strongly affected by heat as in the case of corn.
3. It is not attacked by crows or wild boars and is not an attractive culture for evasion. Thus, it contributes to the safety of fodder production.
4. Based on the results achieved so far, we believe that sorghum paste will constitute a good fodder for the animal husbandry sector in the context of unfavorable climate changes in recent years. Sorghum paste will be tested on the farm on groups of animals and the results will be published in a future session.

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RESEARCH ON WEED CONTROL OF GRAIN SORGHUM CROP

CERCETĂRI PRIVIND CONTROLUL BURUIENILOR DIN CULTURA SORGULUI PENTRU BOABE

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Abstract.
*The research was carried out in the period 2019-2020, in the conditions of the sandy soils of southern Oltenia and aimed at the control of weeds in the grain sorghum culture, by applying chemical products, alone or associated with other herbicides and with mechanical control measures. The obtained results revealed an average degree of weeding during the sorghum vegetation period, noted on the EWRS scale with values between 1.3-8.1, the maximum being recorded in the control variant (unherbicided and not hoeing mechanics). The best results were obtained in the variants in which the weeds were controlled by chemical methods with pre-emergent and post-emergent herbicides + 2 mechanical hoeing in the vegetation, the weeds being destroyed in a proportion of 79-84%, compared to the control variant. Plant height and leaf area index were distinctly significantly negatively correlated with increasing weediness (r=-0.823**; r=-0.980**). Treating the sorghum crop with the product Dual Gold 960, applied pre-emergent at a dose of 1.5 l/ha, in combination with a post-emergence herbicide with one of the products Buctril Universal, applied at a dose of 1 l/ha, Trek P334 SE, in dose of 3.5 l/ha or Dicopur Top 464 SL, in a dose of 1 l/ha and associated with two mechanical weedings production increases of 143-168% were achieved as, compared to the non-herbicated control and without hoeing mechanics in the vegetation.*
Keywords: *sorghum, herbicides, selectivity, efficacy, weeds, production*

Rezumat
*Cercetările s-au efectuat în perioada 2019-2020, în condițiile solurilor nisipoase din sudul Oltenia și au vizat controlul buruienilor din cultura sorgului pentru boabe prin aplicarea unor produse chimice, singure sau asociate cu alte erbicide și cu măsuri de combatere mecanică. Rezultatele obținute au relevat un grad mediu de îmburuienare pe perioada de vegetație a sorgului, notat pe scara EWRS cu valori cuprinse între 1,3-8,1, maximul fiind înregistrat in varianta martor (neerbicidat și neprășit). Cele mai bune rezultate au fost obținute în variantele în care buruienile au fost controlate prin metode chimice cu erbicidare preemergentă și postemergentă + 2 prășile mecanice in vegetație, buruienile fiind distruse în proporție de 79-84%, comparativ cu varianta martor. Talia plantei și suprafața foliară s-au corelat distinct semnificativ negativ cu creșterea gradului de îmburuienare (r=-0,823**; r=-0,980**). Erbicidarea culturii de sorg cu produsul Dual Gold 960, aplicat preemergent în doză de 1,5 l/ha, în combinație cu o erbicidare postemergentă cu unul dintre produsele Buctril Universal, aplicat în doză de 1 l/ha, Trek P334 SE, în doză de 3,5 l/ha sau Dicopur Top 464 SL, în doză de 1 l/ha și cu efectuarea a 2 prașile mecanice, a realizat sporuri de producție de 143-168%, față de martorul neerbicidat și nelucrat mecanic în vegetație.*

Cuvinte cheie: *sorg, erbicide, selectivitate, eficacitate, buruieni, producție*

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is a cereal adapted to hot and dry climates, native to Equatorial Africa, being the main bread grain in Africa, Southern Europe, Central America and Southern Asia. Although 80% of the world's sorghum production is under drought conditions, water stress at the vegetative stage can reduce yield by over 36%, and water stress at the reproductive stage can reduce yield by over 55% (Assefa et al., 2010). A recent study showed that sorghum has the yield and economic advantage over maize in dry regions due to better drought and high temperature tolerance (Starggenborg et al., 2008, Prasad et al., 2021). Sorghum grains have a high nutritional value with 70-80% carbohydrates, 11-13% protein, 2-5% fat, 1-3% fiber and 1-2% ash. Sorghum grain proteins do not contain gluten, making it a special food for people suffering from celiac disease (gluten intolerance),

including diabetic patients (Prasad and Staggenborg, 2010). Reducing the degree of weeding of crops is a major objective in obtaining good results in agriculture and can be achieved primarily by improving the management of plant cultivation, i.e. by reintroducing "good practices in agriculture" (soil quality works, quality seeds with rapid emergence, crop rotation, weed-free maintenance of the land between crops, elimination of sources of infestation etc.).

Tillage technology can influence weed population dynamics and consequently the choice of appropriate weed management practices. In this sense, the research carried out by Govindasamy et al., (2020) at Texas A&M University, College Station, showed that on the land worked by the conventional method for 36 years, the degree of weeding in the sorghum crop was lower , compared to the use of the no-till method, where a high percentage of perennial weeds (*Sorghum halepense* (L.) Pers.) was found. In grain sorghum, weed management is a key challenge to achieve economic production, but available herbicides are very limited, especially those applied post-emergence to control monocot weeds (Fromme et al., 2012, Werle et al., 2017, Bagavathiannan et al., 2018).

Worldwide research has shown that the use of herbicides increases the coefficient of utilization by cultivated plants of improved vegetation factors: solar energy, nutrition space, fertilizing elements, irrigation, drying etc. (Pannacci et al., 2018). To exploit the genetic potential of sorghum, in addition to other technological factors, an integrated sustainable weed management approach is emphasized, which includes a strategic application of preemergence and postemergence herbicides used in combination with non-chemical practices. Research in Arkansas showed that sorghum yield was 50% lower on land where no chemical weed control treatments were applied (Bararpour et al., 2019).

Development of an integrated management plan for weed control in sorghum may become necessary if herbicide-intolerant sorghum hybrids are sown, which limit chemical control options and therefore increase the rate of germination and emergence of sorghum weeds. Studies conducted by Grichar et al. (2005) highlight the optimal timing of application of atrazine, pendimethalin, and trifluralin herbicides, applied alone or in a mixture, by testing at different developmental stages of weeds and sorghum. The best results were obtained by applying the herbicides based on *atrazine* and *trifluralin* in the early development phase of the 7 cm plants, the weeds being controlled in a percentage of 76-100%, depending on the climatic conditions of the year. The same results were obtained in the state of Gujarat on the west coast of India (Verma et. al., 2018).

The quality of sorghum production can be significantly reduced when the degree of weeding is not controlled (Protein=9.94%), compared to the application of various weed control methods where the protein in the grain is 10.31-10.63%, function of treatment (Verma et. al., 2017). The chemical control of weeds creates favorable premises for the complete mechanization of agricultural crops, at the same time facilitating the increase of the efficiency of some agrophytotechnical measures and their culture parameters (Matei, 2011, Drăghici et al., 2019). The high degree of weeding on sandy soils is determined by the fact that weed seeds retain their generative capacity very easily and for a long time, as a result of increased aeration and lower soil moisture. On these lands, the high porosity and the use of irrigation water provide favorable conditions for the growth and development of cultivated plants, but also for weeds (Pintilie et al., 1972, Matei et al., 2021).

MATERIAL AND METHOD

The research was carried out during 2019-2020 at the Research-Development Station for Plant Culture on the also Dăbuleni Sands and aimed at preventing and combating weeds in grain sorghum culture and aimed at the prevention and control of weeds in the grain sorghum crop, by applying some chemical products, alone or associated with other herbicides and mechanical control measures, compared with a control variant (not herbicided and not hoeing mechanics). The experiment included 9 experimental variants (Table 1), located in the field according to the method of randomized blocks in 3 repetitions.

The sorghum culture was placed in an irrigated system, on a sandy soil poorly supplied with nitrogen (0.04% and 0.12%), well supplied with phosphorus (54-10 ppm), with a state of low to medium exchangeable potassium supply (26-64 ppm), and in organic matter (organic carbon =0.20-0.63%) and a pH in water with values between 4.64 and 5.63, which shows a moderately to weakly acidic reaction acid. The sorghum culture was established between April 24 (2019) - April 29 (2020), by sowing the ES ALIZE hybrid, treated with CONCEP III, ensuring a seeding of 25 germinable grains/m^2 and a plant nutrition regime of $N_{150}P_{80}K_{80}$. The pre-emergent herbicides were applied immediately after sowing the sorghum crop, without exceeding the 5-day period from the preparation of the seed bed so that the weeds do not germinate. The application of post-emergent herbicides was carried out in the period between weed seed germination and the 4-6 leaf phase of the weeds, in the last decade of May.

During the vegetation period of the sorghum plants, determinations were made regarding the degree of weeding, the selectivity and effectiveness of the herbicides, the height of the plant, the diameter of the stem and leaf area index. At harvest, the quantity and physical quality of grain production were determined. The obtained research results were analyzed by the method of analysis of variance (ANOVA) and with the help of mathematical functions.

Table 1. Experimental variants for weed control in grain sorghum // Variante experimentale pentru combaterea buruienilor la sorgul pentru boabe

No.var.	The experimental variant	Active substance	Application time	Dose (l/ha)
1.	Control	-	-	-
2.	Trek P34 SE	*pendimetalin 64 g/l + terbutilazin 270 g/l*	Postemergence	3.5
3.	Gardoprim Plus Gold 500 SC	*S-metolaclor 312,5 g/l + terbutilazin 197,5 g/l*	Preemergence	3.5
4.	Buctril universal	*bromoxilin 280 g/l + acid 2,4 D (ester) 280 g/l*	Postemergence	1
5.	Dicopur Top 464 SL	*sare de dimetilamină 40 g/l + dicamba 120 g/l*	Postemergence	1
6.	Dual Gold 960 EC	*S-metolaclor 960 g/l*	Preemergence	1.5
7	Dual Gold 960 EC + Trek P34 SE + Hoeing mechanic	*S-metolaclor 960 g/l* *pendimetalin 64 g/l + terbutilazin 270 g/l*	Preemergence Postemergence	1.5 3.5
8	Dual Gold 960 EC + Buctril universal+ Hoeing mechanic	*S-metolaclor 960 g/l* *bromoxilin 280 g/l + acid 2,4 D (ester) 280 g/l*	Preemergence Postemergence	1.5 1
9	Dual Gold 960 EC + Dicopur Top 464 SL+ Hoeing mechanic	*S-metolaclor 960 g/l* *sare de dimetilamină 40 g/l + dicamba 120 g/l*	Preemergence Postemergence	1.5 1

RESULTS AND DISCUSSION

Analyzing the climatic conditions recorded at the weather station of the Research-Development Station for Plant Culture on Sands Dabuleni (R&DSPCS Dabuleni), the emphasis of the thermal-hydric stress in the area is highlighted, through the increase of 1.46 ^{0}C in the average air temperature during the period April - September 2019-2020, compared to the multi-year average (Table 2). The drought intensified in the months of July and August, when the average air temperature recorded values of 24.15-25.15 ^{0}C, with an increase of 0.91-2.55 ^{0}C, compared to the multi-year average. The amount of rainfall of 224.4 mm, recorded during the plant's consumption period (May-August), was unevenly distributed and insufficient for the growth and development of sorghum plants, necessitating the application of 4 waterings with a rate of 250 m^3 water/ ha.

Regarding the tolerance of the sorghum plant to the chemical products tested, tolerance assessed by grades in the range 1-9, according to the scale established by the European Weed Research Society (*EWRS*), the results obtained showed a good selectivity for the sorghum plants of the herbicides

Gardoprim Plus Gold 500 SC, *Buctril universal* and *Dual Gold 960 EC* (Table 3). A slight phytotoxic effect, presented in the form of burns on the leaves, was manifested with the post-emergence application of the products *Trek P334 SE* and *Dicopur Top 464 SL*, an effect that faded with the development of the leaf apparatus.

Table 2. Climatic conditions recorded at the weather station* of R&DSPCS Dabuleni during the sorghum vegetation period // Condiții climatice înregistrate la stația meteo* a SCDCPN Dăbuleni în perioada de vegetație a sorgului

Climatic conditions		Calendar month						Period	
		April	May	Junie	July	August	September	April - September	May -August
Average 2019-2020	Average monthly air temperature (^{0}C)	12.8	17.55	22.7	24.15	25.15	20.6	20.49	22.39
	Maximum monthly air temperature (^{0}C)	29	33	35	37.6	38.4	37.3	38.4	38.4
	Minimum monthly air temperature (^{0}C)	-2	4.7	6.7	11	12.9	3.5	-2	4.7
	Rainfall (mm)	32.5	57.3	71.5	63.9	31.5	25.1	281.8	224.2
	Relative air humidity (%)	73	75	78	65	60.5	59	68.42	69.63
Multiannual average 1956-2020	Average monthly air temperature (^{0}C)	11.93	16.93	21.53	23.24	22.6	17.94	19.03	21.08
	Sum of monthly rainfall (mm)	46.82	62.88	70.43	55.19	36.88	45.3	317.5	225.38
Deviation from the multi-year average	Average monthly air temperature (^{0}C)	0.87	0.62	1.17	0.91	2.55	2.66	1.46	1.31
	Rainfall (mm)	-14.32	-5.58	1.07	8.71	-5.38	-20.2	-35.7	-1.18

**AgroExpert from Adcon Telemetry SRL Romania*

Table 3. Selectivity of herbicides against sorghum plants// Selectivitatea erbicidelor față de plantele de sorg

The experimental variant	Dose (l/ha)	Application time	Herbicide selectivity at:			
			15 days from application	30 days from application	45 days from application	60 days from application
Martor (neerbicidat, nelucrat)	-	-	-	-	-	-
Trek P334 SE	3.5	Postemergence	2	1	1	1
Gardoprim Plus Gold 500 SC	3.5	Preemergence	1	1	1	1
Buctril universal	1	Postemergence	1	1	1	1
Dicopur Top 464 SL	1	Postemergence	2	1	1	1
Dual Gold 960 EC	1.5	Preemergence	1	1	1	1

The determinations regarding the efficacy of the products tested in the control of weeds in the sorghum culture, which were carried out dynamically starting with the price of post-emergence herbicide, then at 15, 30, 45 and 60 days after application, they showed that the unilateral use of herbicides did not ensure a good effectiveness in weed control, the degree of weediness at 60 days after application being noted on the EWRS scale with grades in the range 4.6-6 (Table 4). It should be noted that the degree of weeding in the control variant remained high throughout the vegetation period, being noted 6 before the post-emergence application of herbicides, reaching a maximum (note 9) after a period of 45 days. The use of the *Buctril universal* product, applied post-emergence at a dose of 1 l/ha, in combination with pre-emergence weedicide with *Dual Gold 960 EC*, applied at a dose of 1.5 l/ha + mechanical works has shown the best results in combating weeds in grain sorghum crop (average grades being 1.3). Also, good results were recorded by the post-emergence application of *Trek P334 SE* and *Dicopur Top 464 SL* products, in the same combination of pre-emergence herbicide + mechanical works (EWRS Notes 1.6-1.7).

Research in Texas showed that the combined application of products based on *pyrasulfotole* and *bromoxynil* resulted in more than 94% control of *Amaranthus ssp*, *Cucumis melo* and *Proboscidea louisianica* (Fromme et al., 2012). The degree of weeding, recorded on sandy soils, is generally much higher, compared to other soils, because sands heat up more easily, and early spring creates an optimal environment for the germination of weed seeds (Șarpe, 1987, Drăghici Iul, 1999). In our study, the degree

of weeding in the untreated version was high throughout the growing season, rated on the EWRS scale with grades ranging from 6-9 and an average of 8.1.

Table 4. Efficacy of herbicides in the control of weeds in grain sorghum crop / /Eficacitatea erbicidelor în combaterea buruienilor din cultura de sorg boabe

The experimental variant	*Dose(l /ha)*	*Application time**	*Efficacy depending on the date of application of post-emergence herbicides*					
			one day before	*at 15 days*	*at 30 days*	*at 45 days*	*at 60 days*	*average*
Control	-	-	6	8	8.3	9	9	8.1
Trek P34 SE	3,5	Postem	6	4	5	5.33	6	5.3
Gardoprim Plus Gold 500 SC	3,5	Preem	2	2	2.3	4	4.6	3.0
Buctril universal	1	Postem	5.3	3	4.6	5	5.6	4.7
Dicopur Top 464 SL	1	Postem	5.3	3	4	4.66	6	4.6
Dual Gold 960 EC	1,5	Preem	2	2.66	3.33	4.66	6	3.7
Dual Gold 960 EC, Trek P34 SE, Hoeing mechanic	1,5 3,5	Preem Postem	2	1	1.3	1.66	2	1.6
Dual Gold 960 EC, Buctril universal, Hoeing mechanic	1,5 1	Preem Postem	2	1	1	1.33	1.3	1.3
Dual Gold 960 EC, Dicopur Top 464 SL, Hoeing mechanic	1,5 1	Preem Postem	2	1	1.6	1.66	2	1.7

**pre=preemergence; post=postemergence*

The spectrum of weeds recorded in the sorghum crop, in the control variant (Figure 1) was highlighted by the abundant presence of the species *Digitaria sanguinalis* (48.11%), followed by *Ambrosia artemisiifolia*, in a percentage of 29.42 %, by *Portulacca oleraceaea* (10.872%), by *Chenopodium album* (7.17%) and by *Amaranthus retroflexus* (4.43%).

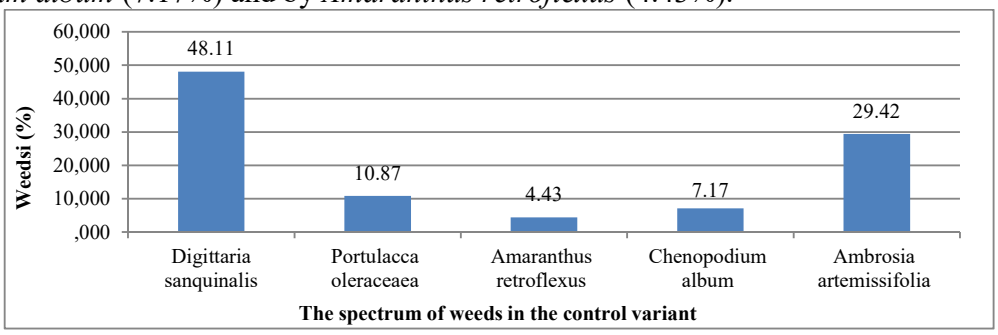


Figure 1. The percentage of weeds recorded in the grain sorghum crop in the control variant / Procentul de buruieni înregistrat în cultura de sorg pentru boabe în varianta martor

The best results regarding efficacy the application of methods to prevent and control the weeding of the grain sorghum crop were obtained by using herbicides with pre-emergent and post-emergent application associated with mechanical methods of maintaining the crop in vegetation through two mechanical works, in which weeds were destroyed in a proportion of 79-84%, compared to the control that presented an average degree of weeding noted on the EWRS scale with a grade of 8.1 (Figure 2).

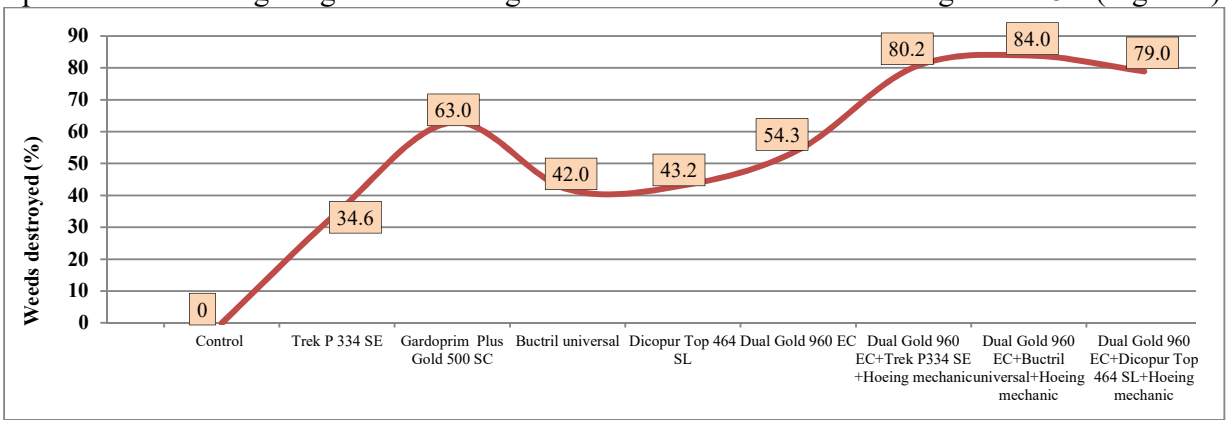


Figure 2. Efficacy methods of prevention and control of weeds during the vegetation period of grain sorghum / Eficacitatea metodelor de prevenire și combatere a buruienilor din perioada de vegetație a sorgului pentru boabe

The determinations made 60 days after the application of post-emergent herbicides revealed the very good efficacy of the combined methods of weed control, by using chemical methods with pre-emergent + post-emergent herbicides and mechanical methods (Figure 3, Photo 1, Photo 2). Thus, compared to the control variant, where the degree of weeding was noted as 9 and a weed biomass amount of 55.11 t/ha was recorded, the positive effect was highlighted in the integrated weed control variants (chemical methods + mechanical methods), where the degree of weeding in the sorghum crop was noted as 1.3-2, being reported 60 days after the post-emergence herbicide only the species *Digitaria sanguinalis* in the amount of 0.6-0.9 t/ha biomass. From the analysis of the effectiveness of the treatment variants on weed species, it can be seen that the products *Gardoprim Plus Gold 500 SC* and *Dual Gold 960 EC* applied alone pre-emergence better controlled the species *Digitaria sanguinalis* and less the species *Ambrosia artemisiifolia*, and the products applied post-emergence, respectively *Trek P334 SE*, *Buctril universal* and *Dicopur Top 464 SL* provided better control of *Ambrosia artemisiifolia* and poorer control of *Digitaria sanguinalis*.

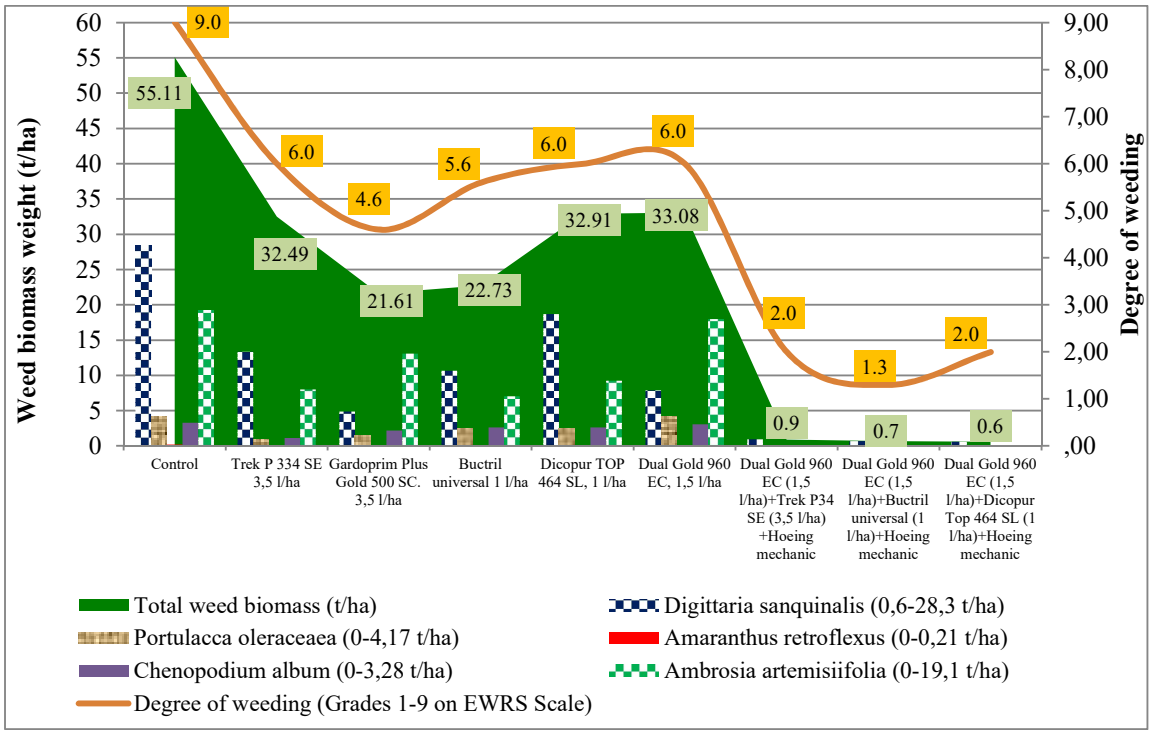


Figure 3. Degree of weediness and weed biomass in sorghum after 60 days from the application of postemergence herbicides / Gradul de îmburuienare și biomasa de buruieni la sorg după 60 zile de la aplicarea erbicidelor postemergente

The analysis of the morphological, physiological and productivity parameters recorded in grain sorghum revealed differentiated values according to the weed prevention and control option (Table 5). The application of measures to prevent and control the weeding of the sorghum crop produced positive effects compared to the non-herbicided and unworked control, where the lowest values were recorded regarding plant height, stem diameter, leaf surface index (LAI), weight of one thousand grains (WTG) and hectoliter weight (HW). Thus, the integrated options for preventing and combating weeds were clearly separated, by using out pre-emergent herbicide with *Dual Gold 960*, applied in a dose of 1.5 l/ha, and in vegetation carrying out post-emergent herbicide with one of the products *Trek P334 SE* (3.5 l/ha), *Buctril universal* (1 l/ha) or *Dicopur Top 464 SL* (1 l/ha), associated with the performance of two mechanical weeding works, which achieved, compared to the control, increases of 29.08-32.66% in the height of the plant, of 41.35-88.77% in the diameter of the stem, of 72.72-78.78% in the leaf surface index, of 67.56-70.81% at the weight of one thousand grains and 17.94-19.08% at the hectoliter weight.

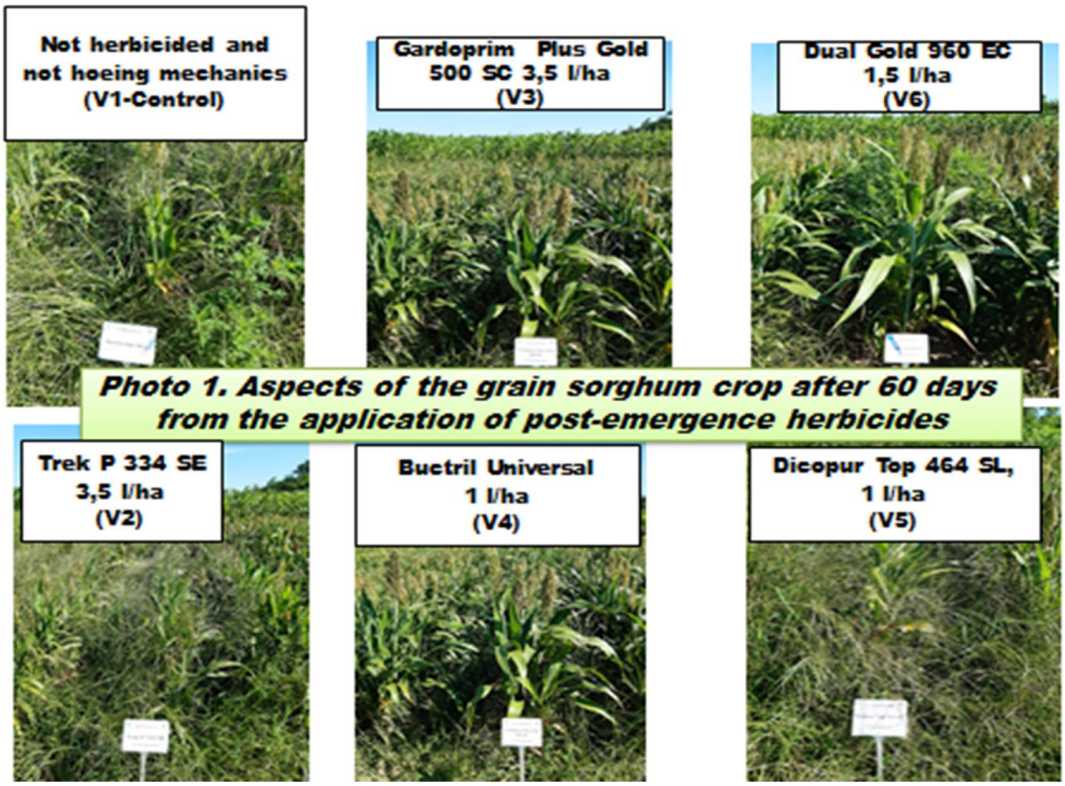


Photo 1. Aspects of the grain sorghum crop after 60 days from the application of post-emergence herbicides

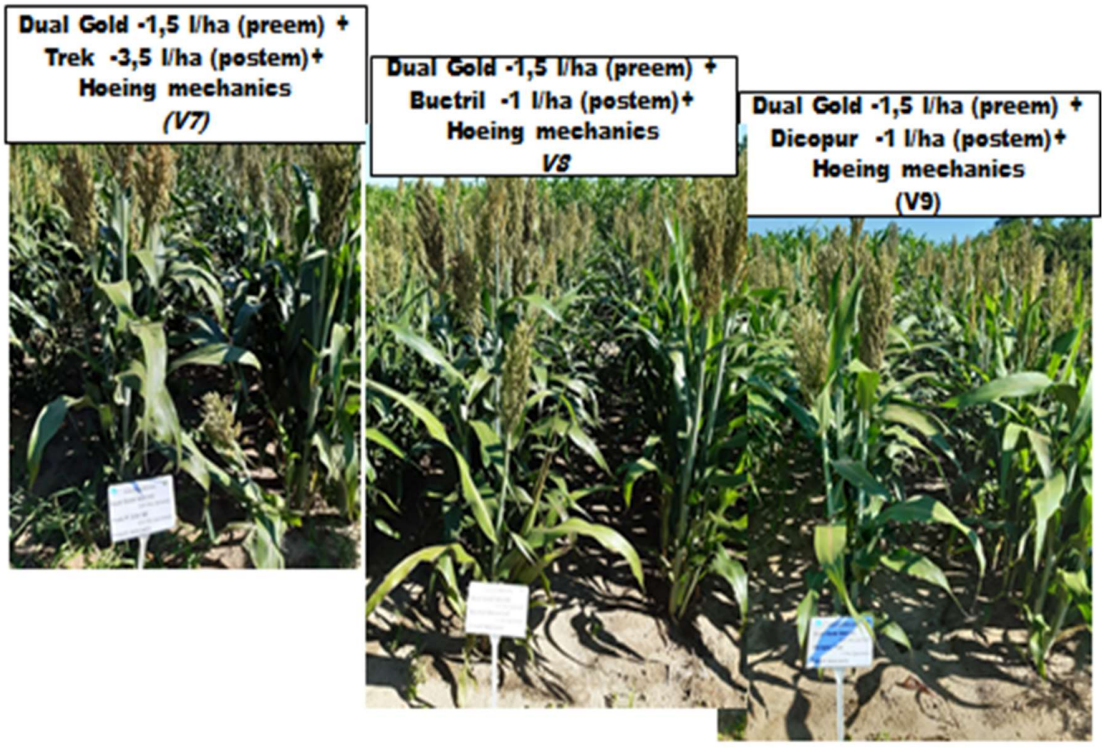


Photo 2. Aspects of the grain sorghum crop after 60 days from the application of post-emergence herbicides

Table 5. Variability of some morphological and productivity parameters in grain sorghum depending on the weed prevention and control option // Variabilitatea unor parametri de morfologie şi productivitate la sorgul pentru boabe în funcţie de varianta prevenire şi combatere a buruienilor

No. var.	The experimental variant	Dose (l/ha) l/ha	The plant height (cm)	The stem diameter (mm)	LAI	WTG (g)	WH (kg/hl)
V1	Control	-	89.4	8	3.3	18.5	61.3
V2	Trek P34 SE	3.5	96.2	8	4.5	29	71
V3	Gardoprim Plus Gold 500 SC	3.5	118.4	10.91	5.1	23	70.3
V4	Buctril universal	1	107.6	8.43	4.6	25	71.6
V5	Dicopur top 464 SL	1	113.8	8.99	4.4	23	70
V6	Dual Gold 960 EC	1.5	124.2	10.43	4.6	23	69.6
V7	Dual Gold 960 EC+ Trek P34 SE + Hoeing mechanic	1.5+ 3.5	115.4	11.31	5.9	31	73
V8	Dual Gold 960 EC + Buctril universal + Hoeing mechanic	1.5 + 1	118.6	11.5	5.9	31.6	73
V9	Dual Gold 960 EC + Dicopur top 464 SL + Hoeing mechanic	1.5 + 1	117.4	14.46	5.7	31	72.3
Difference from control (%) of combined treatments applied in V7, V8, V9			*29.08 - 32.66*	*41.35 - 80.77*	*72.72 - 78.78*	*67.56 - 70.81*	*17.9 - 19.08*

The literature mentions that a weed-free sorghum crop can develop normally, generating a leaf apparatus that has positive implications in the realization of production components (Pannacci et al., 2018, Johnson et al., 2023). From the graphic representation (Figure 4) of the interaction of the degree of weeding of the sorghum crop with the growth of the plant and the development of the leaf apparatus, presented with the help of mathematical functions, negative correlations are highlighted, which show a distinctly significant decrease in plant height and leaf surface with the increase in the degree of weeding (r=-0.823**; r=-0.980**).

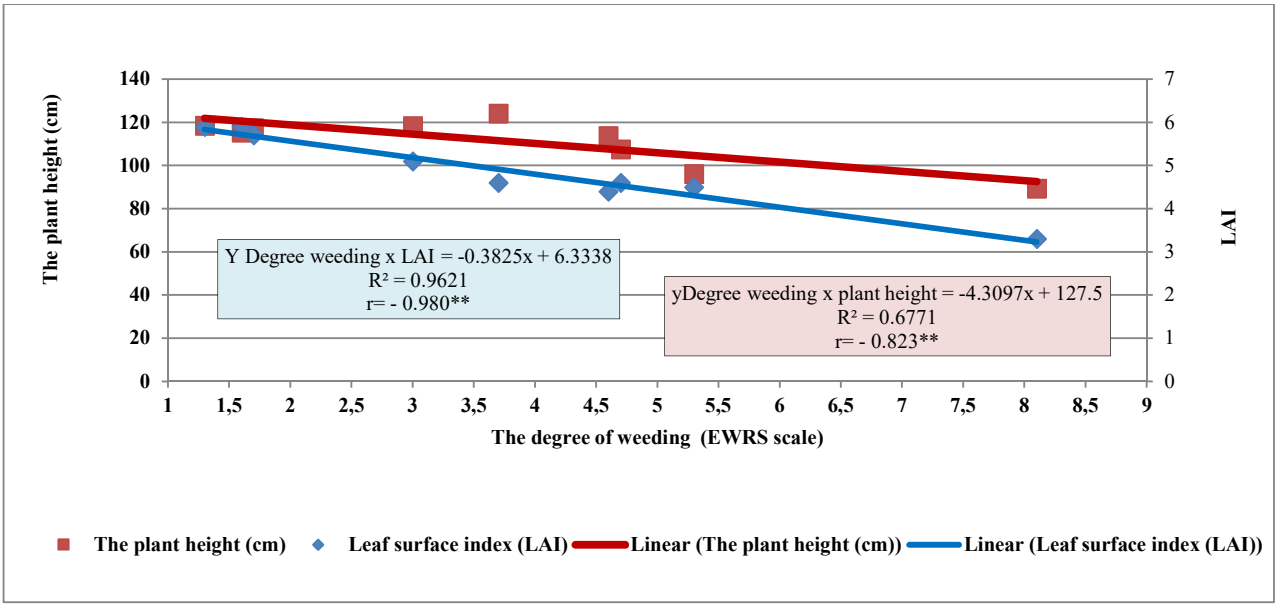


Figure 4. Correlations between the degree of weeding and the development of the sorghum plant // Corelaţii între gradul de îmburuienare şi dezvoltarea plantei de sorg

Having a good ability to efficiently exploit natural resources, sorghum can achieve high yields in ecological conditions less favorable to other cereals (Antohe, 2007, Drăghici, 1999). The production results obtained in grain sorghum, cultivated under sandy soil conditions, were between 2488.3-6678.3

kg/ha, being differentiated by the variant of prevention and control of weeds in the crop (Table 6). Compared to the control variant, which recorded a minimum production of 2488.3 kg/ha, all herbicides brought statistically guaranteed production increases.

Table 6. Influence of herbicide application on grain yield of grain sorghum grown under sandy soil conditions/ / Influența aplicării erbicidelor asupra producției de boabe obținută la sorgul pentru boabe cultivat în condițiile solurilor nisipoase

The experimental variant	*Dose(l/ha)*	*Application time*	Grain Yield		Difference from control	
			kg/ha	%	kg/ha	Significance
Control	-	-	2488.3	100	Control	Control
Trek P34 SE	3.5	Postemergence	4387.4	176.3	1899.1	**
Gardoprim Plus Gold 500 SC	3.5	Preemergence	4369.0	175.6	1880.7	**
Buctril universal	1	Postemergence	4150.3	166.8	1662.0	*
Dicopur top 464 SL	1	Postemergence	3893.1	156.5	1404.8	*
Dual Gold 960 EC	1.5	Preemergence	3854.4	154.9	1366.1	*
Dual Gold 960 EC+ Trek P34 SE+ Hoeing mechanic	1.5 3.5	Preemergence Postemergence	6238.4	250.7	3750.1	***
Dual Gold 960 EC+ Buctril universal+ Hoeing mechanic	1.5 1	Preemergence Postemergence	6678.3	268.4	4190.0	***
Dual Gold 960 EC+ Dicopur top 464 SL+ Hoeing mechanic	1.5 1	Preemergence Postemergence	6048.7	243.1	3560.4	***

LSD 5%= 1245.5; LSD 1% = 1812.7; LSD 0.1% =2717.4

The best results were obtained by controlling the weeding of the sorghum crop with chemical+mechanical methods. Thus, the herbicide treatement of the sorghum crop with the product *Dual Gold 960*, applied pre-emergent at a dose of 1.5 l/ha, in combination with a post-emergent herbicide with one of the *Buctril Universal* products, applied at a dose of 1 l/ha, *Trek P334 SE*, in a dose of 3.5 l/ha or *Dicopur Top 464 SL*, in a dose of 1 l/ha and with association of 2 mechanical harrowings in the vegetation, led to production increases of 143-168%, compared to the control, the differences being very significant, from a statistical point of view.

The statistical analysis of the influence of the degree of weeding during the sorghum vegetation period on grain production and on the physical quality of the grains (Figure 5), highlighted distinctly significant negative correlations between the degree of weeding and grain production (r= (-)0,913**) and weight hectoliter (r = (-)0,879**) and a significantly negative correlation with weight of one thousand grains (r = (-)0,766**). The obtained results demonstrated that technology elements significantly influence the production potential of grain sorghum (Matei, 2011, Narges et al., 2013). Research in India has shown that weeds are a major problem in increasing productivity in sorghum, with losses ranging from 15-97%, depending on the nature and density of weeds (Verma et al., 2019).

CONCLUSION

1. Pre-emergent application of Dual Gold 960, at a dose of 1.5 l/ha, in combination with a post-emergence herbicide with one of the products Buctril Universal, at a dose of 1 l/ha, Trek P334 SE, at a dose of 3, 5 l/ha or Dicopur Top 464 SL, in a dose of 1 l/ha and in association performance of 2 mechanical weedings during the vegetation of the plant led to production increases of 143-168%, compared to the non-treated control and mechanically unworked in vegetation.

ACKNOWLEDGEMENT

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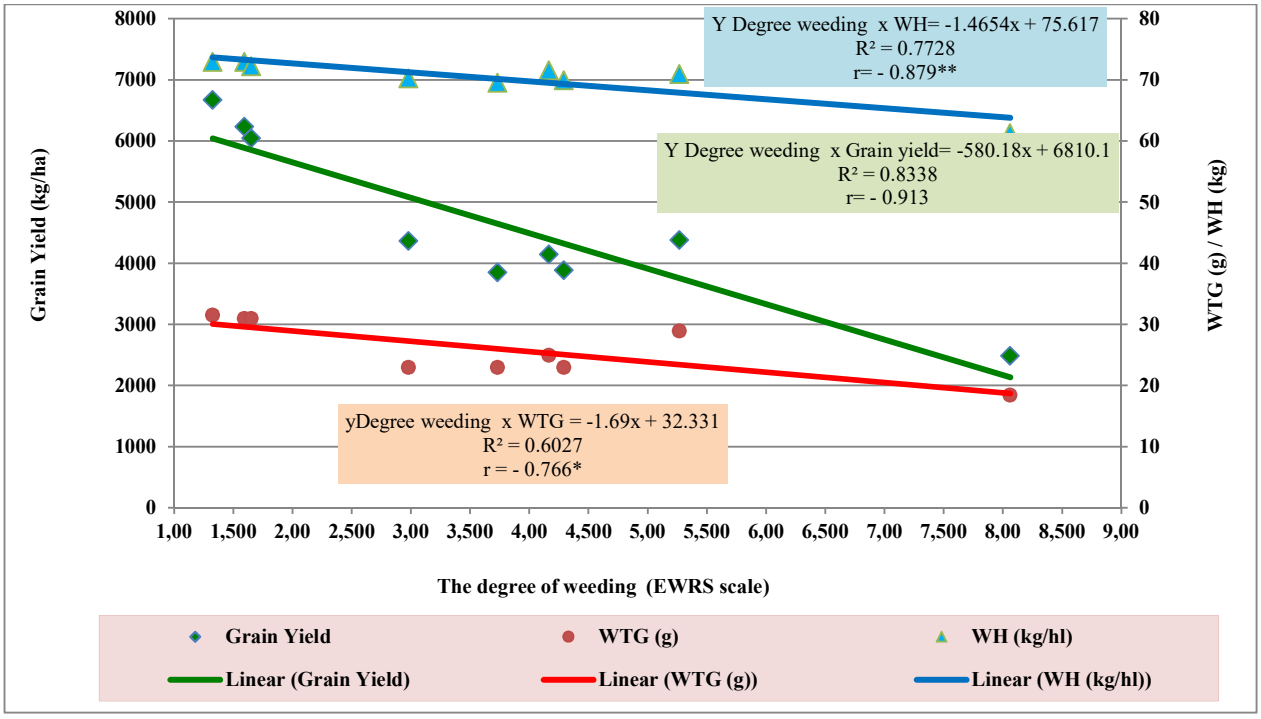


Figure 5. Correlations between the degree of weeding and the productivity of the sorghum plant //Corelații între gradul de îmburuienare și productivitatea plantei de sorg

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THE IMPACT OF XENOTOXIC SUBSTANCES ON SOME ECOPHYSIOLOGICAL INDICES DURING THE FLOWERING PHASE OF BROAD BEAN CROP (*Vicia faba L. var. major Harz*) IN THE ECOLOGICAL CONDITIONS OF THE NORTH OF SUCEAVA COUNTY

IMPACTUL SUBSTANŢELOR XENOTOXICE ASUPRA UNOR INDICI ECOFIZIOLOGICI ÎN FAZA DE ÎNFLORIRE LA CULTURA DE BOB (*Vicia faba L. var. major Harz*) ÎN CONDIȚIILE ECOLOGICE DIN NORDUL JUDEȚULUI SUCEAVA

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Abstract

The research was carried out at SCDA Suceava, in the pedoclimatic conditions of the Pojorâta Center during 2017-2018, on a lithic alluvial soil, located on the first terrace of the Moldova River, at an altitude of 700 m. One of the main factors in this experience, constituted the use of a xenobiotic product, called Tender 960 EC, herbicide from the IV toxicity group and its implications on the microbiological activity of the soil. The following research variants were pursued:V_1- untreated unsown; V_2 - manually sown; V_3- treated 1 l/ha before sowing; V_4- 1.5 l/ha before sowing; V_5- 1 l/ha before the plants sprouting; V_6- 1.5 l/ha before the plants sprouting; V_7- 1 l/ha after sprouting; V_8- 1.5 l/ha after sprouting, sown at 50 cm between rows; V_9- untreated unsown; V_{10}- manually sown; V_{11}- 1,5 l/ha before sprouting, sown at 30 cm between rows. This paper analyzes the action exerted by the herbicide mentioned above in the fight against weeds in broad beans culture on some ecophysiological indices (the content of dry matter, total water and carbohydrates) in the flowering phenophase of the plants. It was found that the accumulation of dry matter, in the flowering phase of the broad bean, in the pedoclimatic conditions of normal years, varies between 20.35 and 32.28%. The highest values of the dry matter content were recorded in the plants of the uncultivated variant not treated with herbicide (31.84-32.28%), and the lowest in the sowed but untreated variants (20.35-21.05%). The rest of the variants show an average accumulation in dry matter between 22.89 and 30.42%. The analysis of the broad bean carbohydrate content, by form and total, was done using the Bertrand-Iljin titrimetric micromethod. The directly reducing carbohydrates (glucose-fructose), the water-soluble carbohydrates (sucrose) and the water-insoluble polysaccharide (starch) were analyzed. Organ analysis of carbohydrates by form and total, indicates an increased content of carbohydrates in reproductive organs and leaves and a lower content in roots and stems. In the varieties treated with herbicide, the lowest content of soluble carbohydrates and the highest content of water-insoluble carbohydrates were achieved.

Keywords: Tender 960 EC, ecophysiological indices, Vicia faba, carbohydrates.

Rezumat

Cercetările au fost efectuate la SCDA Suceava, în condiţiile pedoclimatice de la Centrul Pojorâta în perioada 2017-2018, pe un sol aluvial litic, situat pe prima terasă a râului Moldova, la altitudinea de 700 m. Unul din principalii factori în această experienţă, l-a constituit utilizarea unui produs xenobiotic, numit Tender 960 EC, erbicid din grupa a IV de toxicitate şi implicaţiile lui asupra activităţii microbiologice ale solului. S-au urmărit următoarele variante de cercetare: V_1 - netratat neprăşit; V_2- prăşit manual; V_3- tratat 1 l/ha înainte de semănat; V_4- 1,5 l/ha înainte de semănat; V_5- 1 lg/ha înainte de răsărire; V_6- 1,5 l/ha înainte de răsărire; V_7- 1 l/ha după răsărire; V_8- 1,5 l/ha după răsărire, semănate la 50 cm între rânduri şi V_9- netratat neprăşit; V_{10}- prăşit manual; V_{11}- 1,5 l/ha înainte de răsărire, semănate la 30 cm între rânduri.

În prezenta lucrare se analizează acţiunea exercitată de erbicidul amintit mai sus în combaterea buruienilor la cultura bobului asupra unor indici ecofiziologici (conţinutului în substanţă uscată, apă totală şi a glucidelor) în fenofaza de înflorire a plantelor. S-a constatat că acumularea de substanţă uscată, în faza de înflorire la bob, în condiţiile pedoclimatice ale anilor normali, variază între 20,35 şi 32,28 %. Cele mai mari valori ale conţinutului de substanţă uscată s-au înregistrat

la plantele din varianta nelucrată netratată cu erbicid (31,84-32,28 %), iar cele mai mici la variantele prăşite dar netratate (20,35-21,05 %). Restul variantelor prezintă o acumulare medie în substanţă uscată cuprinsă între 22,89 şi 30,42 %. Analiza conţinutului de glucide la bob, pe forme şi totale, s-a făcut folosind micrometoda titrimetrică Bertrand-Iljin. S-au analizat glucidele direct reducătoare (glucoză- fructoză), glucidele solubile în apă (zaharoză) şi poliglucidul insolubil în apă (amidonul).Analiza pe organe a glucidelor pe forme şi totale, indică un conţinut sporit de glucide în organele de reproducere şi în frunze şi un conţinut mai mic în rădăcini şi tulpini. În variantele tratate cu erbicid, s-a realizat cel mai scăzut conţinut de glucide solubile şi cel mai ridicat conţinut de glucide insolubile în apă.
Cuvinte cheie : Tender 960 EC, indici ecofiziologici, Vicia faba, glucide.

INTRODUCTION

The growth of cultivated plants, respectively of broad bean, is characterized by the summation of favorable climatic factors as well as agrotechnical ones. During growth, the largest amount of organic matter is accumulate due to the photosynthesis process (Sălăgeanu N., Atanasiu L., 1981). This process is well highlighted, practically, by the accumulation of dry matter and total carbohydrates.

The thermal regime greatly influences the accumulation of dry matter, or what is called net photosynthesis. The bean is one of the species that can withstand lower temperatures, which is why it is recommended to be cultivated in the colder and wetter areas of the country.

Carbohydrates have an essential energy role, constituting the source of energy used in the process of breathing and therefore life maintenance. They also represent the main form of organic substances under which reserve substances are deposited in most cultivated and spontaneous plants. If we also take into account the fact that both soluble and insoluble forms of carbohydrates are preferred nutrients in food, we understand why the content in carbohydrates is an index of the quality of cultivated plants.

MATERIAL AND METHOD

The research was carried out in the pedoclimatic conditions of Pojorâta, during 2017-2018, on a lithic alluvial soil, located on the first terrace of the Moldova river, at an altitude of 700 m. It focused on the influence of weed control methods at bean (manual work and herbicide use) on the content of dry matter, total water and carbohydrates in the flowering phenophase of the plants.

Eleven research variants are studied: uncultivated - untreated, manually worked, variants sown at two distances between rows (30 cm and 50 cm) and 7 variants treated with herbicide. Two herbicide doses (1 and 1.5 l/ha) were studied, applied in three periods (before sowing, before sprouting and after the sprouting of beans plants), in variants sown at a distance of 50 cm between rows and 10 cm per row, ensuring 200,000 germinable grains per ha and a variant sown 30 cm between rows and 10 cm per row applied before sunrise, ensuring 300,000 germinable grains per ha.

The analysis of the broad bean carbohydrate content, by form and total, was done using the Bertrand-Iljin titrimetric micromethod. The directly reducing carbohydrates (glucose-fructose), the water-soluble carbohydrates (sucrose) and the water-insoluble polysaccharide (starch) were analyzed.

From a meteorological point of view, the multiannual average temperature in this area was 6.4°C for the entire year and 12.7°C during the vegetation period, and the multiannual average precipitation recorded values of 726.2 for the entire year and 531.0 for vegetation period (tab.1). Temperatures recorded values equal to normal throughout the year and exceeded normal during the vegetation period by 0.8°C. The year 2018 was normal in terms of precipitation both throughout the year and during the vegetation period , but temperatures were significantly above normal, by 2.5°C for the whole year and by 3.4°C during the growing season.

Table 1. Climatic conditions during the experiment period.// Condiţiile climatice din perioada de experimentare.

Specification	Rainfall (mm)		Temperature (° C)	
	annual	IV- IX	annual	IV – IX
2017	699,5	637,9	6,4	13,5
2018	757,7	520,8	8,9	16,1
Multiannual average	726,2	531,0	6,4	12,7

RESULTS AND DISCUSSION

a. Dry matter

From table 2, it can be seen that the accumulation of dry matter, in the flowering phase of the broad bean, in the pedoclimatic conditions of normal years, varies between 20.35 and 32.28%. The highest values of the dry matter content were recorded in the plants of the uncultivated variant not treated with herbicide (31.84-32.28%), and the lowest values in the sown but untreated variants (20.35-21.05%). The rest of the variants show an average accumulation in dry matter between 22.89 and 30.42%.

The cause of this behavior can be attributed to the fact that, in their struggle for survival, the plants from the untreated version have a greater osmotic force to extract water and mineral salts from the soil, and their tissues are more strongly sclerified and lignified, precisely to reduce water loss from plants. Under these conditions, the percentage of dry matter is slightly higher compared to the variants where a different agricultural technique was applied.

The plants from the variants to which weeding works and herbicide were applied due to the aeration of the soil, the water retention power in the loosened soil was higher, and the plants had a more optimal water regime, with a higher percentage of water (67.18 - 79.65 %) compared to the uncultivated version, and less dry matter.

b. Carbohydrates

In the analyzed organs of the bean, carbohydrates have a share of over 40-50% of the total dry substance accumulated, thus occupying an important place from a quantitative and qualitative point of view.

Carbohydrates have an essential energy role, constituting the source of energy used in the process of breathing and therefore of life maintenance. They also represent the main form of organic substances under which reserve substances are deposited in most cultivated and spontaneous plants.

If we also take into account the fact that both soluble and insoluble forms of carbohydrates are preferred nutrients in food, we understand why the content in carbohydrates is an index of the quality of cultivated plants.

The variant analysis of the homogenized aerial organs reveals the fact that large amounts of soluble carbohydrates are present in the grain (tab. 3). These have a value preponderance over the forms of insoluble carbohydrates in the unprocessed and processed variants. In the variants in which the herbicide was given in higher doses and administered especially after the emergence of the plants, the value of soluble carbohydrates obviously decreases in the plants, a fact also illustrated by the ratio between the soluble forms and the insoluble forms which in these variants becomes subunit (0.44 - 0.58). Even so the value of total carbohydrates is the highest (50.31-57.03 %).

Comparing various forms of analyzed carbohydrates, we find that the highest values are generally recorded for insoluble forms (starch), followed by directly reducing sugars (glucose, fructose, sorbose) and then sucrose (diglucose soluble in water). The ratio of soluble / insoluble carbohydrates is over unity in the uncultivated and sown variants and below unity in all variants treated with herbicide. This proves that the xenobiotical product used induces to an unforeseen extent both the metabolism of sugary substances in plants, increasing it slightly also through the accumulation of dry matter.

For the qualitative analysis of the broad beans from all the studied variants, a homogenate of the aerial (stem, leaves, inflorescences) and underground (roots) parts of the plant was used, brought to the flour stage by raising.

Table 2. The influence of the method of weed control on the dry matter content of broad beans plants. // Influența modului de combatere a buruienilor asupra conținutului în substanță uscată a plantelor de bob.

Variant	Distance beetwen rows (cm)	Dry matter (%)	Total water (%)
no hoeing - unteatred	50	31,84	68,16
manual hoeing	50	20,35	79,65
1 l/ha before soweing	50	29,33	70,67
1.5 l/ha before soweing	50	22,89	77,11
1 l/ha before sprouting	50	25,11	74,89
1.5 l/ha before sprouting	50	24,48	75,52
1 l/ha after sprouting	50	30,42	68,58
1.5 l/ha after sprouting	50	28,30	71,70
no hoeing - unteatred	30	32,28	67,72
manual hoeing	30	21,05	78,95
1.5 l/ha before sprouting	30	23,82	67,18

Table 3.The influence of the method of weed control on the carbohydrate content of broad bean // Influența modului de combatere a buruienilor asupra conținutului în glucide a plantelor de bob

Variant	**Distance beetwen rows (cm)**	**Forms of carbohydrates g / 100 g s.u.**				**Total carbohydrates sol. + insol.**	**Total report sol/insol.**
		directly reducing	**soluble in water**	**totally soluble**	**insoluble in water**		
no hoeing - unteatred	50	20,05	4,08	24,13	21,06	45,19	1,14
manual hoeing	50	19,49	4,59	24,08	18,87	42,95	1,28
1 l/ha before soweing	50	18,05	5,49	23,54	24,05	47,59	0,98
1.5 l/ha before soweing	50	12,52	7,56	20,08	21,96	42,04	0,91
1 l/ha before sprouting	50	13,84	5,73	19,57	23,87	43,44	0,82
1.5 l/ha before sprouting	50	11,84	5,95	17,79	28,31	46,10	0,63
1 l/ha after sprouting	50	12,85	4,81	17,66	39,37	57,03	0,44
1.5 l/ha after sprouting	50	14,09	4,47	18,56	31,75	50,31	0,58
no hoeing - unteatred	30	16,43	7,02	23,45	21,06	44,51	1,11
manual hoeing	30	14,28	5,73	20,01	19,45	39,46	1,03
1.5 l/ha before sprouting	30	14,08	5,23	19,31	20,93	40,24	0,92

Analyzing various forms of carbohydrates and their total (table 4, figure1), we find that the smallest amounts are present in the roots, and in the inflorescences and reproductive organs, in formation, the maximum amounts are present (105 mg/1 g. dry substance). In the form of carbohydrates, directly reducing sugars are in the highest amount in inflorescences and forming berries (25 mg), while the roots and stems have the same concentration (20 mg glucose). Water-soluble sugars, namely sucrose, have the highest value in leaves (50 mg), followed by flowers (45 mg), stems (40 mg) and roots (35 mg / 1 g s.u.).

Table 4. The content of carbohydrate forms in the organs of broad beans plants// Conținutul formelor de glucide din organele plantelor de bob

Specification	Roots	Stems	Leaves	Inflorescence
directly reducing carbohydrates	20	30	15	25
soluble carbohydrates	35	40	50	45
insoluble carbohydrates	30	30	30	35
total	85	90	95	105

directly reducing carbohydrates: glucose, fructose, sorbose; soluble carbohydrates: sucrose; insoluble carbohydrates: amide.

CONCLUSION

1. The highest dry matter content was recorded in the unworked - untreated variants and the lowest in the sown or treated variants. The total water content is inversely related to the dry matter content;
2. The highest content of total carbohydrates was found in the plants from the herbicide-treated variants and the lowest in the untreated variants;

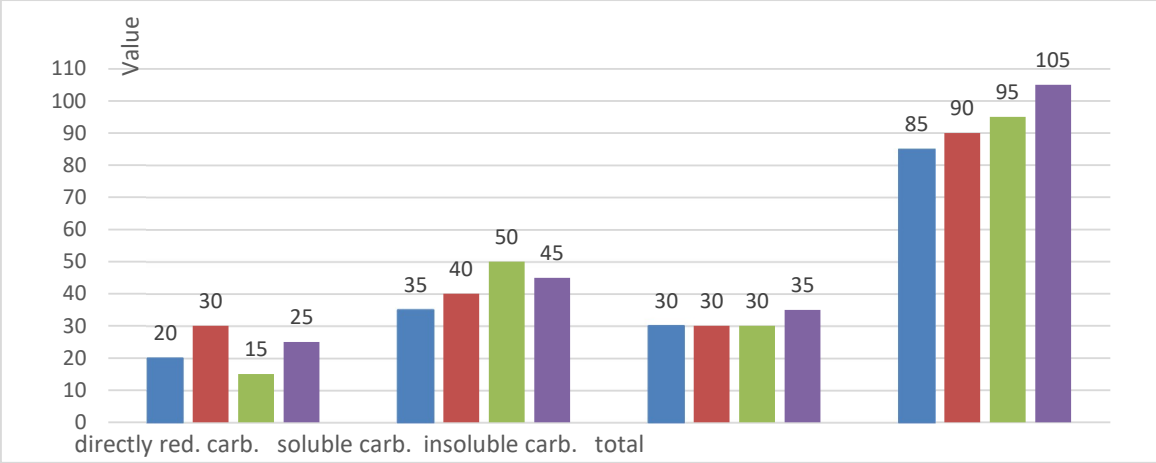


Figure 1. The content of carbohydrate forms in the organs of broad beans plants (mg/1 g dry substance) // Conținutul formelor de glucide din organele plantelor de bob (mg/1 g substanță uscată)

3. All vital processes happen normally if there is a percentage of water of 70-80% in the cells, our analyzes highlighted an optimal percentage of total water that favored metabolic processes of synthesis and polymerization of organic substances and especially of carbohydrates;
4. The organ analysis of carbohydrates by form and total, indicates an increased content of carbohydrates in reproductive organs and leaves and a lower content in roots and stems;
5. In the variants treated with herbicide, the lowest content of soluble carbohydrates and the highest content of water-insoluble carbohydrates were achieved;
6. The highest content of total carbohydrates was achieved in the plants from the varieties treated with herbicide;
7. Carbohydrate content as a qualitative indicator of broad bean plants can be used to detect the best agro technologies measures that can be applied in intensive broad bean culture, as well as to detect the variety with the most intensive growth, but also with the highest concentration of carbohydrates, both in the vegetative apparatus of the plant and in the grains.

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RESEARCH ON AGRICULTURAL LANDUSE UNDER THE CONDITIONS OF DEGRADATION THROUGH GULLIES AND LANDSLIDES AND SOLUTIONS FOR ANTI-EROSION ORGANIZATION AND SUSTAINABLE AGRICULTURAL EXPLOITATION

CERCETĂRI PRIVIND UTILIZAREA TERENURILOR AGRICOLE ÎN CONDIȚIILE DEGRADĂRII PRIN RÂNGĂȘI ȘI ALUNĂRI DE TEREN ȘI SOLUȚII DE ORGANIZARE ANTIEROZIONARE ȘI EXPLOATARE AGRICOLE DURABILĂ

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Abstract:
Starting from 2006, at SCDCES "MM" Perieni, research was carried out on the way agricultural land is used in the current ownership conditions and degradation through gullies and landslides in watersheds, or perimeters, of different sizes, and solutions for anti-erosion organization and sustainable agricultural exploitation were proposed to local authorities and farmers. The categories of anti-erosion works proposed belong to two groups: a) works of a larger scale, which require the intervention of the State, such as land reclamation works, modeling works, landscaping of slopes with landslides, torrent or gully stabilization works, etc., afforestation and planting of protection forest belts; b) works of a smaller scale, which are within the reach of farmers or administrators, such as: drawing grassy strips between stripcrops, establishing grassed waterways on the bottom of valleys or ephemeral gullies, correcting the routes of agricultural exploitation roads, etc. In the period 2021-2024, research is carried out in the framework of the project entitled "Solutions for anti-erosion organization of the territory and sustainable agricultural exploitation based on the inventory of landslides and gullies" in four hydrographic micro-watersheds. This article presents the results of the research carried out in the first two stages of the project, in the Tomești and Trestiana-Conizoaia hydrographic micro-watersheds, within the Bârlad Tableland.
Keywords: ***Geographical Information Systems, GPS measurements, gullies, landslides, landuse, land reclamation, sustainable agriculture.***

Rezumat:
Începând cu anul 2006, la SCDCES „MM" Perieni s-au efectuat cercetări privind modul de utilizare a terenurilor agricole în condițiile actuale de proprietate și degradare prin rigole și alunecări de teren în bazine hidrografice, sau perimetre, de diferite dimensiuni și soluții de organizare antierozională și exploatarea agricolă durabilă au fost propuse autorităților locale și fermierilor. Categoriile de lucrări antieroziune propuse aparțin a două grupe: a) lucrări de anvergură mai mare, care necesită intervenția statului, precum lucrări de reabilitare a terenurilor, lucrări de modelare, amenajarea versanților cu alunecări de teren, lucrări de stabilizare a torenților sau a rigolei, etc., împădurirea și plantarea de centuri forestiere de protecție; b) lucrări de anvergură mai mică, care sunt la îndemâna fermierilor sau administratorilor, precum: trasarea fâșiilor înierbate între culturi, înființarea căilor navigabile înierbate pe fundul văilor sau rigole efemere, corectarea traseelor drumurilor de exploatare agricolă etc. în perioada 2021-2024, cercetarea se desfășoară în cadrul proiectului intitulat „Soluții pentru organizarea antieroziune a teritoriului și exploatarea agricolă durabilă pe baza inventarierii alunecărilor de teren și rigole" în patru microbazine hidrografice. Acest articol prezintă rezultatele cercetărilor efectuate în primele două etape ale proiectului, în microbazinele hidrografice Tomești și Trestiana-Conizoaia, din cadrul Podișului Bârladului.
Cuvinte cheie: Sisteme de informații geografice, măsurători GPS, rigole, alunecări de teren, utilizare a terenurilor, recuperare a terenurilor, agricultură durabilă

INTRODUCTION

The relief conditions, the geological structure and the climatic conditions of the Bârlad Tableland lead to the idea that more than 70% of the territory's surface is theoretically prone to degradation through water erosion, gullies and/or landslides. According to the total erosion zoning map drawn up by Moțoc M. (1975), in Vaslui County, an area that mostly overlaps the Bârlad Tableland, the total erosion is 20-30 t/ha/year (Figure 1) .

According to Moţoc M. (1984) sheet erosion participates with 36%, deep erosion with 31%, and the remaining 33% is due to the combination of landslides – deep erosion and landslides in the forest fund – bank and thalweg erosion.

Maria Rădoane et al. in 1992 and 1995 inventoried a number of over 9,000 gullies in the Moldavian Plateau and made a map of the gully density. In that map, the authors singled out two distinct areas:

- one located in the middle basin of the Jijia and in the upper part of the Bahluieţ watershed (predominantly clayey domain);
- the other one, with the greatest susceptibility to gullying, located roughly in the Bârlad Tableland (predominantly sandy area) includes the Tutova Rolling Hills, the Fălciu Hills and the Covurlui Hills.

The application of Law 18/1991 on the reallotment of agricultural land to the previous landowners had several negative consequences, among which we mention:

- A huge fragmentation of agricultural land, through the appearance of 48 million individual plots on a surface of 9 million hectares of arable land, in 4.2 million agricultural holdings with an average surface of 2.2 ha, and consisting of 5-16 small plots, sometimes sparsely distributed, isolated from each other (Gavrilescu and Giurcă, 2000), made the new small owners economically unviable.

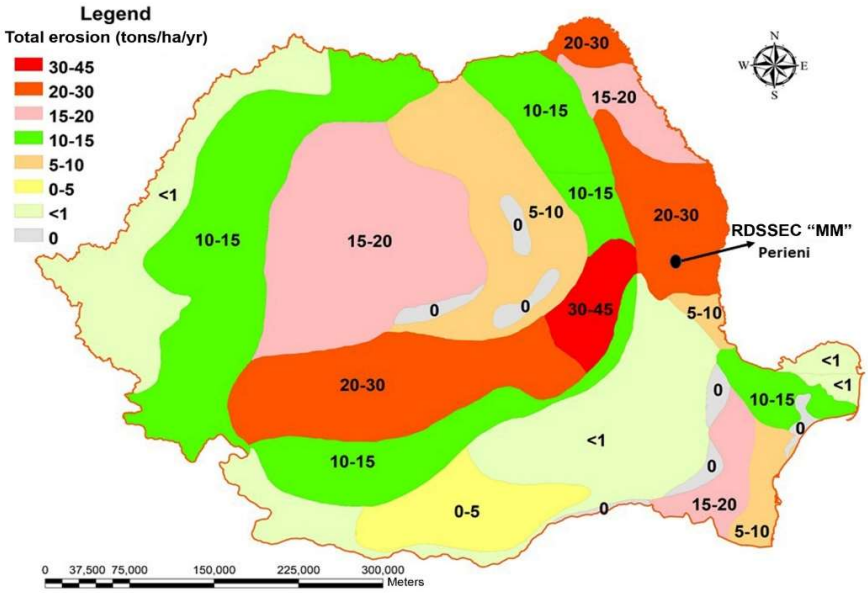


Figure 1. Zoning of total erosion (tons/ha/year) in Romania (Moţoc M., 1984)// Zonarea eroziunii totale (tone/ha/an) în România (Moţoc M., 1984)

- The effective realottment on previous sites, which in Romania, according to tradition, most often meant the location of small, narrow and long plots in the up-and-down-hill direction, made it almost impossible for small farmers to exploit the agricultural landowners in an anti-erosion system. The fact led to the destruction of the terraces and other pre-existing landscaping works. Generally speaking, in our country, the anti-erosion exploitation systems include as main measures: the execution of agricultural works in the direction of contours (level curves), stripcrops with the alternation of some good protective plants with some less good protective ones and terraces of different types, which, even in rich countries are considered too expensive. In the case of gullies or landslides, if the chosen solution is not afforestation, the work required for stabilization can be much more expensive.

In the period 2021-2024, the research is carried out in the framework of the project entitled "*Solutions for anti-erosion organization of the territory and sustainable agricultural exploitation based on the inventory of landslides and gullies*" in four micro-watersheds, namely: in 2021 - Tomeşti (2,127ha), in 2022 - Trestiana-Conizoaia (5,096ha), in 2023 Cârjăoani (1,847ha) and in 2024 - Valea Seacă. This

article presents the results of the research carried out in the first two stages of the project, in the Tomești and Trestiana-Conizoaia hydrographic micro-watersheds, in the Bârlad Tableland.

From an administrative-territorial point of view, the Tomești hydrographic watershed is located, in different proportions, on the territory of two communes: Pogana and Iana, in the southern half of Vaslui county. In total, the perimeter of Tomești includes a territory with an area of 2,126.7 hectares. The Trestiana hydrographic watershed is located, in different proportions, on the territory of four communes: Fruntișeni (3,279.4 ha), Grivița (1,112.4 ha), Vinderei (508.4 ha) and Zorleni (195.4 ha), in the southern half of Vaslui County. In total, the Trestiana perimeter includes a territory with an area of 5,095.7 hectares.

MATERIAL AND METHOD

A first objective of the research was the creation of a geographic information system with data from the selected basins/perimeters regarding the relief (physical-geographical conditions), the hydrographic network and vegetation, the categories of land use and the current mode of agricultural land exploitation, based on which to propose appropriate solutions for anti-erosion organization and sustainable agricultural exploitation.

Database. The following cartographic materials and documentary information were used to carry out the activities provided for in these projects:

- topographic maps in scale 1:5,000, purchased from O.C.P.I. Vaslui;
- topographic maps on a scale of 1:25,000 drawn up by the Military Topographic Directorate, existing in the archive of the research station, edition 1982-1984 (with historical value);
- geological maps prepared by I. Atanasiu, N. Macarovici, P. Jeanrenaud, Bica Ionesi and C. Ghenea (1961, 1965, 1971);
- geological map of Romania, Geological Institute of Romania (1968), scale 1:200,000, Bârlad and Iași sheets, with related explanatory notes;
- geomorphological map of Romania, Posea Gr., Badea I., Scale 1:400,000, 1980, Didactic and Pedagogical Ed., Bucharest;
- climatic data from Bârlad and Perieni weather stations (ADCON-BEIA automatic weather station from RDSSEC "MM" Perieni);
- the cadastral maps related to the communes in the perimeter, on a scale of 1:10,000, drawn up by specialists from OCPI Vaslui (1982-1983);
- soil erosion data obtained from the runoff control plots from RDSSEC "MM" Perieni;
- Google Earth Pro images with a resolution of 0.5 m georeferenced in Dealul Piscului Stereo 1970 system .

The Method used was that of geographic information systems, of three-dimensional databases in which each pixel of each digital map - layer - has behind it (metadata) all the necessary information (relief, soil, climate, etc.). The physical interface was the GIS program ArcGIS 9.3.1, ESRI (Environmental Systems Research Institute), ArcEditor license with Spatial Analyst, 3D Analyst, Geostatistical Analyst extensions. Practically, all available maps and plans were scanned, georeferenced, initially in the system used in Romania, Stereo '70 Dealul Piscului. All this has been updated, where appropriate, by high-precision and high-productivity measurements carried out with the Thales Z-Max.Net professional GPS equipment, and then added to the information obtained in the many field trips to the town halls of the communes in perimeter, but especially from farmers in the area.

A very important stage in the spatial modeling of land degradation processes, within the geographic information system, consisted in creating the digital elevation model of the terrain (figure 2, a and b), obtained by scanning, importing, georeferencing and digitizing the level curves (contours) from ANCPI topographical maps at a scale of 1:5,000.

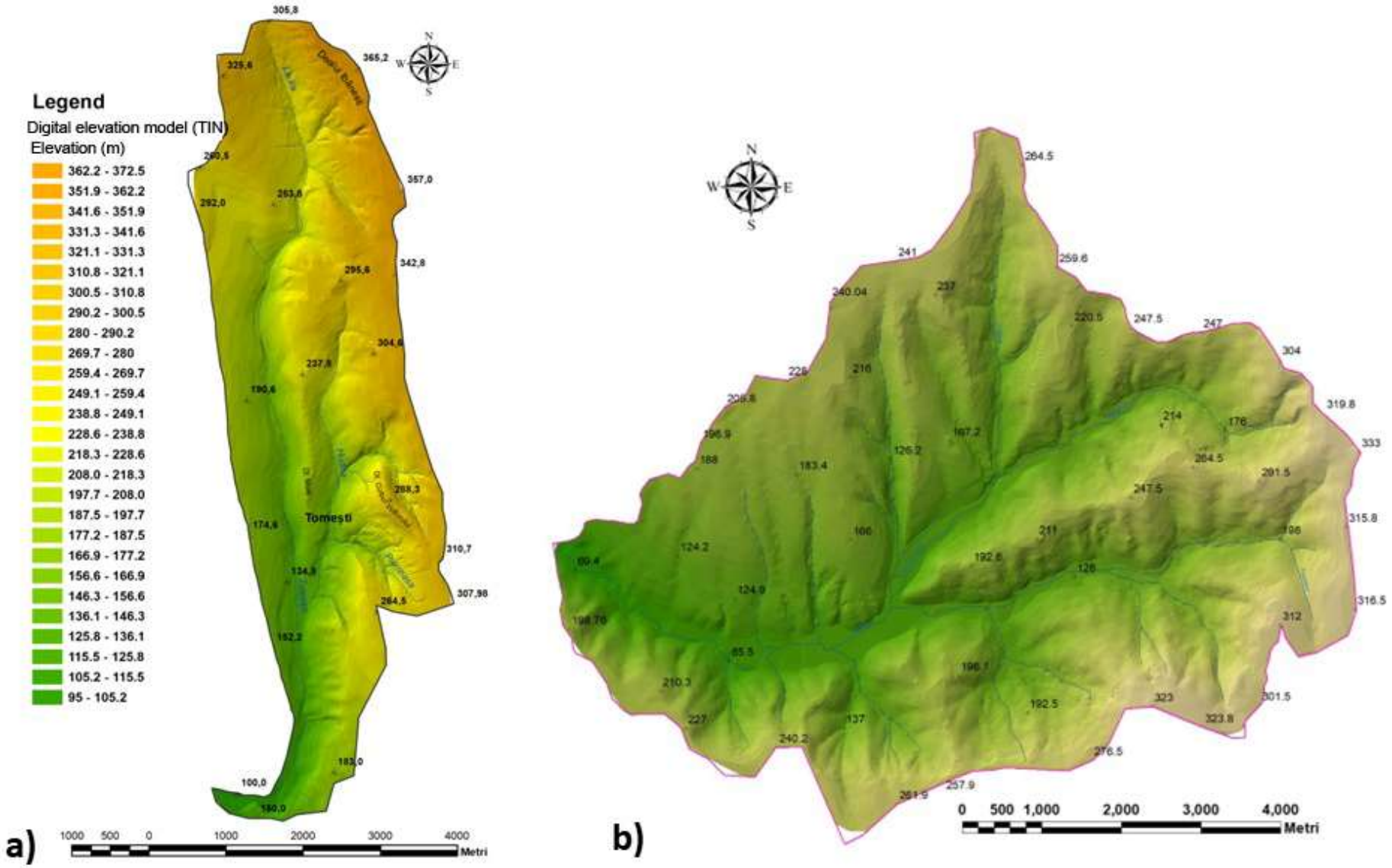


Figure 2. Digital elevation model TIN (Triangulated Irregular Network) of Tomești watershed and Trestiana-Conizoaia watershed
(Barlad Tableland, Vaslui County)// Model digital de elevație TIN (Triangulated Iregular Network) al bazinului hidrografic Tomești și al bazinului hidrografic Trestiana-Conizoaia (Podis Barlad, județul Vaslui)

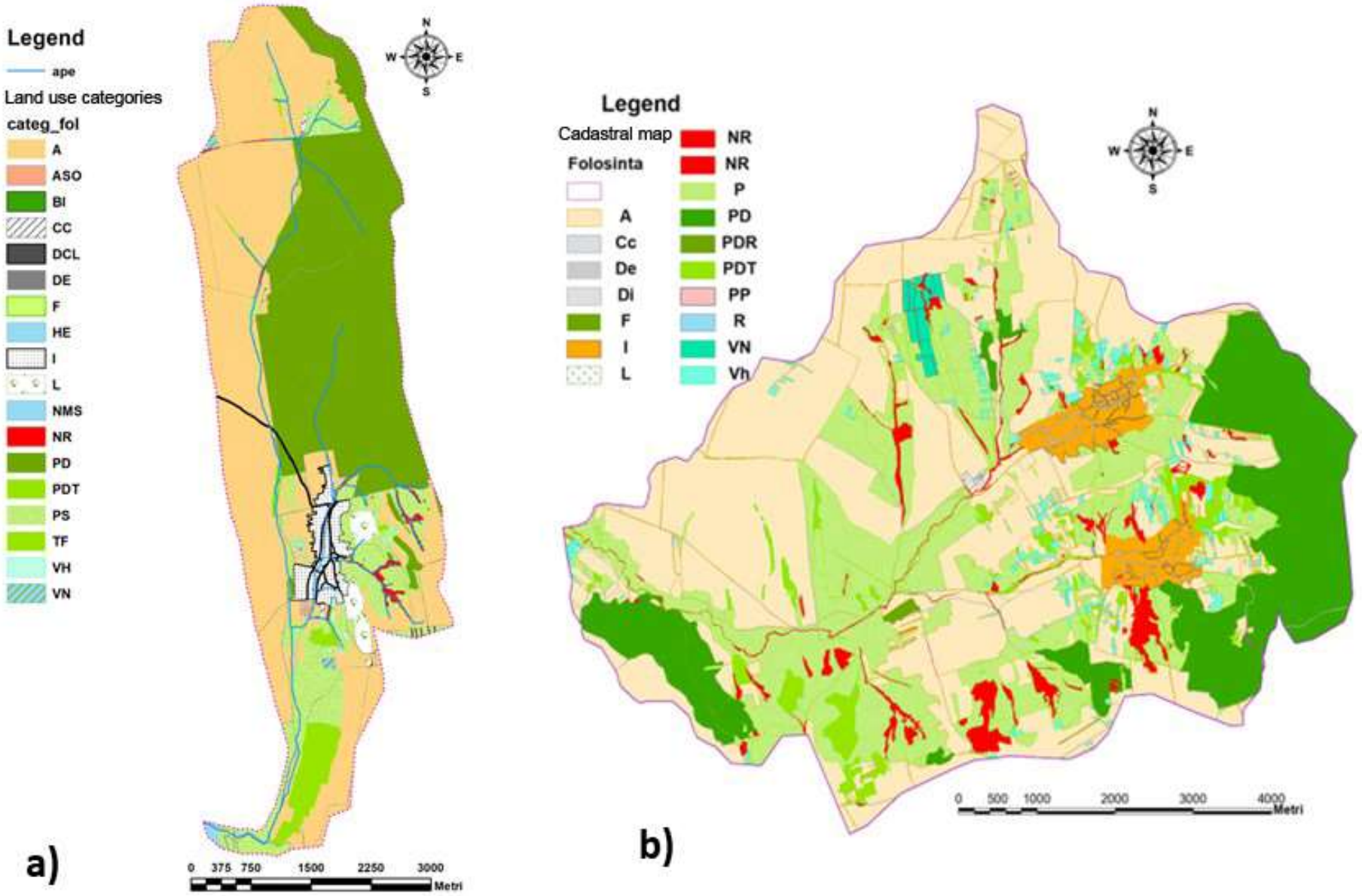


Figure 3. Cadastral maps of landuse categories in Tomeşti watershed and Trestiana-Conizoaia watershed (Bârlad Tableland, Vaslui County)// Hărți cadastrale ale categoriilor de folosință în bazinul hidrografic Tomești și Trestiana-Conizoaia

The digitization was done manually with the ArcGIS Editor program. During this operation, each level curve was assigned an altitudinal value, resulting in a vector layer, used in the actual realization of the numerical terrain model through the TIN (Triangulated Irregular Network) interpolation operation, which we believe reproduces the way more faithful to the relief forms, because it uses only the digitized elevation points, without rounding or embellishing the entered data in any way.

From figure 2, even at a simple examination it can be seen that the numerical model of the land, with this rather high resolution, obtained by interpolation using the TIN method, very faithfully recreates/reflects the relief of the land stripped of vegetation and any works human activities, allowing very easy and clear delimitation of forms of land degradation (erosion in depth and landslides).

Another particularly important stage in the creation of the Geographical Information System was the preparation of the cadastral map in digital format (figure 3 a and b and tables 1 and 2), which illustrates the categories of land use in the studied perimeter.

For this purpose, the most recent (1982-1983) cadastral maps, on a scale of 1:10,000, related to the areas of the five communes, were purchased from the Real Estate Registry and Publicity Office (OCPI) in Vaslui. Although at first sight the respective maps appear to be too old, they have proven to be particularly interesting and important for achieving the objectives of the project by reflecting the real situation before 1989, i.e. from the period that can be considered to be reference, because the maximum level of anti-erosion management of agricultural land had been reached, at a figure of almost two million hectares designed for anti-erosion. In addition to the grassy strips, from the map in figure 6, other elements of comparison can be extracted for which the cadastral maps used as a basis can be considered as a reference, such as:

- the situation of the vineyards and orchards of that period;
- the situation of forests and pastures;
- the situation of lands degraded by deep erosion (gullies) and/or landslides, which appear on cadastral maps as non-productive lands. In this sense, we can mention that these maps were not necessarily intended to reflect these degradation processes, but they can be considered to be extremely rigorous from this point of view, since the specialists who drew them up had to be very careful, instead, to the situation of the adjacent agricultural lands.

Inventory of gullies and landslides in the Tomești and Trestiana watersheds.

As part of this activity, we proposed to, first of all, carry out an inventory of the gullies and landslides in the two micro-watersheds. We must mention from the beginning that the problem of inventorying gullies and landslides is not as easy as it might seem, for several reasons:

- as Rădoane Maria, Ioniţă I., Rădoane N. have observed in several published works, regarding gullies there is a very large number (of the order of dozens) of definitions of these forms of deep erosion;
- both regarding the identification of ravines and landslides, there is a lot of subjectivity that results from the specifics of the activity or training of the specialists who do the identification (so, for example, the geomorphologist who knows the paleogeography or the geology of the area will be tempted to exaggerate the extent of the landslide phenomena and revenation, the military topographer is influenced by the imperatives of knowing the particularities of the land for military activities, and the cadastral surveyor is influenced by the constraints of the property regime or the categories of land use, etc.). For this reason, the results of the inventories differ, sometimes very much from one specialist to another;
- the degree of vegetation coverage, but especially the nature of the vegetation carpet, can sometimes be an indication of the geomorphology of the area (for example in the case of sliding crown/scarps, landslides or wooded gullies), but most of the time it is an obstacle to identification of gullies or landslides;

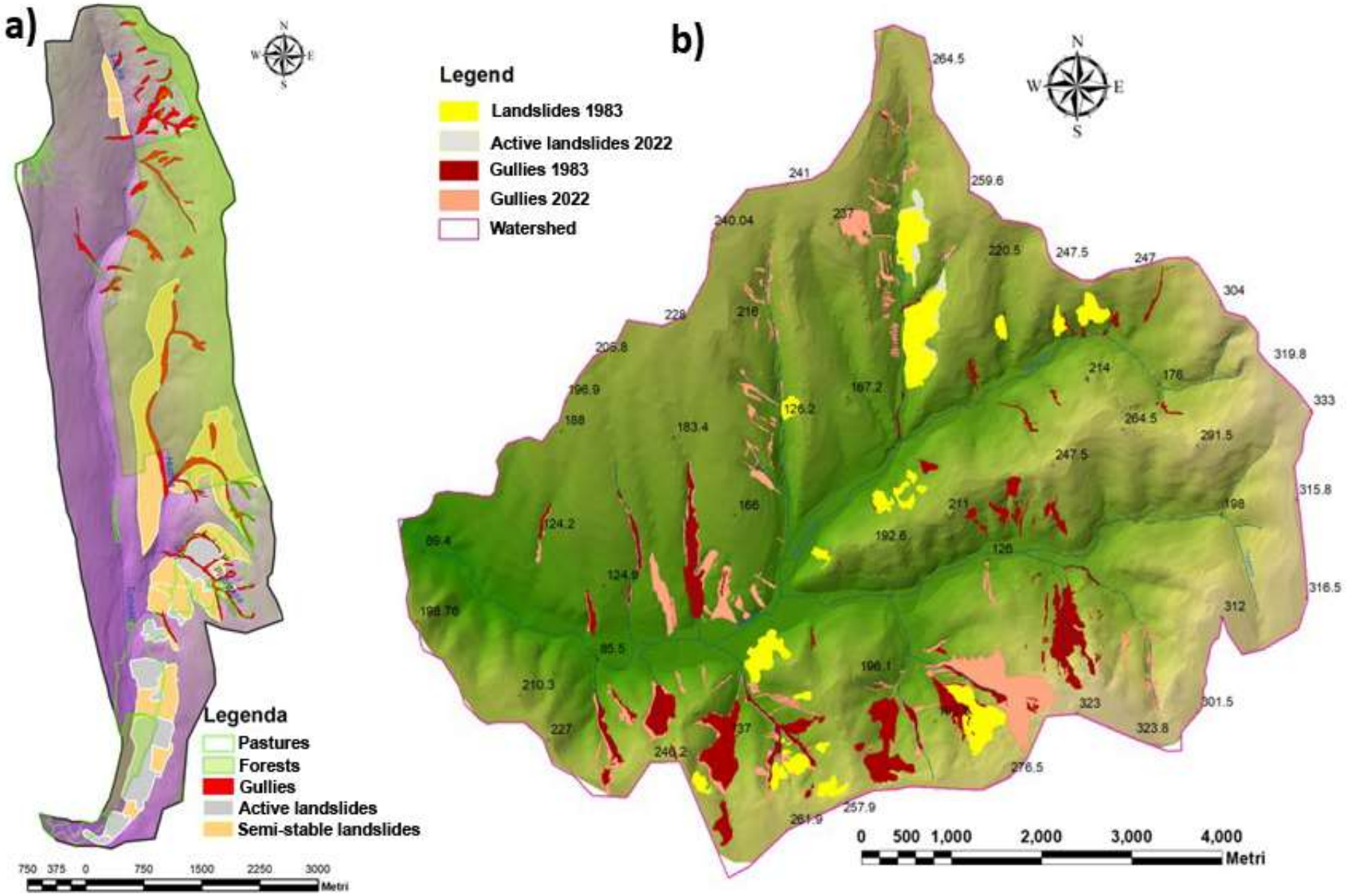


Figure 4. Gullies and landslides in 1982/83 and 2022 in Tomești and Trestiana-Conizoaia watersheds (Bârlad tableland, Vaslui County)// Ravene și alunecări de teren în 1982/83 și 2022 în bazinele hidrografice Tomești și Trestiana-Conizoaia (podisul Bârladului, județul Vaslui)

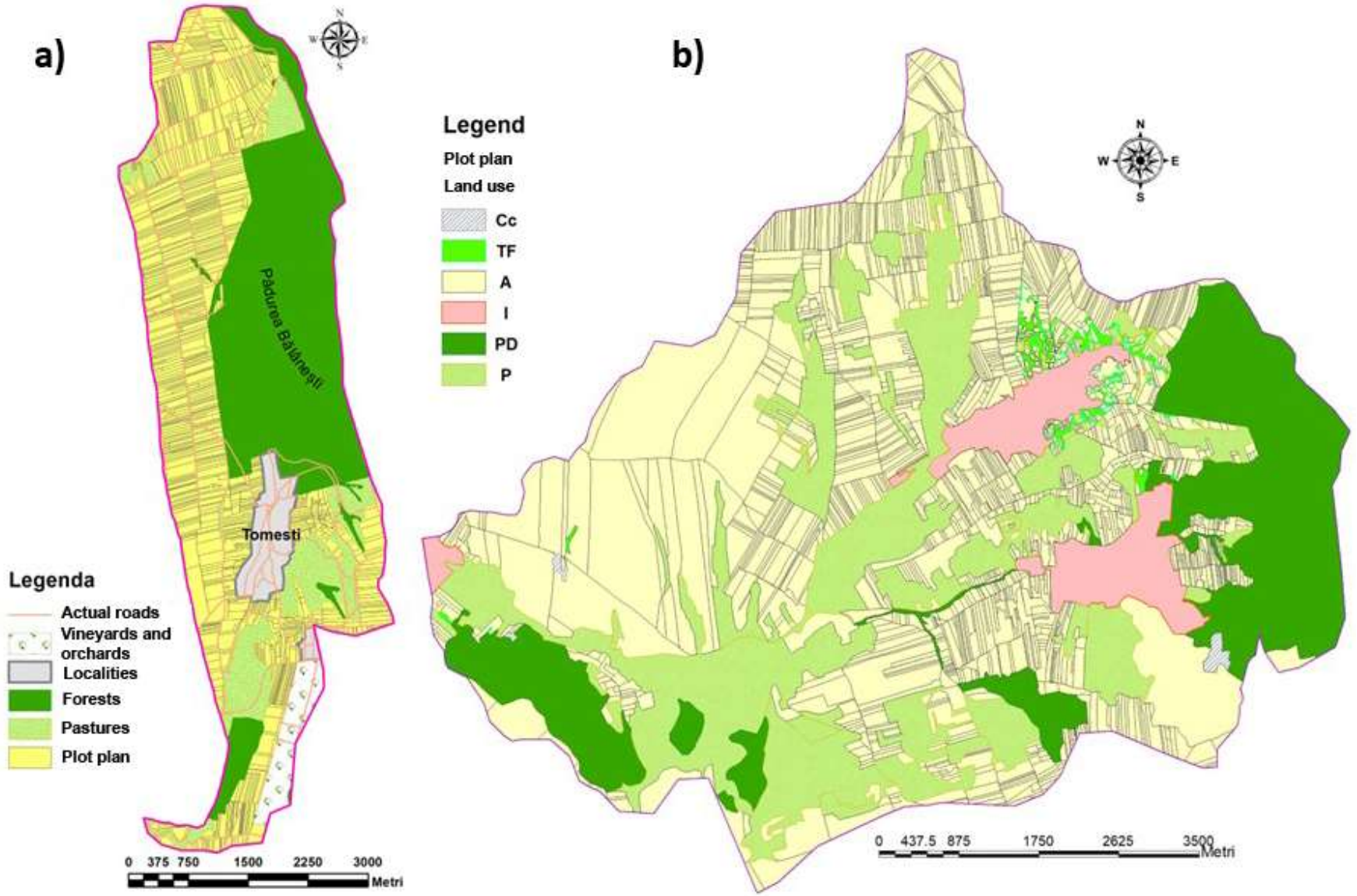


Figure 5. Actual Plot plans of Tomești and Trestiana-Conizoaia watersheds (Bârlad Tableland, Vaslui County)// Planuri parcelare actuale ale bazinelor hidrografice Tomești și Trestiana-Conizoaia (Podisul Bârladului, județul Vaslui)

- in the absence of detailed point-by-point measurements (generally unjustified or in any case very expensive due to economic and financial reasons in regional studies) of long duration regarding the dynamics (the speed of movement of the earth masses) of the processes of subsidence or sliding, the assessments regarding the current state of land degradation forms) active, semi-active, semi-stabilized, stabilized) are subjective.

For these reasons, among all the maps obtained from different documentary sources, we chose and used in the project the maps suitable for the intended purpose (establishing solutions for agricultural exploitation), using the information from geology-geomorphology and those from the cadastral maps and the own parcel plan (Figure 5, a and b).

The actual inventory was carried out by digitization on the basis of several documentary materials (topographical maps on a scale of 1:5,000, O.C.P.I. Vaslui from 1960, topographical maps on a scale of 1:25,000 drawn up by D.T.M., existing in the archive of the unit, edition 1982-1984, the cadastral plans related to the communes in the perimeter, on a scale of 1:10,000, purchased from OCPI Vaslui (1982-1983 – the most recent). All these were validated by field observations and measurements carried out with the Magellan Thales Z-Max.Net professional GPS equipment.

RESULTS AND DISCUSSION

From table no. 1 it can be seen that the main categories of land use in Tomești b.h. are: arable land (43.43% of the entire area of the watershed), forest (37.13%) and pasture (11.88%).

Table 1 . Landuse categories in Tomesti watershed, according to OCPI Vaslui cadastral maps from 1982-1983.// Categoriile de folosință în bazinul hidrografic Tomești, conform hărților cadastrale OCPI Vaslui din 1982-1983.

Crt. No.	Land use category	Area (ha)	Area (%)
1	Arable land	923.61	43,43
2	Grassed strips	0.30	0,01
3	Buildings	3.14	0,15
4	Communal road	6.46	0,30
5	Technological road	17.25	0,81
6	Fodder	3.11	0,15
7	Pool	5.61	0,26
8	Intravilan	63.99	3,01
9	Orchards	29.26	1,38
10	Swamp	1.16	0,05
11	Gullies	15.33	0,72
12	Forest	780.35	36,69
13	Young forest	9.28	0,44
14	Pasture	252.64	11,88
15	Bushes	5.39	0,25
16	Hybrid vineyard	9.92	0,47
	TOTAL	2126.80	100.00

In table no. 2 shows the situation of land use categories, expressed quantitatively in hectares, kilometers, or percentage, as the case may be. From table no. 2 it can be seen that the main categories of land use in B. h. Trestiana are: arable (44.08% of the entire area of the watershed), pasture (26.22%) and forest (16.25%).

Table 2 .Landuse categories in Tomesti watershed, according to OCPI Vaslui cadastral maps from 1982-1983.// Categoriile de folosință în bazinul hidrografic Tomești, conform hărților cadastrale OCPI Vaslui din anii 1982-1983.//

Nr. crt.	Categoria de folosință	Aria (ha)	Aria (%)
1	**Arable land**	**2,246.04**	**44.08**
2	**Pasture**	**1,336.01**	**26.22**

Nr. crt.	Categoria de folosință	Aria (ha)	Aria (%)
3	**Forest**	**828.24**	**16.25**
4	Intravilan	175.28	3.44
5	Young forest	154.11	3.02
6	Gullies	148.64	2.92
7	Hybrid vineyard	94.01	1.84
8	Technological Road	53.16	1.04
9	Noble vineyard	34.32	0.67
10	Intravilan road	17.29	0.34
11	Constructions	5.63	0.11
12	Fodder	2.46	0.05
13	Orchards	0.51	0.01
TOTAL		**5,095.7**	**100**

Tables 3 and 4 show some of the inventory data of ravines and landslides. In the perimeter of Tomești, 41 gullies were inventoried, whose channels occupy 71.71 ha, i.e. 3% of the area of the hydrographic basin.

Table 3. Summary of gullies inventory in Tomesti watershed in 2021// Rezumatul inventarului rigolelor din bazinul hidrografic Tomești în 2021

	No. Of gullies	Area (ha)	Length* (m)	Width** average/gully (m)	Depth** average/gully (m)	Width/ depth Ratio	Volume (m^3)
MIN		0.04	86,86	1.89	1,16	1.63	95,22
MAX		12.27	3,115,23	143.32	9,14	15.68	134,535,88
AVE-RAGE		1.75	552,87	9.45***	5,15	1.83	13,453,40
TOTAL	**41**	**71.71**	**22,667,78**				**551,589.41**
		3%	from/of Tomești watershed area.				

*Total length of channels - in the case of arborescent gullies, the total length of all tributaries.
**In military/topographic sense.
***Weighted average.

In table no. 3 we present some morphometric parameters of practical importance: the width and depth of the gully channels in a topographic and/or military sense (as they are inscribed in the form of that ratio on the topographic plans to see if they can be crossed on foot or with different vehicles), as and depth/width ratio. In table no. 3 I also introduced the morphometric index used in hydrology for the characterization of watercourse beds, calculated as the ratio between the width and depth of the ravine (Width to Depth Ratio).

The width/depth ratio is a key indicator for understanding the distribution of available energy in a channel and the ability of various discharges occurring in the channel to move, dislodge or transport sediment. Subunit values characterize loessoid terrains where narrow and deep ravines develop with rather vertical walls.

The unit values of the geomorphological indicator width/depth ratio would suggest that regardless of the morphometric dimensions, the cross-sections of the gullies remain approximately equilateral, a fact specific to predominantly sandy lands. The above-unit values indicate a predominantly clay substrate, due to which the ravines, in their evolution, extend laterally rather than in depth.

Determinations regarding the morphometric characteristics of gullies (especially width and depth), as well as those based on the width/depth index, especially in the case of so many ephemeral hillside gullies, are of great practical importance and are by no means just considerations descriptive, because the respective index allows the assessment of the impact of erosion in depth on the quality of agricultural lands and provides indications on the possibility/impossibility of overpassing them through ordinary works with the tractor, or not. The volume of soil and bedrock evacuated from the actual channels of the gullies was estimated at approx. 551,589.41 m^3.

Tables 4 and 5 show some of the inventory data of the gullies from the Trestiana watershed, where 193 gullies were inventoried, whose channels occupy 208.97 ha, i.e. 4% of the area of the hydrographic basin.

Table 4. Summary of gullies inventory in Trestiana watershed in 2022// Rezumatul inventarului rigolelor din bazinul hidrografic Tomești în 2022

	No. of gullies	Area (ha)	Length* (m)	Width** average/gully (m)	Depth** average/gully (m)	Width/ Depth Ratio	Volume (m^3)
MIN		0.02	11,30	3.46	1,38	2.50	
MAX		48,36	1,754.88	546,02	12.21	44.71	
AVERAGE		1,08	9.15	2,84***	0.07	4,06	
TOTAL	**193**	**208.97**	**54,441,65**				**98,820.15**
		4%	From/of Trestiana watershed area.				

*Total length of channels - in the case of arborescent gullies, the total length of all tributaries.
**In military/topographic sense.
***Weighted average.

From table no. 5 it can be seen that the number of ravines in Trestiana watershed increased in 2022 compared to 1983 from 123 to 193, which percentage means from 3.5 to 4.1% of the area of the hydrographic basin.

Table no. 5. Summary of gullies inventory in Trestiana watershed in the years 1983 and 2022// Rezumatul inventarului rigolelor din bazinul hidrografic Tomești în anii 1983 ți 2022

Category	Number / Count	Total area (ha)	(%) from total watershed
Gullies 1983	123	177.57	**3.5**
Gullies 2022	193	208.97	**4.1**

From table no. 6 it can be seen that the area affected by active landslides in 2021 is 95.74 ha, which represents 5% of the area of Tomești commune, and semi-stable landslides occupy 244.69 ha, which represents 12% of the area the watershed. In total, both landslide categories occupy 17% of the watershed area.

Table 6. Summary of landslides inventory in Tomesti watershed in the year 2021// Rezumatul inventarului alunecărilor de teren în bazinul hidrografic Tomești în anul 2021

Category	Number	Total area (ha)	(%) from total watershed	Minimum aria (ha)	Maximum area (ha)	Average aria (ha)
Active landslides	13	95.74	**4,5**	0.48	40,7	7.36
Semi-stable landslides	19	244,69	**12**	0.38	81,13	12.88
	32	**340,43**	**17%**			

According to our previous research (Hurjui C., 2001), in Vaslui county the area affected by landslides is 5.61%. In the perimeter of Tomești, the area affected by landslides (95.74 ha) is lower than the value for the county, namely 4.5%. However, the landslides identified by us as semi-stable are of equal importance when it comes to anti-erosion landscaping solutions and then the figure to be considered is 17%.

In figure 4a, where both gullies and landslides are represented, it can be seen that when gullies and landslides occur together, their action is the most destructive. That is why the area of the slope above the village of Tomești is the most spectacular and, curiously, has never been landscaped. In figure 4a, both the gullies and landslides, as well as the areas covered with pastures and/or forests, are represented, in order to be able to see to what extent the gullies and/or landslides are covered with protective vegetation.

From table no. 7 it can be seen that in 2022 the area affected by active landslides is 46.66 ha, which represents only 0.92% of the area of Trestiana. Considering the data on landslides from the year 1983 (23 in number with an area of 120.54 ha), this does not mean that the number of landslides has decreased/shrunk, but only that all those landslides inventoried for the year 1983 are currently semi-stabilized, as can also be seen from figure 4b and that only two are apparently active. In the centralizer of the data about the ravines in the Trestiana perimeter in 2022 (table no. 4) the much higher values of the width/depth ratio indicate the clayey nature of the geological substrate (even if we did not encounter relevant outcrops), a fact that explains the very large surface dimensions of of ravines, but also of

landslides and, at the same time, their superficiality. Therefore, it can be seen that such lands are maintained and exploited only as pasture.

Table 7. Summary of landslides inventory in Trestiana watershed in the year 2022// Rezumatul inventarului alunecărilor de teren în bazinul hidrografic Tomești în anul 2022

Category	Number	Total area (ha)	(%) from total watershed	Minimum area (ha)	Maximum area (ha)	Average area (ha)
Landslides 1983	23	120,54	**2,4**	0,225	30,63	5,24
Active landslides 2022	2	46,66	**0,92**	19,78	26,87	23,33

In the Trestiana perimeter, the area affected by landslides (95.74 ha) is lower than the value per county, namely 2.4% (1983 - 2022). However, the landslides identified by us as being semi-stable and shallow in terms of the depth of the sliding surfaces, have an equally great importance when it comes to anti-erosion management solutions, as they are, anyway, degraded lands.

Solutions for anti-erosion organization of agricultural lands in Tomești and Trestiana watersheds. Having accumulated all the information presented up to this point, it was possible to centralize the data from different sources and establish the anti-erosion management solutions for the agricultural lands in the Tomești and Trestiana watersheds (Figure 6 and tables no. 8 and 9). It was also necessary to draw up an individual parcel/plot plan for each micro-watershed by digitizing the plots apparently cultivated with the same crop, on orthophotoplans in a scale of 1:5,000, which we validated through discussions with farmers and local authorities (mayors) in during field visits. (figure 5 a and b).

The plot plans drawn up in the years 2021 and 2022 illustrate to a certain extent the current ownership structure of agricultural land, but especially the way in which agricultural land is worked.

Table 8. Actual and designed / provisioned landuse categories and anti-erosion works in Tomesti watershed // Categorii de folosinta a terenurilor actuale si proiectate/prevazute si lucrari antieroziune in bazinul hidrografic Tomesti

Land use category / work / measure	Existent on ANCPI cadastral maps, 1982 (m or ha)	Existent in 2021 (m or ha)	Designed/Provisioned (m or ha)
Grass strips	1,114 m	16,321 m	-
Forests	789.63 ha	791.27 ha	97.82 ha*
Forest protective belts	-	-	12.272 m
Pastures	252.64 ha	281.90 ha	-
Technological roads (earthen roads)	36,999 m	57,124 m	1,677 m**

* Afforestation is proposed to be carried out on degraded pastures.
** Roads proposed to be withdrawn into serpentines.

Table 9. Actual and designed / provisioned landuse categories and anti-erosion works in Trestiana watershed // Categoriile de utilizare a terenurilor actuale și proiectate/prevăzute și lucrări antieroziune în bazinul hidrografic Trestiana

Land use category / work / measure	Existent on ANCPI cadastral maps, 1982 (m or ha)	Existent in 2022 (m or ha)	Designed/Provisioned (m or ha)
Grass strips	2,874.65 m	2,182.33 m	7,109.00 m
Forests	828.24 ha	613.98 ha	73.58 ha*
Forest protective belts	623.46 m	623.46 m	13,750 m
Pastures	1,336.01 ha	2,159.08 ha	-
Technological roads (earthen roads)	83,822.00 m	79,873.00 m	2,403 m**

* Afforestation is proposed to be carried out on degraded pastures.
** Roads proposed to be withdrawn into serpentines.

The categories of anti-erosion works proposed (designed) belong to two groups:

a) larger-scale, more expensive structural works that require the intervention of the State or private economic-financial institutions, such as land improvement works, modeling works, landscaping of slopes with landslides, stabilization works of torrents, of gullies, etc., afforestation and even the planting of forest protection belts;

b) smaller-scale, less expensive works that are within the reach of farmers (administrators of commercial companies with an agricultural profile), such as: correction/modelling of torrents, streams, ogashes,

stabilization by various techniques of the banks and bottoms of gullies, tracing/drawing (with the help of specialists such as those from SCDCES "MM" Perieni) grassy strips to separate the strip crops, establishing grassed waterways along the valleys or ogashes, correcting some routes of roads for agricultural exploitation, etc.

CONCLUSIONS

1. The main categories of land use in B.h. Tomești, according to the cadastral map, are: arable land (43.43% of the entire area of the basin), forest (37.13%) and pasture (11.88%).
2. In the perimeter of Tomești, 41 gullies were inventoried, whose channels occupy 71.71 ha, i.e. 3% of the area of the hydrographic basin.
3. The area affected by active landslides in 2021 was 95.74 ha, which represents 5% of the area of Tomești district, and semi-stable landslides occupy 244.69 ha, which represents 12% of the area of the basin. In total, both landslide categories occupy 17% of the basin area.
4. After analyzing all influencing factors, within the anti-erosion organization solutions of the territory, the following were proposed/designed for b. h. Tomești:
   - Afforestation of areas totaling 97.82 ha instead of degraded pastures;
   - Drawing and planting protective forest curtains in a total length of 12,272 m linear meters;
   - The re-drawing of technological roads from their current form, to serpentines, in a total length of 1,677 linear meters.
5. The main categories of land use in. Trestiana watershed, according to the cadastral map, are: arable land (44.08% of the entire area of the basin), pasture (26.22%) and forest (16.25%).
6. In 2022, a number of 193 gullies occupy an area of 208.97 ha, i.e. 4.1% of the area of Trestiana watershed, and a number of 25 landslides (23 semi-stabilized and identified since 1983 and two apparently active in 2022), occupies an area of 167.20 ha, i.e. 3.3% of the area of b. h. Trestiana. Gullies and landslides occupy a relatively large area, but are shallow.
7. The area covered by forests decreased drastically (!) in Trestiana watershed, from 828.24 ha (16.25%), to 613.98 ha (12.05%);
8. The area covered with pastures increased from 1,336.01 ha (26.22%) to 2,159.08 ha (42.37%).
9. The terrains in the arable land category comprise a number of **1,728 parcels/plots (high fragmentation)**, each with an **average area of 1.21 ha** and are oriented in the hill-valley direction. The area of arable land decreased from the beginning of the 80s to the present, from 2,246.04 ha (44.08%), to 2,082.28 ha (40.86%), in favor of the so-called pastures, which the researchers westerners call them **abandoned lands**.
10. After analyzing all influencing factors, within the anti-erosion organization solutions of the territory, the following were proposed/designed:
    - Tracing/drawing/laying and/or rehabilitating grass strips in length of 7,109.00 linear meters;
    - Afforestation of areas totaling 73.58 ha instead of degraded pastures;
    - Drawing and planting protective forest belts in a total length of 13,750.00 linear meters;
    - The re-drawing of technological roads from their current form, to serpentines, in a total length of 2,403.00 linear meters.

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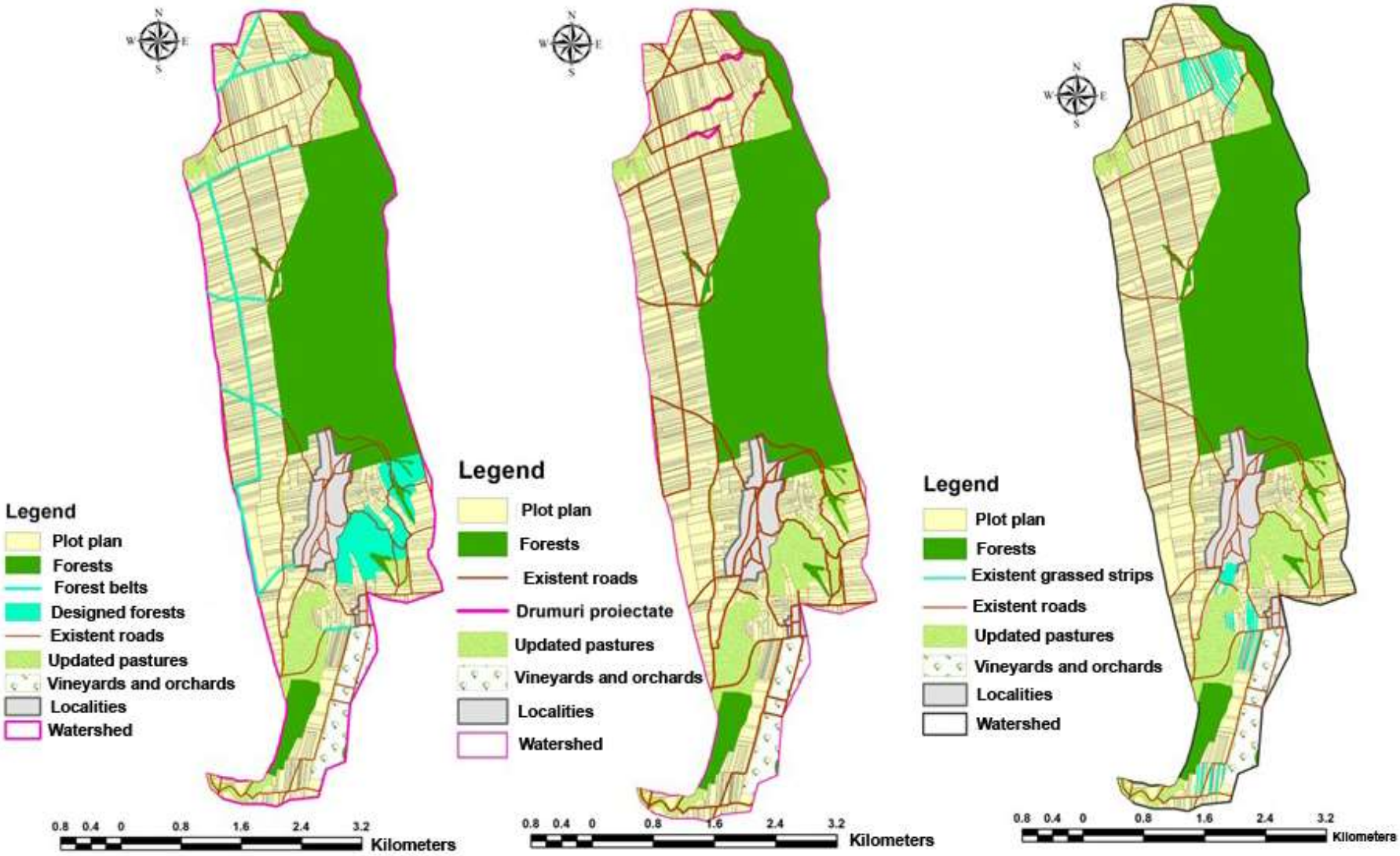


Figure 6. Actual and designed / provisioned landuse categories and anti-erosion works in Tomesti watershed.// Categorii de folosinta a terenurilor actuale si proiectate/prevazute si lucrari antieroziune in bazinul hidrografic Tomesti

ASPECTS OF WEED CONTROL IN WHEAT CROP
ASPECTE ALE CONTROLULUI BURUIENILOR DIN CULTURA GRÂULUI

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Summary
The study of the relationship between wheat and weed species is important in finding out the measures by which the competition can be reduced. Fighting only with herbicides gradually lost its importance due to the new requirements for agriculture, due to the accumulation of noxes in the soil. The luvosol contains large amounts of weed seeds reserves, and their biomass from wheat was on average 6.7 t/ha. Weed development can be stopped, not by eradication, but at levels where crop losses no longer occur. Herbicides used in wheat crop gave good results after April-May. The dose reduction in the wheat crop will be done gradually, with care, namely 25% for 2,4-D acid and 25-50% for chlorsulfuron. Late application of hormones is very critical because it is possible to destroy grain production (Ionescu, 2000). Further research is needed for other herbicides. In wheat, herbicides reduced the degree of coverage (GA) from 60% to 24%, and wheat production increased by approx. 1-2 t/ha. SU type products have a very good control over weeds, due to their broad spectrum.
Key words: *weeds, wheat, herbicides, luvosol environment, selectivity*

Rezumat
Studiul relaţiei dintre grâu şi speciile de buruieni este important în aflarea măsurilor prin care se poate reduce competiţia. Combaterea numai prin erbicide şi-a pierdut treptat din importanţă datorită noilor cerinţe pentru agricultură, datorită acumulării noxelor în sol. Luvosolul conţine cantităţi mari din rezervele de buruieni, iar biomasa acestora din grâu a fost în medie de 6.7 t/ha. Dezvoltarea buruienilor poate fi stopată, nu prin eradicare, dar reducerea la nivele la care nu mai au loc pierderi de recoltă. Erbicidele folosite în grâu au dat rezultate bune după perioada aprilie-mai. Reducerea dozelor în cultura grâului se va face treptat, cu grijă şi-anume de 25 % la acidul 2,4-D şi 25-50 % la chlorsulfuron. Aplicarea târzie a insecticidelor hormonale este foarte critică deoarece este posibilă distrugerea producţiei de boabe (Ionescu, 2000). Pentru alte erbicide sunt necesare cercetări în continuare. În grâu erbicidele au redus gradul acoperire (GA) de la 60% la 24%, iar producţia de grâu a crescut cu cca 1-2 t/ha. Produse de tip SU au un foarte bun control asupra buruienilor, datorită spectrului larg al acestora.
Cuvinte cheie*: buruieni, grâu, erbicide, mediul luvosolului, selectivitate*

INTRODUCTION

Winter wheat, a crop considered "dense", with a high degree of soil coverage, gives the image of a slightly lower annual weeding degree, of course in comparison to fallow plants, like "rare". In appearance, things are like this, but if we analyze the state of wheat weeding through its structure, some important aspects can be found, which every farmer should take into account. The species, both annual and perennial ones that make up the floristic spectrum, are characteristic of wheat. It is considered that the vegetation period of wheat is relatively long (autumn wheat covers practically all 4 seasons of the year). During this time, unwanted species sprout at a very diverse level, as follows: some weeds are specific for autumn, others for spring, others for autumn-spring, and others for early summer (Anghel, 1972). The particularly high virulence of some dicotyledonous species on wheat is also taken into account, then the resistance to varying degrees of some species to some herbicides, etc.

Considering these various and very important aspects, it is considered that even for the winter wheat crop, the best results in reducing the degree of weeding are obtained by accepting and promoting the integrated management of weeds (MIB) (Şarpe, 1983; Barberi, 2002). Regardless of the wheat cultivation area, the appearance of certain weed species can be observed even around the emergence of crop plants. In these autumn conditions, with the gradual decrease in the length of the day and the temperatures, both the installation and the coexistence between the wheat plants and the autumn weeds take place. The degree of infestation in this period of the year with autumn weeds, as

well as with walking weeds, can be from a relatively low one (here crop rotation plays an important role) to a very high one (in the case of monoculture). However, in the autumn period, a real danger of affecting the wheat crop cannot be appreciated, because with the appearance of negative temperatures, some sprouted weeds are destroyed.

The spring period is considered the most critical for autumn wheat, because the degree of weeding in late spring produces the most obvious influences of weeds on the physiology and morphology of wheat plants (Berca, 1994; Auld, 1996; Ionescu, 2000). In order to avoid the concrete damage that weeds cause to wheat, the most appropriate herbicides will be chosen and applied. The best time to apply the active ingredients is when "wheat is at the end of twinning and beginning of rooting, and the weeds are at the rosette stage" (Sharpe, 1983). In the course of several decades, a multitude of herbicides specific to the floristic spectrum of the autumn wheat crop, as well as different periods of application, have been obtained and used. Very diverse and complex research carried out both in other parts and in our country, highlighted the best strategies, of interweaving with chemical and non-chemical means (mainly cultural methods). Their goal was to reduce the levels of weeding to the lowest possible levels, to increase productivity, to reduce the cost price per unit of product: mainly grains and secondary straw. In the new conditions, to protect the agricultural environment and to ensure its sustainability (for the recent and long-term perspective), the application of new rules, also valid for winter wheat, is required (Altieri, 1995).

Currently, the idea is accepted that for the concrete development of an appropriate MIB for each agricultural area, experiments as diverse as possible, of an ecological, physiological, morphological etc. View point are necessary. namely for each method considered as the best for reducing the degree of weeding. This paper presents a series of results obtained through ecology and weed control studies characteristic of the Luvicsoil in the south of the country. From their presentation, it is hoped that for the present and the future, wheat culture will be included in new European parameters.

MATERIAL AND METHOD

In one of the multi-year studies several series of researches were carried out which generally included aspects of winter wheat weeding. Thus, on the one hand, weed species from the categories: annual dicots, perennial dicots and annual monocots were quantitatively determined. Perennial monocots were present sporadically and mostly in the form of small, dispersed and irregular hearts. Based on the data obtained from the weeds that accompanied the wheat annually, the correlation was established between the degree of coverage of the unwanted vegetal carpet and the losses of useful wheat production. Another direction of research refers to how the high degree of infestation influenced the growth and development of wheat plants. The analyzed variants were placed at the beginning of the experiments, namely as untreated controls within the experiments with herbicides established for the entire national network by the Fundulea Institute, including in Pitesti.

Another specific researched direction refers to the exclusive use of chemical methods - with the help of herbicides, to reduce the degree of weeding, but also to protect the soil and wheat plants. Of course, after a relatively large number of years (about 5-6 decades), permanent improvements were found - both from the companies, but also from the researchers in the field, so that the practitioners had and have at their disposal the most modern, effective and cheap variants that can be very easily adapted to specific situations in own soils cultivated with winter wheat. In separate experiments, several herbicides were studied - classical, but also promising, with the aim of addressing the new EU issue of reducing herbicide doses, regardless of the active substance, the crop and the European area. In the present case, in wheat, several lower proportions were experienced for two herbicides: 2,4-D acid (a hormonal herbicide) and chlorsulfuron (SU). The experimental variants were placed in the station's research field, according to the Latin rectangle method, in 4 repetitions, with a surface area of 25 m^2 each.

The plant samples (weeds and wheat) were collected in the different moments of the vegetation, as well as in the maturity phase with the metric frame, from all repetitions. The dry

substance was obtained each time by oven drying, according to the common method (Clawson), 6-8 hours at 105^0C. The statistical processing was done by the variance analysis method (Anova test), and the Excel program was used to express the average data. The varieties of wheat used were different from year to year, but in the vast majority of them the creations of the station prevailed, namely: Albota and Trivale.

RESULTS AND DISCUSSION

Considering the specific degree of competition of wheat with weeds, especially in the early moments of the spring vegetation, it was considered appropriate to study its infestation with specific species, in the natural conditions of the luvosol eco-environment. From the multitude of species present in a crop area (Anghel, 1972), most of them usually cause obvious damage (Rusu, 2010) to wheat. The interaction between the two camps: wheat and the vegetal carpet, can be studied either separately depending on a chosen weed, or a close botanical group of weeds, or for the whole unwanted vegetal carpet (Mortensen, 2000). When considering the means of weed control in a crop, it is preferable that the weeding be considered primarily as a whole.

Natural weeding of the wheat crop. From the respective experiences, all the present species were harvested in the final phase, namely with the metric frame. After they were all weighed together, a separation was made into three categories: DA- annual dicots, DP- perennial dicots and MA- annual monocots. The way the weeds in the untreated control evolved quantitatively is shown in figure 1. The graph shows that the weeds had quite different annual values in terms of quantity. The smaller amounts were obtained on the drier climate, while the higher amounts, over 8-10 t/ha s.u. they were formed in wet, favorable years. The annual structure between the three categories of weeds was also different, fluctuating, demonstrating the variability of appearance, the evolution of their vegetation in relation to the degree of interspecific competitiveness (Sagar, 1968; Wilson, 1998; Christensen, 2009) and what ultimately results. The structure shows the dominance of annual dicotyledons constantly represented by: *Matricaria inodora, Galeopsis tetrahit, Centaurea cyanus, Viola arvensis*.

The perennial dicotyledons, permanently present in the wheat vegetation, as well as in the final phase - at harvest, were represented by *Cirsium arvense, Sonchus arvensis* and *Convolvulus arvensis*. The annual monocots of the type: *Echinochloa crusgalli, Digitaria sanguinalis, Setaria glauca*- as dominant, appeared towards the end of the wheat vegetation and only on the background of the penetration of sunlight among the almost ripe (mature) wheat plants.

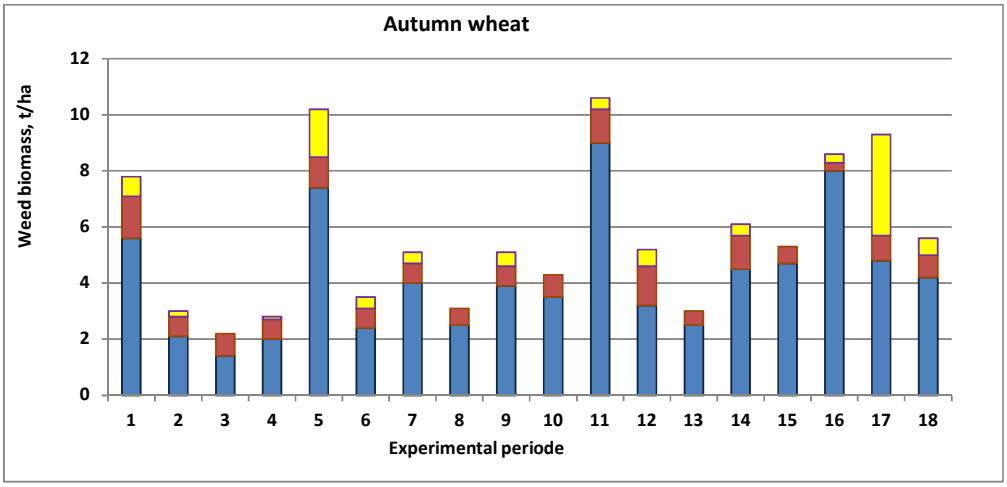


Figure 1. Weeds biomass evolution in time: blue - AD, red - PD, yellow - AM] // Evoluţia biomasei buruienilor în funcţie de categoria botanică: (albastru- dicotile anuale; roşu- dicotile perene; galben- monocotile anuale

[

A direct relationship was established between the degree of weeding of the weed complex and the production of wheat grains obtained depending on the different degrees of weeding (figure 2). From the graph it can be seen that if the weeds formed between 0.5 and 1 t/ha s.u., the damage started to be clearly highlighted. At higher amounts of weeds: between 5 and 7 t/ha wheat production was drastically reduced, to below 1 t/ha s.u. grains.

The impact weeds that appeari both in autumn and especially in spring can produce on wheat (Wilson, 1998), is shown below (Figure 3). The average rate of accumulation shows delays, it takes place at much lower values, and the period of deposition of nutrients in the grains has been reduced.

Overall, average natural wheat weeding reduced biomass accumulation to about one-third (1/3) of normal.

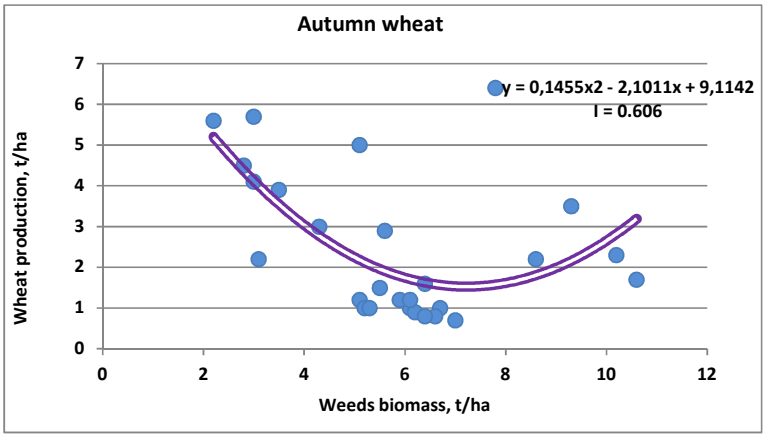


Figure 2. Relationship between weeds biomass and wheat loss of yield// Relaţia dintre gradul de îmburuienare şi pierderile de producţie la grâul de toamnă

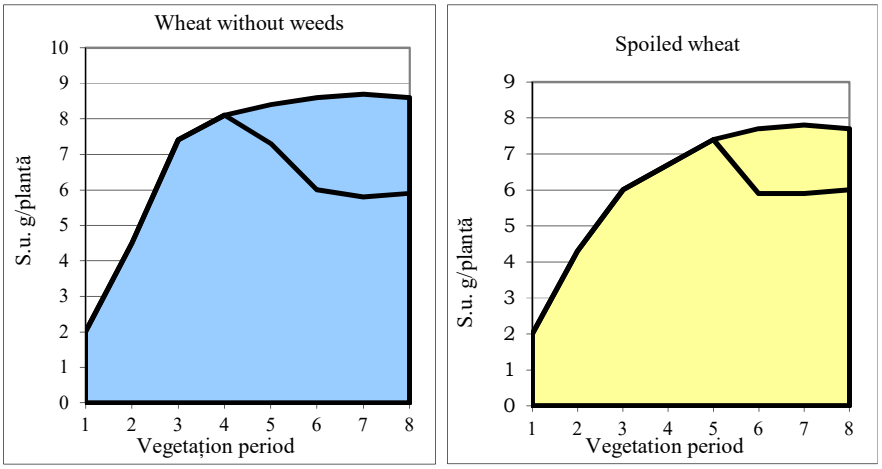


Figure 3.Diagrams of wheat plant biomass formation, without weed competition (left) and under condition of weed encroachment (right) // Diagramele formării biomasei plantei de grâu, fara concurenţa buruienilor (stânga) şi în condiţii de îmburuienare (dreapta); (1-2= aprilie, 3-5= mai, 6-8= iunie)

Chemical methods to reduce the degree of weeding in wheat culture. As is known, currently there is a veritable arsenal of wheat-specific herbicidal active substances, characterized by high degrees of effectiveness and selectivity. Both the companies in the field and the research can offer the best and appropriate options for weed control in the wheat culture. The specialized European Commission accepted some products applied to the crop to be reduced as much as possible. In our country, too, some variants of reducing herbicides on wheat have been tried, with little conclusive results. In the present example (figure 4), the effectiveness of two treatments in different doses is presented: 0- without herbicide, 25%, 50%, 75% and 100% of the normal doses. The evolution of the effectiveness expressed by GA of no controlled weeds demonstrates that wheat can be dispensed with

in the case of 2,4-D acid, but only in certain special conditions of reduced weeding as a result of a very successful agrotechnical crop, at the recommended dose, at 75% of this, while with chlorsulfuron, in the same special agri-technics successful conditions, the dose can sometimes be reduced by 25-50%. Research of this kind is promising and will have to be carried out in as many weeding conditions as possible and in as many ecological areas as possible.

Another aspect that is not yet fully clarified refers to the timing of herbicide application in the winter wheat crop. The researches carried out everywhere with herbicides - especially hormonals ones, but also with different varieties, have highlighted the danger of physiological damage to wheat plants and especially to the ear when the respective application is made in advanced phases of the vegetation, around the earing of the wheat. In the conditions of the season, it is true that only in the years when nature sensitized the plants, the hormonal herbicides of the 2,4-D acid type together with dicamba (figure 5), demonstrated approximately the destruction of grain production.

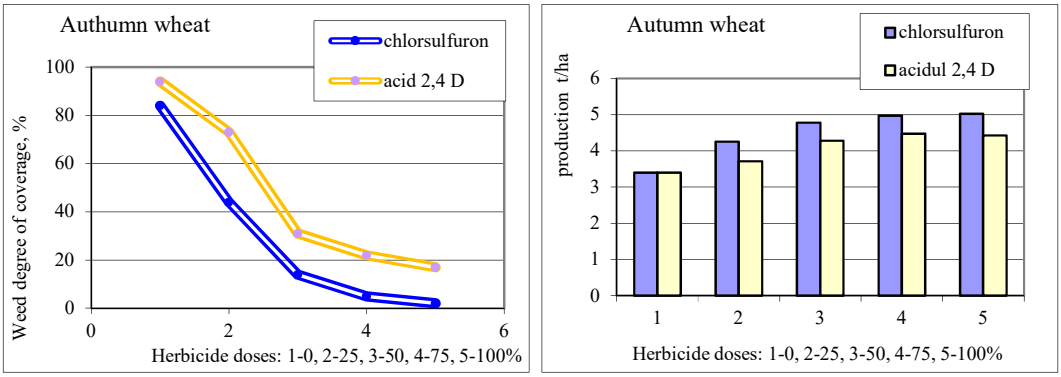


Figure 4. The efficacy of specific herbicides function of different doses, in winter wheat crop// Eficacitatea erbicidelor specifice grâului în funcţie de doza aplicată

The data obtained over several years demonstrate the fact that herbicides used appropriately in wheat culture have led to very favorable results in reducing the degree of weeding specific to luvicsoil. Thus, the grain production gained as a result of the herbicide increased by about 15%, while the final degree of coverage (GA) with the unwanted vegetation decreased from about 60% (multiannual average), to about 14-18% (figure 6).

Through a comparison between the different chemical methods (herbicides), both more classic and newer, slight differences were found (figure 7). The differentiation took place as a result of the fact that new products, such as those in the SU class (sulfonylureas), have a wider spectrum of dicotyledonous control (DA and DP). Some of the SUs, as in the case of chlorsulfuron, ensure safe environment, combating both through plants and through the soil.

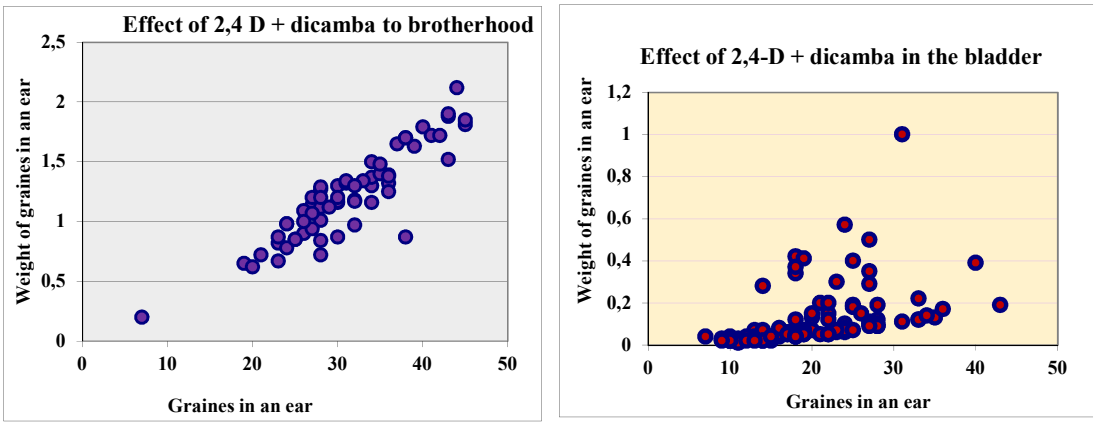


Figura 5. Correlations between grains number and grains weight from wheat treated with herbicide 2,4-D + dicamba, late application // Corelaţii între numărul şi greutatea medie aboabelor (g) din spicul mediu de grâu prin aplicarea târzie a erbicidului 2,4-D+dicamba

Water regime and weeding. In a study over several years, the correlation of the total bimass of weeds was analyzed in this area where the rainfall regime is quite high, including during the wheat vegetation period (figure 8). The graph clearly shows the connection between the rains that fell on the wheat vegetation and the unwanted biomass formed.

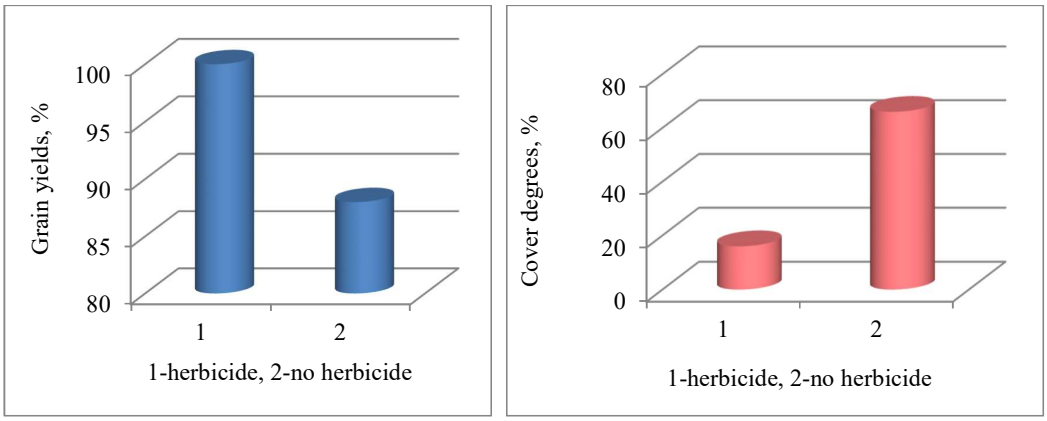


Figure 6. The herbicide efficacy by wheat grain yields and by covered degrees of weeds at harvesting // Influenţa erbicidării asupra producţiei de boabe de grâu şi asupra gradului de acoperire a buruienilor, la recoltare

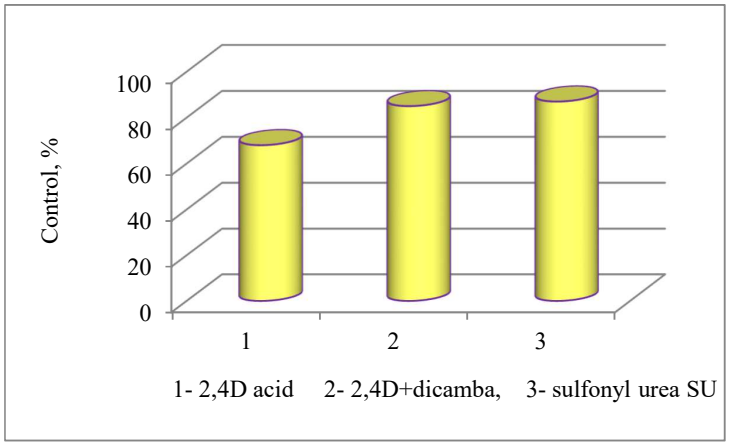


Figure 7. The weeds control degrees from wheat crop with differing herbicides// Gradul de combatere al buruienilor din cultura grâului cu diferite erbicide

CONCLUSIONS

1. Wheat is weeded every year with characteristic species, at levels considered to be moderately high. The main causes are: the high reserve of seeds in the soil and the relatively low power of wheat to compete with weeds. The multi-year study of natural weeding, however, resulted in fluctuating biomass values due to climatic causes - drought or rainy regime. The limits between which the total biomass of weeds was located were 2.1 t/ha dry weight and 11.0 t/ha dry weight.
2. The structure of the weeds was as follows: 75% annual dicotyledons - very competitive with wheat, 16% perennial dicotyledons and 9% annual monocotyledons, out of a multiannual average of 6.7 t/ha dry weight. With this structure, it was expected that the production loss of wheat grains would be approximately at relatively low levels.
3. Chemical control of weeds through appropriate herbicides achieves very good protection resulting in a clean field until harvest. In the crop dense by wheat, weeds can appear later, usually through reinfestations, as in the case of sweet chamomile, but from an economic point of view, they no longer cause significant damage. Towards the maturity of wheat, annual monocots also appear.

4. The problem of reducing herbicide doses is current and requires new experiments, in as diverse ecological conditions as possible. From the data obtained so far in the station, it was found that the researched products protected the wheat against weeds, in slightly smaller doses, namely with only 25-rare 50% reduction as compared to the normal (legal) doses.
5. Regardless of the option of the method - chemical, cultural, biological etc., it will be considered to reduce the weeding of the wheat crop to the levels where economic damages are no longer produced. This recommendation was accepted in cases where it is no longer necessary to eradicate all weed species, namely for reason of preserving biodiversity in agricultural fields.

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THE VARIATION OF SOME MORPHOLOGICAL CHARACTERISTICS OF THE A.50-15 WHEAT LINE UNDER CURRENT CLIMATE CONDITIONS

VARIAȚIA UNOR CARACTERE MORFOLOGICE ALE LINIEI DE GRÂU A.50-15 ÎN CONDIȚII CLIMATICE ACTUALE

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Rezumat

The morphological characteristics expressed in different climatic conditions, and especially those of drought, could be used in the progress of the improvement of the new lines and varieties of winter wheat. The wide genetic dowry and growing conditions of wheat usually lead to the characteristic expression of plant morphology in close connection with periods of drought. In the last three years, the A.50-15 line has encountered different situations, namely from the most favorable to the drought. Thus, the year 2019 was more favorable from a climatic point of view, the year 2020 was the most favorable environment, and the year 2021 the least favorable. Against this diverse background of ensuring the amounts of precipitation, morphological characteristics of the plants of this new line were manifested specifically. Thus, the dimensions of the straw were expressed relatively evenly in the three years, the dominant ones being those at 60-65 cm. The thickness of the straw at the base was 3 mm in the second year (2020), compared to the other two years, by 3.5-4 mm. The length of the ear had a dominant value of 8.5 cm in the favorable year and 8 cm in the two less favorable years. The weight of the ear expressed the modal value at 4 g in the first year, while in the other two years it stood at 2-2.5 g. The number of grains in the ear had the modal value at 75 in the first year, being 30 grains higher. much more than the other two years. The weight of the grains in one of their ears was dominant at 3 g in the first year and at 2 g in recent years. The number of spikelets dominated at 19 in the years with drought effect and 21 in the favorable year. The aristas had dominant lengths of 7-8 cm in all three years. The grain length was 7 mm in the dry years and 6.5 in the more favorable year. The thickness of the grain was similar to 3 mm, with a slight tendency to increase in the favorable year. The mass of a thousand grains dominated by 38 g in 2020 and 42 g in the other two years. The correlations obtained between all the determined morphological characters demonstrated specific variability both by very significant positive and by negative values in all three degrees of significance. However, very close positive links show a relatively high level of adaptability of this new wheat line to the changing climate. The statistical indices of the studied morphological characters demonstrated, especially those with productive justification, at average values of the coefficients of variation, namely between 10% and 20%. These aspects in fact demonstrate the good adaptability to these climatic conditions with more and more obvious accents of drought.

Keywords: ***grains, spike, spikelets, variability, wheat***

Rezumat

Caracteristicile morfologice exprimate în diferite condiții climatice, și în special cele de secetă, ar putea fi utilizate în progresul ameliorării noilor linii și soiuri de grâu de toamnă. Zestrea genetică largă și condițiile de creștere ale grâului conduc de obicei la expresia caracteristică a morfologiei plantelor în strânsă legătură cu perioadele de secetă. În ultimii trei ani, linia A.50-15 a întâmpinat situații diferite, și anume de la cele mai favorabile secetei. Astfel, anul 2019 a fost mai favorabil din punct de vedere climatic, anul 2020 a fost cel mai favorabil mediu, iar anul 2021 cel mai puțin favorabil. Pe acest fundal divers de asigurare a cantităților de precipitații s-au manifestat în mod specific caracteristicile morfologice ale plantelor acestei noi linii. Astfel, dimensiunile paiului s-au exprimat relativ uniform în cei trei ani, cei dominanti fiind cei de la 60-65 cm. Grosimea paiului la bază a fost de 3 mm în al doilea an (2020), față de ceilalți doi ani, cand a avut 3,5-4,0 mm. Lungimea spicului a avut o valoare dominantă de 8,5 cm în anul favorabil și 8 cm în cei doi ani mai puțin favorabili. Greutatea spicului a exprimat valoarea modală la 4 g în primul an, în timp ce în ceilalți doi ani a fost de 2,0-2,5 g. Numărul de boabe din spic a avut valoarea modală de 75 în primul an, fiind cu 30 de boabe mai mare, mult mai mult decât în ceilalți doi ani. Greutatea boabelor într-unul dintre spicele lor a fost dominantă la 3 g în primul an și la 2 g în ultimii ani. Numărul de spiculete a dominat la 19 în anii cu efect de secetă și 21 în anul favorabil. Aristocrații au avut lungimi dominante de 7-8 cm în toți cei trei ani. Lungimea boabelor a fost de 7 mm în anii secetoși și de 6,5 mm în anul mai favorabil. Grosimea bobului a fost similară cu 3 mm, cu o ușoară tendință de creștere în anul favorabil. Masa a o mie de boabe a dominat de 38 g în 2020 și 42 g în ceilalți doi ani. Corelațiile obținute între toate caracterele morfologice determinate au demonstrat variabilitate specifică atât prin valori pozitive foarte semnificative, cât și prin valori negative în toate cele trei grade de semnificație. Cu toate acestea, legăturile pozitive foarte strânse

arată un nivel relativ ridicat de adaptabilitate a acestei noi linii de grâu la schimbările climatice. Indicii statistici ai caracterelor morfologice studiate au inregistrat, în special cei cu justificare productivă, valori medii ale coeficienților de variație între 10% și 20%. Aceste aspecte demonstreaza de fapt buna adaptabilitate la aceste conditii climatice cu accente din ce in ce mai evidente de seceta.
Cuvinte cheie: boabe, țeapă, spiculeți, variabilitate, grâu

INTRODUCTION

Cultivated for a long time (Bonjean & Angus, 2001; Curtis et al., 2002), 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) is one of the most important crops (Farooq et al., 2015; Ihsan et al., 2016). *Triticum* derives from *threshing* (*bruising*), sorting, and summer *aestivum*. The threshing wheat is also called *spelta.* With the genes from *Aegilops tauschii*, they give the bread wheat the cold resistance it needs in temperate climates. As a surface, wheat occupies the first place, being met in a multitude of conditions (Reddy & Raghavendra, 2006; Tester & Langridge, 2010). The purpose of its cultivation is the production of grains (Fang & Xiong, 2015) used in the production of bread. The nutrient content of the grains is diverse and balanced, having a special importance in human food. Over time, the plant has undergone obvious evolutions, namely through various new characters (Bray, 1997; Wasson et al., 2021), and recently (Nikolaeva et al., 2010; Petrov et al., 2018) with significant resistance for drought (Fischer et al., 2005; Săulescu et al., 2006). Thus, wheat is considered one of the most diverse cereals from a genetic point of view (Kulkarni et al, 2017), it has types of autumn and spring, with coated grains but also bare. The genome is hexaploid 2n = 6x (6x7) = 42, with six sets of AuAuBBDD chromosomes. The diploid form 2n = 2x = 14 has 2x7 chromosomes, one from each parent, and through hybridization we have reached to the evolved forms tetraploid (2n = 4x = 28 chromosomes) and hexaploids. The variety of this new line is *erythrospermum* Körn, with white, awn spike, glabrous glume and red bean/ grain. It also contains the modern *RHt* gene, introduced by Borlaug (1960s) from the *Norin 10* variety of Japanese origin. It determines short stems, suitable for rich fertilization and mechanized harvesting, both necessary for new, very productive creations. The inflorescence of the plant is a terminal spike, distich, 4-18 cm long, with sessile spikelets, caught solitary on the rachis, in a zig-zag pattern. The spikelet is 10-15 mm long, being compressed laterally with two glume and several flowers. The glume is the tip like a short, blunt tooth, but also a 3-5 cm edge. Each flower has a palea and a lemma.

Depending on the variety, the lemma extends in the form of an awn, or as a hood. When the palea and the lemma adhere to the grain, it becomes so dressed. The bean (caryopsis) is ellipsoidal in shape, with a central channel on one side. The bean is 4-12 mm long and 1.5-4.0 mm thick. The mass of one thousand grains (MTG) is between 15-60 g. The plant generally forms stems with heights between 50 cm and 150 cm. The research carried out to observe the variation of some characters of the new wheat line plants in different water deficit conditions, included: the stem through the total length of the straw, the length and thickness of the basal internode (upper third), the sub-apical internode length and the apical internode length, length and weight of spike, number of spikelets / spike, length of outer glume, length of lower palea (lemma) and length of awns, number of grains / spike, weight, mass of one thousand grains (MTG) and grain size by length and thickness.

MATERIALS AND METHODS

The variants have been cultivated in the last three years with the new line A.50-15. The experience was set up according to the block method, with variants of 25 m^2 in 4 repetitions. The technology used was the one recommended by the station. At full maturity, 25 plants / strains from each repetition were randomly selected (100 in total), cut and brought to the laboratory. The 100 stems were measured and determined: total straw length, basal internode length and thickness, sub-apical internode length, apical internode length, spike length and weight, number of spikelets in spike, length of glume, palea and awn, the number of grains in an ear and their weight, the mass of one thousand grains (MTG), as well as the dimensions of the grains: length and thickness. The morphological characters obtained were analyzed by the method of histograms (or frequency

polygons, PF%). In their expression, the class intervals established according to the specific string of values obtained were used.

The study highlighted several aspects, namely: the highest frequencies, the limits of the intervals of variability of the studied characters and the specificity of each character of the wheat ecotype in the analyzed area. The correlations were established between the analyzed characters, with the help of which their tendencies within this ecotype could be observed. Excel was used to express values. The significance of the correlation coefficients was obtained by comparing with the r_{max} values (Weber, 1961) for the levels of 5%, 1% and 0.1% of the transgression probabilities. In the statistical calculation of all the values obtained, the analysis of variance (Anova test) on the variation strings was used. Statistical parameters were calculated using the formulas: $\bar{a} = \Sigma x / n$, where $\bar{a}$ = 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% (coefficient of variation) = $S / \bar{a}.100$.

RESULTS AND DISCUSSIONS

Climatic characterization of autumn wheat vegetation. In general, the cultivation area has a favorable climate throughout the beginning from vegetation to flowering (Figure 1 - Walter-Lieth climate-gram). Rainfall in the autumn should ensure optimal emergence and tillering. The rains that fall in the spring and until the wheat matures should be favorable, which would lead to the normal morphological manifestation of each genotype. On the other hand, this continuous favorable rhythm began to be more and more fragmented by periods, either with some excesses or with drought, sometimes quite prolonged. Under these conditions, each new cultivar reacts morphologically through certain trends. Knowing them could define certain degrees of tolerance and even the resistance of the respective line to the climatic accentuations (drought) that have been taking place lately (Farooq et al, 2014). On the other hand, the comparison between evaporation transpiration potential- ETP and the rainfall regime in the wheat vegetation highlights the possibilities that the plant had both in the formation of the plant habitus and the respective production elements (Figure 2).

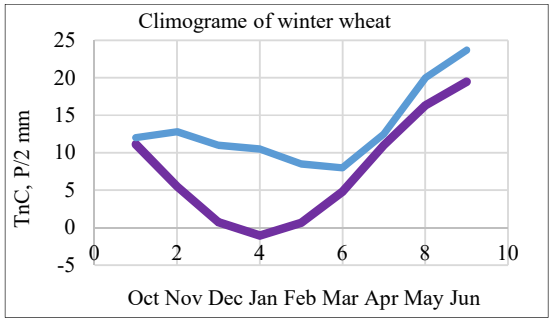


Figure 1. Climograma Walter-Lieth // Walter Lieth Clinograma

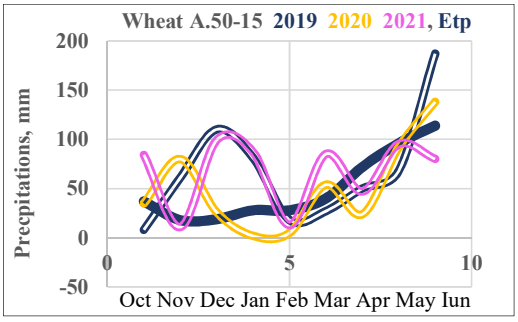


Figure 2. Precipitații anuale și valorile ETP// Annual precipitation and ETP values

As the volume of precipitation approached the necessary, for consumption (Fang & Xiong, 2015), the favorability of that year was more obvious. Of the three years analyzed, 2019 was the closest condition needed. The rich autumn precipitations favored on the one hand the passage of the first vegetation period of the wheat, and on the other hand there was the accumulation of an important reserve of water in the soil for the resumption of vegetation. An important peak was noted this year in June, which contributed to the formation of morphological features of the plant in general and of the ear in particular, namely at high levels. The year 2020 started well with rains in November, after which the drought set in April was also dry. When deposited in the grain, the A.50-5 line had precipitation closer to the required in May and June, which ensured a good level of grain formation. In the last year, the quantities of water that fell for the first period were closer than necessary and were maintained until March, after which the drought set in. Finally, lower amounts of water affected

the deposition of nutrients in the grains (Fischer et al., 2005). In conclusion, the first year of cultivation had better favorability, and the next two years had relatively lower degrees of favorability.

Variability in wheat straw size. The wheat stalk or straw consists of several internodes (5-7) with increasing lengths towards the ear. In general, the straw has lengths between 50 cm and 150 cm, with lower values for new creations obtained for intensification conditions. The measurements showed that in the three years of cultivation the straw at this new line of winter wheat was between 55 and 80 cm. The dominant frequencies were the straws of 60-65 cm, very close in the three years (Figure 3). Due to these dimensions of wheat straw, line A.50-15 can be considered to meet both the qualifier of evolved and intensive. The diameter of the basal internode had modal values between 3 and 4 mm, being thinner in the second year, average in the last year and thicker in the first year (Figure 4).

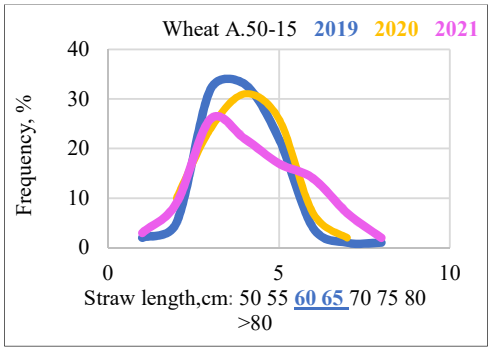


Figure 3. Straw length frequencies // Frecvențele lungimii paiului

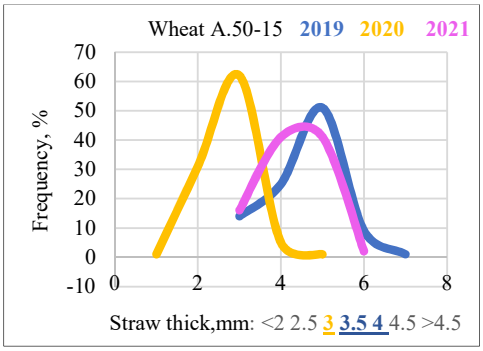


Figure 4. Straw thickness frequencies // Frecvențele grosimii paiului

Variability of wheat ears. The appearance and size of the ear of this wheat line are characteristic. Thus, its length had dominant values of 8 cm in years with insufficient rainfall and 8.5 cm in the first year (2019). In general, its variability was between 6.5 cm and 11.5 cm (Figure 5). The biomass of the analyzed ears was generally between 1.5 g and 4.5 g (Figure 6). The high values expressed weights of 2-2.5 g in the two years with drier periods and 4 g in the more climatically favorable year.

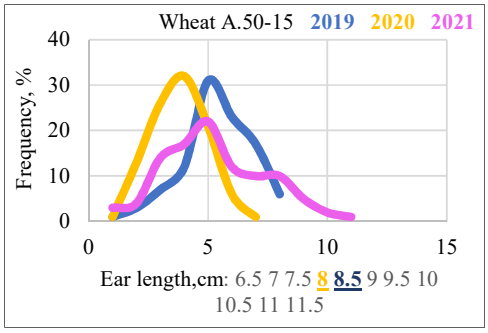


Figure 5. Ear lenght frecvency //Frecvențele lungimii spicului

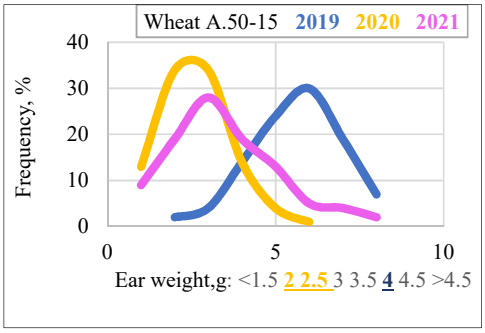
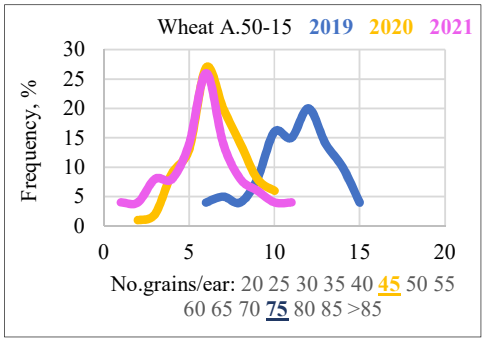


Figure 6. Ear weight frecvency // Frecvențele masei spicului

The ears formed grains depending on the climate favorability (Săulescu et al., 2006), expressed by the amounts of precipitation distributed during the nine months of vegetation. Their number had a relatively wide range, namely between 20 and over 85 from a spike of line A.50-15. The highest frequencies were obtained at 45 grains / ear in the last two years and 75 grains in the very favorable year (first year) (Figure 7). The weight of these grains generally followed the same mode of expression due to the different favorability of the three years of cultivation. The limits of variability over the whole research period were between 1 g and over 3.5 g (Figure 8).



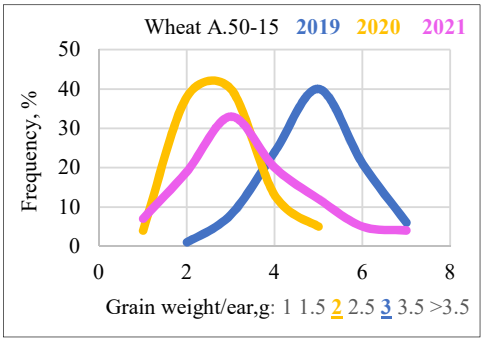
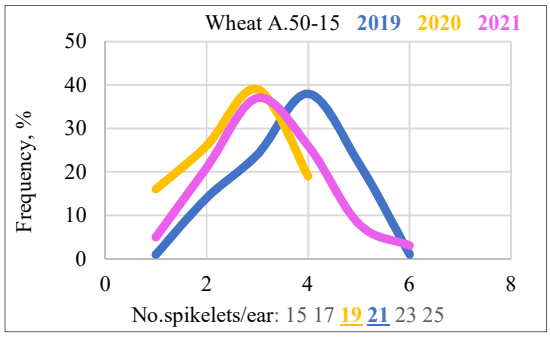


Figure 7. Frecvențele no.boabe/spic //No.grains/ear frecvency **Fgure 8Grain weight / ear frecvency**//Frecvențele masei boabelor/spic

They dominant ears had grains weight 2 g in the years 2020-2021 and 3 g in the more favorable year 2019. In the expression of these values of the number and weight of the grains a morphological importance had the number of spikelets formed in each ear of line A.50-15. Thus, in the three years, spikelets formed between 15 and 25 / ear. The high values were 19 spikelets in the last two years of culture and 21 spikelets in the first year (Figure 9). Among the pieces of the spike are the length of the awn, which could generally express a relatively specific character of the new lines obtained in the conditions of the research station. The limits between which the lengths of the awn were formed, were 4 and 11 cm (Figure 10). Dominant were the lengths of 7 cm in years with periods of drought and 8 cm in the most favorable year.

Variability in the size and absolute mass of wheat grains. The grain size also had some characteristics. Thus, the grain length was generally between 5 and 8 mm. In the favorable year 2019 the dominant grains measured 6.5 mm, while in the other two years the grains had dominated by lengths of 7 mm (Figure 11). The grain thickness was between the general limits of 2.4 mm and 3.8 mm (Figure 12) The modal value for the whole period was 3 mm grain thickness.

The mass of one thousand grains (MTG) showed slight differences depending on the favorability of the three years of cultivation. In general, the values ranged from 22 to 50 g (some grain biomass exceeded 50 g) (Figure 13). The dominant values were 38 g in the year with longer periods of drought (2020) and 42 g in the first and third agricultural year. The present data show that line A.50-15 forms grains with relatively high biomass, a positive premise for productive performance (figure 14).



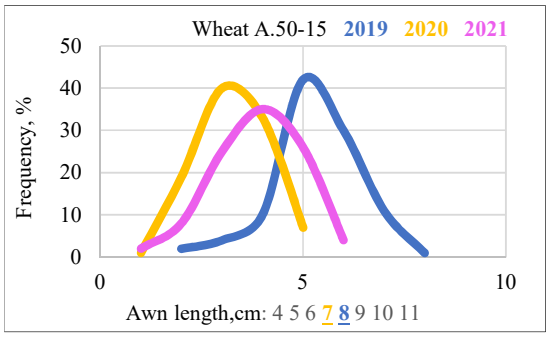


Figure 9. No. Spikelets/ear frecvency //Frecvențele no. de spiculețe/spic **Figure 10.Awn lenght frecvency**// Frecvențele lungimii aristei

Correlations between the main morphological characters. If we analyze the whole set of correlations between all the analyzed characters, we find both positive and negative situations. Very obvious positive correlations were observed between the characteristics of the spike: length, number of spikelets, number of grains, grain weight and the base diameter of the straw. Insignificant positive correlations were observed between the straw length and some characteristics of the production capacity per ear. The absolute mass of the grains was positively correlated with the weight of the ear and the grains, as well as with the dimensions of the grains (Table 1).

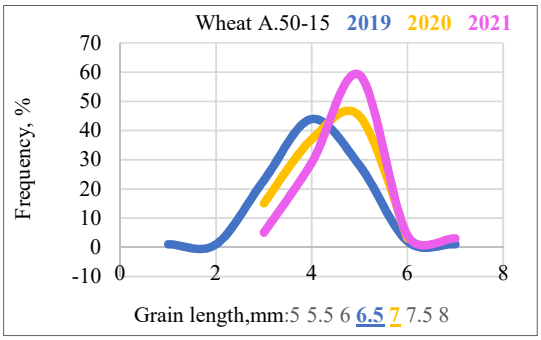


Figura 11. Grain lenght frecvency//Frecvențele lungimii bobului

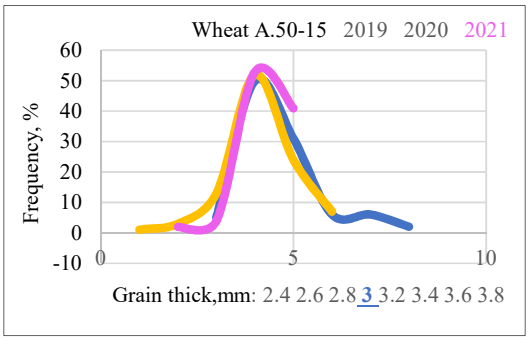


Figura 12. Geain thick frecvengy //Frecvențele grosimii bobului

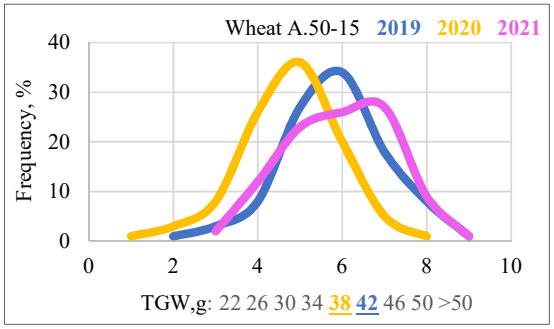


Figura 13. TGW frecvency//Frecvențele masei a o mie de boabe



Figura 14. Line A.50-15 flowering period // Linia A.50-15 perioada înfloritului

Statistical analysis of morphological characters variability at the wheat line A.50-15. The results obtained in the morphological analysis of some characters in winter wheat showed specificity. Thus, the length of the straw measured 62-64 cm. Variability demonstrated low to medium coefficients. The internode at the base measured 10-11 cm, with a small to large variation, and its thickness was 2.7-3.6 mm (10-11% variation). The sub-apical internode had an average length of 15-20 cm (2-14% variability), and the apical internode measured 22-32 cm (highly variable). These data show that this wheat line has a medium to small port, suitable for intensification.

Table 1. Correlations between the main characters of the new wheat line A.50-15 // Corelații între principalele caractere ale noii linii de grâu A.50-15

Caracterul	Basis int. cm	Sub-apical cm	Apical, cm	Basis ø, mm	Ear L. cm	Ear W. g	No. spikelets	Glume mm	Palea mm	Awn cm	No. grains	G. boabe	Grain L. mm	G.rain T. mm	MTG g
	2019														
Straw size cm	.425	.325	.342	.032	-.075	.041	-.028	-.252	.057	-.037	-.106	.102	-.008	.145	.372
Internode 3	1	.211	-.134	.332	.115	.973	.220	-.022	.207	.042	.258	.334	.192	.065	.170
Internode 2		1	.052	-.036	-.098	.008	-.228	-.151	-.022	.038	-.153	.031	-.017	.095	.305
Apical int.,cm			1	-.120	.077	-.001	-.090	-.118	-.096	-.020	-.121	.173	-.070	-.060	.258
Ø basis, mm				1	.450	.612	.543	.235	.302	.017	.642	.563	.150	.090	.003
Length ear, cm					1	.619	.615	.586	.146	.179	.696	.529	.163	.020	-.117
Weight ear, g						1	.628	.212	.205	.119	.867	.970	.139	.113	.334
No. spikelets							1	.193	.277	.094	.617	.547	.007	.080	-.238
Glume, mm								1	-.107	.256	.315	.132	.061	.080	-.258
Palea, mm									1	.245	.306	.154	.331	-.050	-.206
Awn, cm										1	.109	.068	.204	.005	-.036
No. grains											1	.797	.060	-.311	-.124
Grains weight g												1	.136	.168	.471
G. length, mm													1	.392	.305
G. thick, mm														1	.170
	2020														
Straw size cm	.521	.525	.288	.166	.078	.141	.135	.014	.005	.022	.093	.155	.205	.182	.204
Internode 3	1	.212	-.248	.062	.062	.116	.048	.010	.044	.048	-.119	-.132	.081	.010	-.074
Internode 2		1	0.442	.310	.297	.331	.202	.047	.051	.086	.168	.349	.336	.338	.449
Apical int.,cm			1	.182	.254	.368	.201	.200	.213	.195	.254	.368	.105	.230	.376
Ø basis, mm				1	.398	.426	.363	.310	.187	.214	.420	.449	.152	.308	.272
Length ear, cm					1	.654	.648	.311	.239	.297	.689	.656	.233	.287	.316
Weight ear, g						1	.711	.351	.327	.531	.873	.967	.457	.531	.610
No. spikelets							1	.339	.214	.412	.815	.676	.262	.392	.192

Caracterul	Basis int. cm	Sub-apical cm	Apical, cm	Basis ø, mm	Ear L. cm	Ear W. g	No. spikelets	Glume mm	Palea mm	Awn cm	No. grains	G. boabe	Grain L. mm	G.rain T. mm	MTG g
Glume, mm								1	**.590**	**.310**	**.412**	**.353**	**.197**	**.244**	**.130**
Palea, mm									1	.067	**.366**	**.326**	**.307**	.082	.141
Awn, cm										1	**.502**	**.509**	**.321**	**.325**	**.287**
No. grains											1	**.844**	**.307**	**.341**	**.237**
Grains weight g												1	**.440**	**.524**	**.709**
G. length, mm													1	**.491**	**.401**
G. thick, mm														1	**.525**
	2021														
Straw size cm	**.731**	**.455**	**.291**	.167	**-.264**	.054	-.047	-.055	.022	.091	-.049	.087	.128	**.247**	**.417**
Internode 3	1	.078	**-.195**	**-.224**	**-.439**	-.181	-.122	-.189	-.041	-.187	**-.194**	-.156	.010	.049	.069
Internode 2		1	.079	-.058	-.036	.088	-.044	.136	.123	.102	-.002	.104	**.225**	.030	**.269**
Apical int.,cm			1	.024	.033	.092	-.047	.028	-.022	.178	.005	.111	.078	**.198**	**.343**
Ø basis, mm				1	**.605**	**.557**	**.603**	**.352**	**.247**	**.343**	**.583**	**.536**	.107	**.229**	.133
Length ear, cm					1	**.776**	**.751**	**.393**	**.413**	**.485**	**.757**	**.745**	**.298**	.187	**.309**
Weight ear, g						1	**.865**	**.358**	**.361**	**.638**	**.935**	**.994**	**.283**	**.437**	**.568**
No. spikelets							1	**.338**	**.355**	**.606**	**.889**	**.841**	**.208**	**.270**	**.295**
Glume, mm								1	**.428**	**.201**	**.316**	**.341**	**.250**	.153	.152
Palea, mm									1	**.258**	**.348**	**.347**	.177	.103	.095
Awn, cm										1	**.633**	**.641**	.183	**.231**	**.395**
No. grains											1	**.935**	**.138**	**.365**	**.276**
Grains weight g												1	**.269**	**.456**	**.580**
G. length, mm													1	.089	**.424**
G. thick, mm														1	**.457**
	DI 5 % = .190							**DL 1 % = .250**			**DL 0.1 % = .320**				

Table 2. Statistical indexes of the straw and ears of the A.50-15 line// Indicii statistici ai paiului și spicelor liniei A.50-15

Indices*	Straw, internods					Ears		
	Length	Basal	Sub-apical	Apical	Ø base	Length	Weight	No. spikelets
	2019							
Mean	**62.24**	**10.81**	**17.28**	**29.69**	**3.61**	**8.47**	**3.57**	**19.88**
s²	112.9	4.70	6.93	322.2	0,130	0.57	0.42	4.45
s	10.62	2.17	2,64	17.95	0,370	0.76	0.65	2.11
VC %	**17.1**	**20.1**	**1.5**	**60.4**	**10.2**	**9.0**	**18.2**	**10.6**
	2020							
Mean	**63.49**	**11.09**	**15.03**	**21.66**	**2.67**	**7.75**	**2.10**	**17.79**
s²	30.66	2.32	2.23	2.85	0.08	0.45	0.29	3.73
s	5.54	1.52	1.49	1.69	0.285	0.67	0.53	1.93
VC %	**8.7**	**13.7**	**9.9**	**7.8**	**10.7**	**8.6**	**25.3**	**10.9**
	2021							
Mean	**64.20**	**9.91**	**19.67**	**32.01**	**3.48**	**8.50**	**2.56**	**18.95**
s²	58.7	19.62	7.03	17.1	0.145	1.35	0.85	5.99
s	7.66	4.43	2.65	4.14	0.38	1.16	0.92	2.45
VC %	**11.9**	**44.7**	**13.5**	**12.9**	**11.0**	**13.7**	**36.0**	**12.9**

S^2, variance, s- standard error, VC- variation coefficient

The length of the spike was 7.8-8.5 cm, with reduced to medium variability. The weight of the ears was 2.10-2.57 g, but with a medium to high variability. The number of spikelets/from ear was 18-20 (11-13% variability) (table 2). The length of the glume was 9-10 mm (8-10% CV), that of the palea (lemma) 9.93-9.96 mm with little variability, and that of the awn 6.3-8.3 cm (low-medium). The number of grains formed in a spike was 45-69 with a medium to high variability. The weight of the grains in a spike was 1.62-2.97 g, with medium-high variability. The grains had the average dimensions of 6.5-6.7 / 2.99-3.07 mm. The variability of grain sizes was small (less than 10%). The mass of one thousand grains was on average 35-40 g with variable variability (Table 3).

Table 3. Statistical indixes of spikelets and grains of A.50-15 line // Indicii statistici ai spiculețelor și boabelor liniei A.50-15

Indices*	Spikelet pieces			Grains				
	Glume	Palea	Awn	No./ear	Greutate	Length	Thick	MTG
	2019							
Mean	**10.21**	**9.96**	**8.31**	**68.81**	**2.97**	**6.76**	**3.07**	**38.86**
s²	1.040	21.72	0.36	128.9	0.27	0.18	0.01	53.24
s	1,020	4.67	0.60	11.36	0.52	0.43	0.10	7.30
VC %	**10.0**	**46.9**	**7.2**	**16.5**	**17.5**	**6.4**	**3.3**	**18.8**
	2020							
Mean	**9.31**	**9.93**	**6.26**	**45.08**	**1.62**	**6.50**	**2.99**	**35.24**
s²	0.54	0.68	0.78	75.3	0.18	0.17	0.03	22.03
s	0.73	0.82	0.88	8.68	0.42	0.41	0.18	4.69
VC %	**7.9**	**8.3**	**14.1**	**19.0**	**26.1**	**6.3**	**6.1**	**13.3**
	2021							
Mean	**9.03**	**9.95**	**6.88**	**49.08**	**1.95**	**6.72**	**3.01**	**39.8**

Indices*	Spikelet pieces			Grains				
	Glume	Palea	Awn	No./ear	Greutate	Length	Thick	MTG
	2019							
s^2	0.57	0.715	1.147	223.2	0.537	0.164	0.019	25.93
s	0.76	0.845	1.071	14.94	0.733	0.405	0.137	5.092
VC %	**8.4**	**8.5**	**15.6**	**30.4**	**37.1**	**6.03**	**4.5**	**12.8**

S^2, variance, s- standard error, VC- variation coefficient

CONCLUSIONS

1. The new winter wheat line, A.50-15, has recently been cultivated during three different climatic years: the first favorable year, the second favorable average year and the third unfavorable year. During these years the precipitation regime was different due to the periods of drought in the vegetation of the plants.
2. The morphological characters thus expressed were specific. Thus, the stem / straw had similar dominant lengths of 60-65 cm. At the height of the plant, the basal internode contributed by 10-11 cm, the sub-apical internode by 15-20 cm, and the apical one by 22-32 cm, with influence depending on the favorability of the culture year. The thickness of the basal internode was 2.7-3.4 mm, which shows a good resistance in the wheat field, especially in the favorable climate year. The 7.8-8.5 cm long spikelets weighed 2.1-3.6 g and is characterized as medium to long and heavy. The number of spikelets of 18-20, shows a density of 0.44 spicules at 1 cm length of the spike. Spikelets pieces: 9-10 mm jokes, 10 mm blades and 6-8 cm edge describe characters specific to the new line studied. The number of grains in an ear was from 45 in the dry year and 69 in the favorable year, weighing 1.62-2.97 g, highlighted a new line of wheat with high production capacity. The mass of a thousand grains was 35-40 g and is considered medium to large. The grains, measuring 6.5-7 mm long and 3 mm thick, describe average characters.
3. Simple correlations were established between all the studied characters, with some differentiations. The correlations between the characters of the ear were very significant positive, which demonstrates the great productive possibilities that this variety has even in the years with periods of drought. The characteristics of the straw were insignificantly correlated, sometimes negatively with the spike and the grains and are explained by the genetic improvement obtained by reducing the size of this variety.
4. The studied statistical indicators showed a new line of wheat with a small to medium port, with medium to long spikes, sufficiently compact, with many grains and with an absolute mass exceeding 42-44 g in climatically favorable years. over 50 ears of grain, weighing up to 3 g, that describe a new, productive variety with good valences and in new periods of drought.

THANKS

We hereby convey our sincere thanks to the entire wheat breeding team at the station. Through the perseverance they showed, they managed to obtain new varieties (Albota and Trivale) in a relatively short period of time, but also whole series of winter wheat lines characterized by tolerance to aluminum ions, characteristics of the special ecology of this type of soil. Here we mention the researchers Enescu Steluța, Popa Florica, Grigore Marian and Voica Maria, whom we thank for the effort and selflessness they showed in this improvement work. The front line, A.50-15 is one of these achievements.

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ANALYSIS OF STEEL TYPE INFLUENCE ON THE STRESS AND SAFETY PARAMETERS FOR THE CONSTRUCTION OF EQUIPMENT FRAMES FITTED WITH CURVED PROFILE DIGGING SPADES

ANALIZA INFLUENȚEI TIPULUI DE OȚEL ASUPRA PARAMETRILOR DE TENSIUNEA ȘI DE SIGURANȚĂ PENTRU CONSTRUCȚIA ECHIPAMENTELOR ECHIPATE CU CAZMALE PROFILATE CURBE

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Abstract:
To establish a new vegetable or cereal culture, a series of works must be carried out, the most important of which is plowing, also called the basic work of the soil, which, as it is carried out in the current technology, has many disadvantages, including the cost of the work, which implies a higher consumption of diesel and the negative effect on the structure of the soil, because the natural process of soil stabilization after plowing usually takes a few weeks. Under these conditions, within the framework of a project implemented under Measure 16: Cooperation, Submeasure 16.1: Support for the establishment and functioning of operational groups (GO), for the development of pilot projects, new products", an eco-innovative technology is promoted that performs the basic work of the soil in vegetable crops with a technical equipment provided with curved profiled plows. The construction of the curved profiled equipment frame was made from UNP (or "Universal NormalProfile"), INP (or "I NormalProfile"), L (or equal-wing angle) profiles following static analysis using SolidWorks software, with emphasis on von Mises stress and safety aspects analysis, for different grades of hot rolled steel: S235 / S275 / S355 according to EN10025. The analysis allowed us to better understand the 3D geometric model of the technical equipment frame's behavior depending on the demands it is subjected to and to verify the behavior of the chosen materials. The purpose of this work is to make available to specialists in the field, a quick tool for solving simple structural problems, without resorting to complex mathematical relationships that are characteristic of strength calculations.
Keywords: Static analysis, finite elements, stress von Mises, factor of safety.

Rezumat:
Pentru înființarea unei noi culturi de legume sau cereale trebuie efectuate o serie de lucrări, dintre care cea mai importantă este aratul, numită lucrarea de bază al solului, care, așa cum se realizează în tehnologia actuală, prezintă multe dezavantaje, inclusiv costul lucrării, care presupune un consum mai mare de motorină și efectul negativ asupra structurii solului, deoarece procesul natural de stabilizare a solului după arat durează de obicei câteva săptămâni. În aceste condiții, în cadrul unui proiect implementat în cadrul Măsurii 16: Cooperare, Submăsura 16.1: Sprijin pentru înființarea și funcționarea grupurilor operaționale (GO), pentru dezvoltarea de proiecte pilot, produse noi", a fost aplicată o tehnologie eco-inovatoare care efectueaza lucrarea de baza a solului în culturile legumicole cu un echipament tehnic prevăzut cu pluguri profilate curbate. Constructia cadrului utilajului profilat curbat a fost realizata din UNP (sau "Profil Normal Universal"), INP (sau "Profil Normal I"), profile L (sau unghi cu aripi egale), în urma analizei statice folosind software-ul SolidWorks, cu accent pe analiza von Mises și a aspectelor de siguranță, pentru diferite calități de oțel laminat la cald: S235 / S275 / S355 conform EN10025. Analiza ne-a permis să înțeleagă mai bine modelul geometric 3D al comportamentului cadrului echipamentului tehnic în funcție de solicitările la care este supus și să verifice comportamentul materialelor alese. Scopul acestei lucrări este de a pune la dispoziție specialiștilor în domeniu, un instrument rapid de rezolvare. probleme structurale simple, fără a recurge la relații matematice complexe care sunt caracteristice calculelor de rezistență.
Cuvinte cheie: Analiză statică, elemente finite, stres von Mises, factor de siguranță.

INTRODUCTION

The effects of ploughing on crop growth and soil properties need to be compared over a long period of time to determine their impact in the short and long term [8].

Today, farmers in Europe are adopting innovative technologies for field horticultural works, aiming mainly at more efficient, economical, less labor-intensive and more economically friendly management [4].

Due to the long-term use of conventional tillage (plowing) internationally, important cereal-growing regions are threatened by soil degradation [5]. At the same time, intensive agriculture, which is increasingly being practiced, accelerates greenhouse gas emissions and the loss of carbon (C) from the soil, especially with aggressive tillage [7].

It is assumed that the adoption of soil conservation works (working with the chisel, plow with spring-tooth and direct seeding) will restore soil fertility, maintain crop yields, and increase sustainability [1].

In these conditions, the authors of the paper propose a new soil working technology using a device equipped with rotary tines, which, when applied in exploitation, leads to the regulation of the productivity of arable lands and energy consumption, conservation of the upper layer of the soil against erosion, and increase of soil fertility [10].

MATERIALS AND METHODS

The research materials and methods consist of the use of the reading sheets of the research phases of the project ECO-INNOVATIVE TECHNOLOGY PROJECT THAT PERFORMS THE BASIC WORK OF THE SOIL IN VEGETABLE CROPS WITH TECHNICAL EQUIPMENT PROVIDED WITH PROFILED CURVED PLOWS supported by non-refundable European funds through the National Rural Development Program (PNDR), Measure 16-Cooperation, sM 16.1. Support for establishing and operating operational groups (GO), for developing pilot projects and new products, according to the financing contract no. C16100000011884200003/20.04.2021.

The SOLIDWORKS software was used for the modeling of the technical equipment frame provided with curved profiled spades, and for the static analysis with finite elements SOLIDWORK SIMIULATION, which are developed by SW Corporation - USA, part of the French company Dassault Systèmes - DS [11].

Using CAD software, the design was modeled during the design stage, along with material choices and physical attributes, and the results of the calculations performed were examined and verified by specialists in the final design stage before the physical frame of the technical equipment was executed.

RESULTS AND DISCUSSION

In Figure 1 is presented an aspect of the 3D geometric model of the technical equipment provided with curved profiled blades for the base work of the soil.

There are several possibilities for modeling mechanical systems containing metal structures in SOLIDWORKS. To create the frame of the technical equipment provided with curved profiled blades for the basic soil work, each entity was drawn separately, and then the entities were assembled using the Assemblies module. For each component, a supplementary information field could be completed containing the name of the respective component, who made it, and other observations. Figure 2 presents the 3D geometric model of the technical equipment frame designed with the help of the SOLIDWORKS program, which was made by assembling previously defined references, a method called top-down design (bottom-up).

After the stage of creating the 3D geometric model, we moved on to the stage of structural analysis with the help of the structural simulation application SOLIDWORKS SIMULATION, which involved importing the geometry of the created model, defining the material, defining the appropriate restrictions for the discretizations, running the program for calculating the Von Mises stress analysis, displacement, relative elongation, safety factor and visualization of the results in the form of diagrams [6].

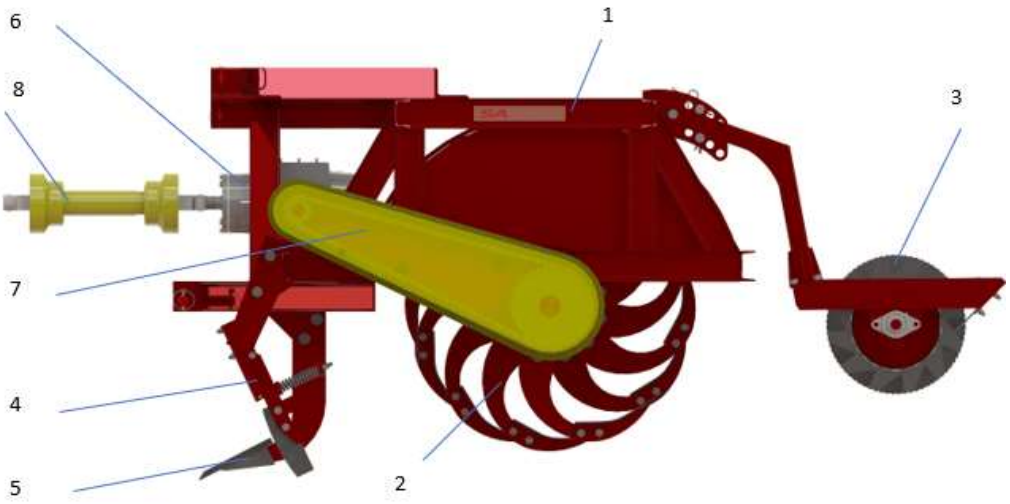


Fig. 1. Technical equipment provided with curved-profiled digging spades for basic soil work, left-side view// Echipament tehnic dotat cu lame de sapat profilate pentru lucrarea de bază a solului-, vedere din partea stângă //1-framework, 2- assembled spade rotor, 3-toothed roller Packer, 4- inclined knife assembly, 5- rippers with chisels, 6- conical reducer 132KW, i=0,55, 7- chain drive 16B-2, 8- cardan transmission T6-65CP // Echipament tehnic prevazut cu cazma de săpat cu profil curbat pentru lucrarea de baza a solului, vedere din stânga 1-cadru, 2- rotor de cazma asamblat, 3-dinți Roller Packer, 4- ansamblu cuțit înclinat, 5- cuțit / daltă, 6- reductor conic 132KW, i=0,55, 7- transmisie cu lanț 16B-2, 8- transmisie cardan T6-65CP

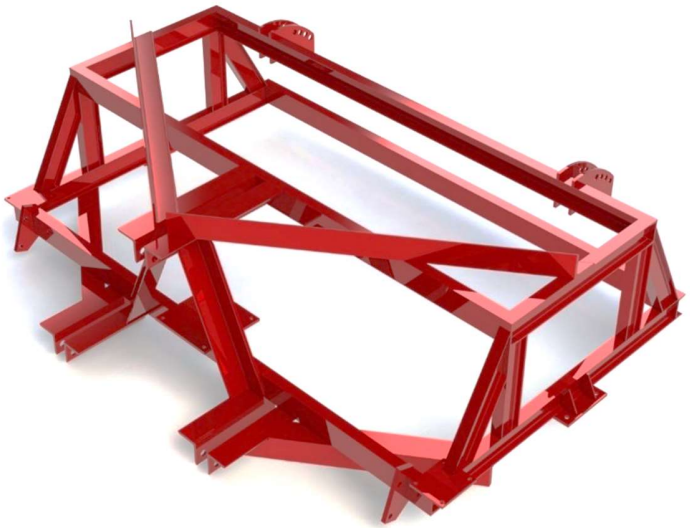
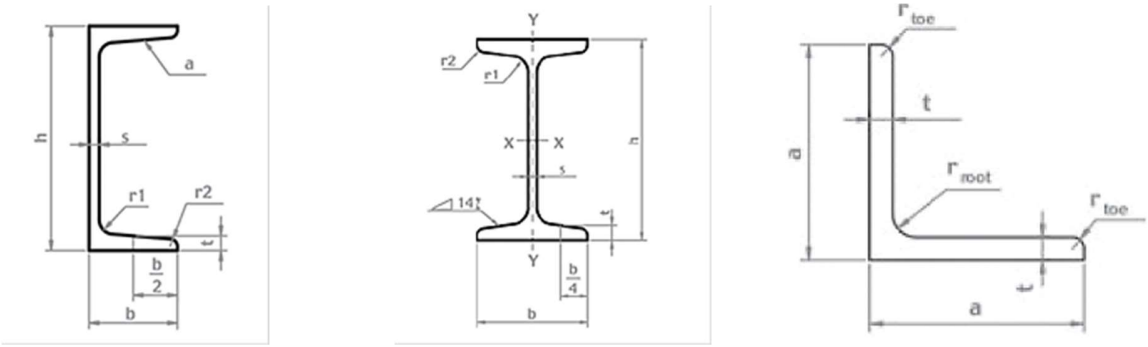


Fig. 2. 3D Geometric model of the technical equipment frame equipped with curved profile drills for basic soil work// Modelul geometric 3D al cadrului echipamentului tehnic echipat cazmale profilate curbe pentru lucrarea de bază al solului

The constructive optimization method involved the following operations:
- selecting the static option as the analysis type, solid for the discretization type and the FFEPlus solver [3].;
- The selection of some materials from the SolidWorks library and the automatic assignment of these properties to the main landmark, the side knife, which is the most subject to wear during use [2].

For any type of finite element analysis using SOLIDWORKS-SIMULATION, it is indispensable to assign a material. The UNP 100 (or "Universal NormalProfile"), INP100 (or "I NormalProfile"), L100 (or equal wing angle) profiles for different qualities of hot-rolled steel: S235JR / S275JR / S355J2G3 according to EN10025 were chosen for the construction of the equipment frame [9].

In Figure 3, the dimensions of the chosen metal profiles are presented.



a. UNP100
h=100 mm, b=50 mm

b. INP 100
h=100 mm, b=50 mm

c. L100
a=100 mm

Fig. 3. Metal profiles chosen for the construction of the equipment frame provided with curved profile spades for basic groundwork// Profile metalice alese pentru construirea cadrului echipamentului prevăzut cu cazmale profilate curbe pentru lucrări de baza ale terenului

For the linear static analysis of the frame, where the stresses and deformations of a loaded structure can be evaluated, the following characteristics were indispensable, as shown in Table 1.

Tabel 1 - Parameters for frame simulation modeling // Parametri pentru simularea modelării cadrului

Name:	**1.0037 (S235JR)**	**1.0044 (S275JR)**	**1.0570 (S355J2G3)**
Model type:	Linear Elastic Isotropic	Linear Elastic Isotropic	Linear Elastic Isotropic
Default failure criterion:	Max von Mises Stress	Max von Mises Stress	Max von Mises Stress
Yield strength:	2.35e+08 N/m^2	2.75e+08 N/m^2	3.15e+08 N/m^2
Tensile strength:	3.6e+08 N/m^2	4.1e+08 N/m^2	4.9e+08 N/m^2
Elastic modulus:	2.1e+11 N/m^2	2.1e+11 N/m^2	2.1e+11 N/m^2
Poisson's ratio:	0.28	0.28	0.28
Mass density:	7800 kg N/m3	7800 kg/m^3	7800 kg/m^3
Shear modulus:	7.9e+10 N/m^2	7.9e+10 N/m^2	7.9e+10 N/m^2
Thermal expansion coefficient:	1.1e-05 /Kelvin	1.1e-05 /Kelvin	1.1e-05 /Kelvin

- S235JR class steel is universal steel and for machine construction, manufactured in accordance with the EN 10025 standard, it can be welded without restrictions, without subsequent heat treatment and without heating;
- S275JR class steel is a structural steel, which meets the requirements of the EN 10025 standard, it can be welded without restrictions, without subsequent heat treatment and without heating;
- S355J2G3 steel is carbon manganese steel. It is characterized by excellent strength and weldability. S355J2G3 is unalloyed structural steel according to EN10025.
- the application of the appropriate load in accordance with the actual mode of operation (from operation), the simulation scenario was adapted accordingly. The load was applied at the points corresponding to the mode of operation;
- using the meshing procedure to decompose the model into discrete elements. In general, a finite element model is defined by a mesh, which is completely made of a geometric arrangement of elements and nodes. Nodes represent points, where characteristics, such as displacements, are calculated;
- running analysis study to calculate stress, factor of safety and displacement, which is based on geometry, material, load, constraint conditions and discretization type.

The 3D geometric model introduced directly into linear static structural analysis supported loads and supports, but the discretization could not be performed only after eliminating some interferences. After running the analysis studies, the results for configurations 1, 2 and 3 could be graphically visualized (Fig. 4, 5 and 6).

Equivalent von Mises stress and equivalent plastic strain show that von Mises stress and strain play a significant role in numerical simulations.

Table 2 presents the minimum and maximum values for the equivalent von Mises stress obtained from the static analysis for the three steel grades of the metal profiles.

Table 2 – The equivalent von Mises stress distribution / N/m²// Echivalentul distribuției stress-ului von Mises/N/m²

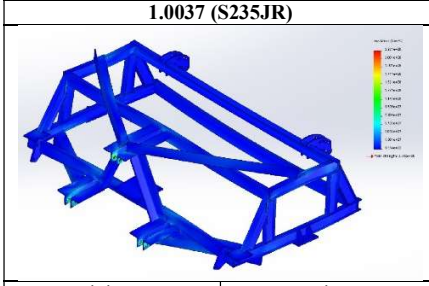
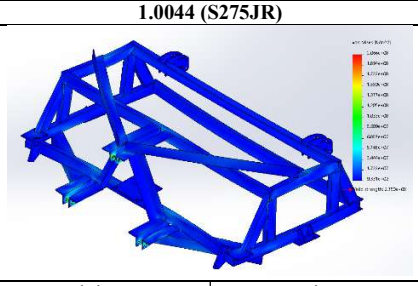
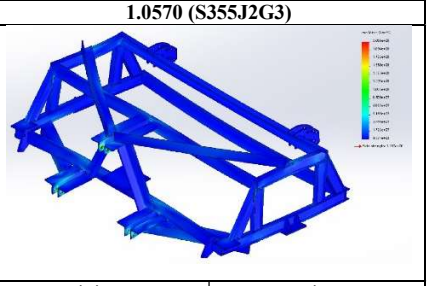
1.0037 (S235JR)		1.0044 (S275JR)		1.0570 (S355J2G3)	
					
Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
9.334e+02	2.281e+08	9.331e+02	2.066e+08	9.331e+02	2.066e+08

Table 3 presents the minimum and maximum values for the specific deformation intensity distribution obtained from the static analysis for the three steel quality variants of the metal profiles.

Table 3- The distribution of specific strain intensity / mm // Distribuția intensității de deformare specifică / mm

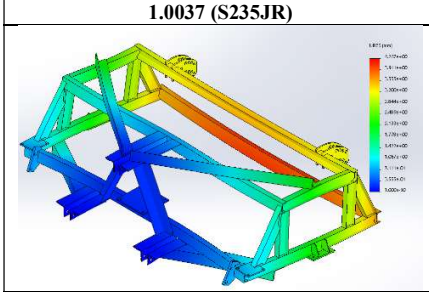
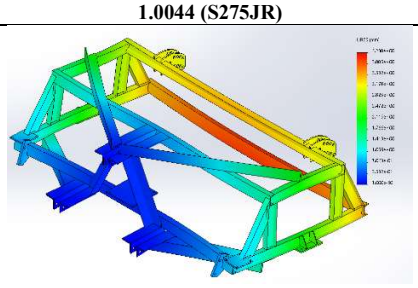
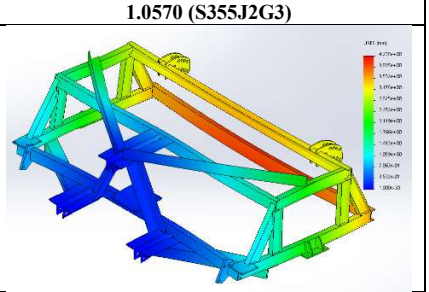
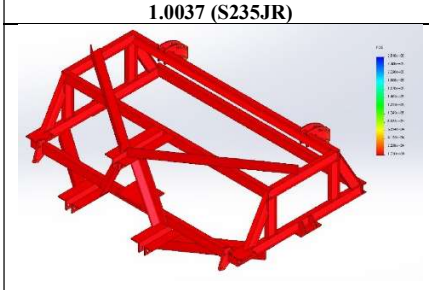
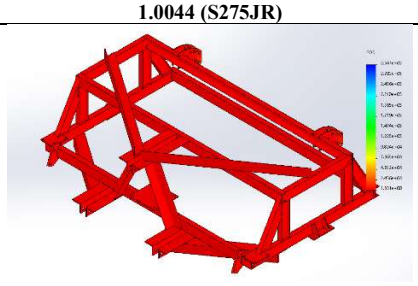
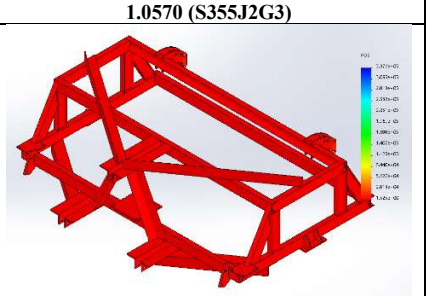
1.0037 (S235JR)		1.0044 (S275JR)		1.0570 (S355J2G3)	
					
Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
0.000e+00	4.267e+00	1.000e-03	4.238e+00	1.000e-03	4.238e+00

Table 4 presents the minimum and maximum values for the power factor distribution obtained from the static analysis for the three steel quality variants of the metal profiles.

Table 4- Power factor distribution //Distribuția factorului de putere

1.0037 (S235JR)		1.0044 (S275JR)		1.0570 (S355J2G3)	
					
Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
1.030e+00	2.518e+05	1.331e+00	2.947e+05	1.525e+00	3376e+05

The results of the analysis on technical-economic criteria for the choice of steel quality of the profiles UNP 100 (or "Universal NormalProfile"), INP100 (or "I NormalProfile"), L100 (or the angle with equal wings) from which the frame of the technical equipment equipped with shovels is manufactured curved profiles for the basic soil work are presented in table 5. The price offer for the profiles was obtained from the company BADUC Str. Vigoniei, no. 5-7, Sector 5, Bucharest.

Table 5- Results of the techno-economic analysis // Rezultatele analizei tehno-economice

Name	Unit of measurement	Configuration value		
		1.0037 (S235JR)	1.0044 (S275JR)	1.0570 (S355J2G3)
The power factor	-	1,030	1,331	1,525
Estimated price	lei / m²	175,94	182,41	192,64
The ratio: Price / power factor	-	170,81	137,79	126,32

The comparison of these indicators led to the choice of the optimal variant (configuration 3 was chosen, which has the lowest ratio Price / Safety Coefficient: 126.32).

The use of S355J2G3 rolled steel profiles for the frame of the technical equipment equipped with curved profile digging spades for basic soil work gives it properties with good mechanical strength, high toughness, very good hardness and a price/quality ratio based on the Win-Win concept of the customer-supplier relationship.

CONCLUSIONS

- Designing the technical equipment frame using hot-rolled metal profiles involved knowledge of the safe, durable, and economical specifications, including the materials, manufacturing technology, and geometric dimensions;
- The authors' proposed technical-economic indicator (material consumption per unit of safety coefficient) for the analysis of the optimal variant, represented by the price ratio and the safety coefficient, contributes to reducing the validation time of the design and manufacturing costs;
- The results of the research presented in the paper offer specialists in the field with a quick tool for solving simple structural problems, without resorting to complex mathematical relationships that are specific to resistance calculation.

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TECHNOLOGICAL SOLUTIONS FOR GRASSLAND IMPROVEMENT IN FAGARAS COUNTRY AREA

SOLUȚII TEHNOLOGICE PENTRU ÎMBUNĂTĂȚIREA PAJIȘTILOR DIN ZONA ȚARA FĂGĂRAȘULUI

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Rezumat:
În lucrare sunt prezentate soluții tehnologice și echipamente tehnice pentru îmbunătățirea pajiștilor permanente prin renovare totală dintr-un dispozitiv experimental al Institutului de Cercetare-Dezvoltare pentru Pajişti Braşov, amplasat în comuna Drăguş, județul Braşov (o locație reprezentativă pentru zona Ţara Făgăraşului). De asemenea, sunt scoase în evidență producţiile şi calitatea furajelor obţinute pe parcursul a 3 ani , 2016, 2017 și 2018, diferențele în producția de substanţă uscată și calitatea furajelor, în cei trei ani între variantele îmbunătățite prin reînsămânțare (V2 şi V3) și varianta martor (V1) fiind distinct sau foarte semnificative. Rezultatele prezentate în această lucrare și interpretarea statistică a acestora arată justețea punerii în practică a tehnologiilor zonale de îmbunătățire prin resămânțare totală, atât din punct de vedere al valorii nutritive, dar și al producției furajelor obținute, comparativ cu varianta martor.

Cuvinte cheie: îmbunătățire, pășuni, tehnologie, reînsămânțare totală, calitate furaj

Abstract:
The paper presents technological solutions and technical equipment for improving the permanent grasslands by total renovation from an experimental field of the Research-Development Institute for Meadows Brasov, located in Drăguş village, Brasov County (a representative location for Ţara Făgăraşului area). Also, are highlighted the productions and the quality of the fodder obtained during 3 years, 2016, 2017 and 2018, the differences in the production of dry matter and the quality of the fodder, in the three years, between the improved variants by reseeding (V2 and V3) and the control variant (V1) being distinct or very significant.
The results presented in this paper and their statistical interpretation show the good reason of the implementation of zonal improvement technologies by total reseeding, both in terms of nutritional value and feed production, compared to the control variant.

Key words: improvement, grassland, technology, total reseeding, feed quality

INTRODUCTION

The Fagaras Depression, also known as "Tara Oltului" is a contact depression, being considered the largest and best individualized among all the contact depressions of Transylvania. It is bounded to the south by the Fagaras Mountains, and to the north by the Olt River and the Transylvanian Plateau. The other limits that outline the surface of this territory are the Persani Mountains to the east and the Transylvanian Plateau, which arches, approaching the mountains, in the vicinity of Olt River from Red Tower, to the west.

The reseeding of permanent grasslands with valuable species and mixtures of perennial grasses and legumes is the main solution to improve and intensify the production of degraded and poorly productive grasslands.

Reseeding the degraded surfaces with mixtures of forage plants is the simplest and most economical measure to combat soil erosion and improve its physical-chemical properties.

From the analysis of the data related to the main agrochemical characteristics of the soil, it is observed that one of the limiting factors of production and quality, predominant in the Brasov area, is soil acidity.

On these soils, ameliorative works to correct its acidity are recommended, carried out before or simultaneously with the operations to improve permanent degraded pastures by total renovation.

Therefore, in the experimental device in the Fagaras (Dragus) Depression, the categorically operation included in the improvement technology is liming, using different assortments and amounts of amendments depending on the soil reaction.

MATERIAL AND METHOD

The experimental assembly of Research-Development Institute for Grasslands , located in UAT(Territorial Administrative Unit) Drăguș (a representative location for Făgăraș Depression), consists of a demonstrative lot (approximately 27 ha) and an experimental field. It allows the dissemination of research results over a relatively large area, located in the same stationary area conditions.

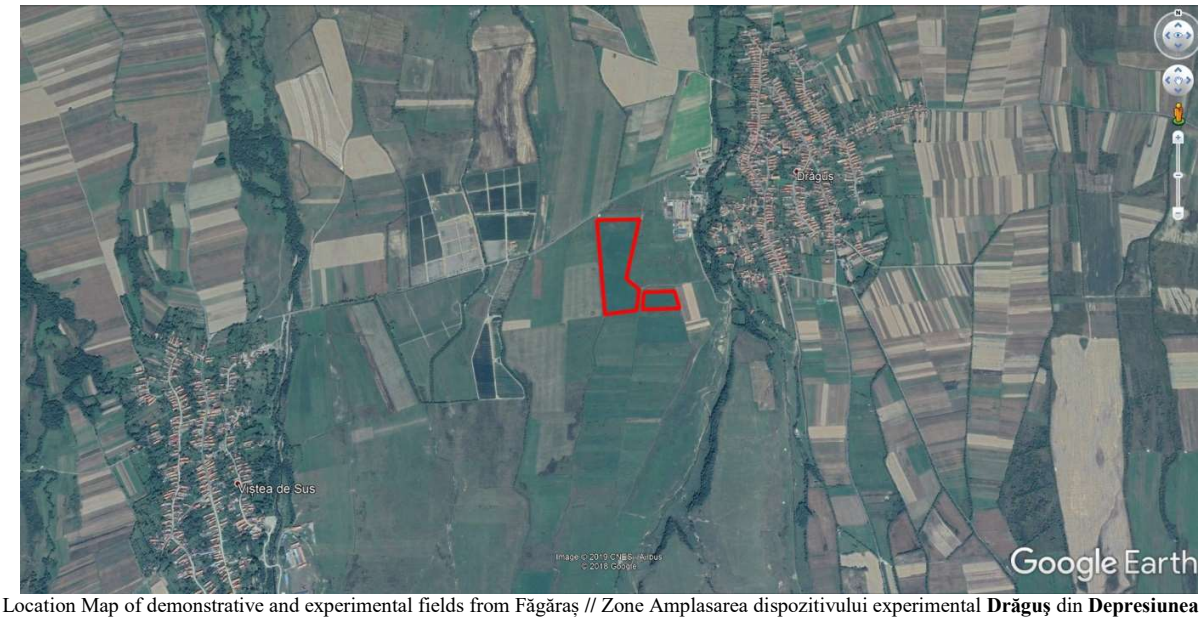


Fig. 1 Location Map of demonstrative and experimental fields from Făgăraș // Zone Amplasarea dispozitivului experimental **Drăguș** din **Depresiunea Făgăraș**

Location: UAT Drăguș
Latitude: 45o 44' 05.66" ... 45o 44' 07.22" N
Longitude: 24o 46' 00.47" ... 24o 46' 30.13" E
Altitude: 516 ... 541 m

The main agrochemical properties of the soil in the Dragus experimental field are presented in table 1.

Table 1. The main agrochemical soil properties of experimental field Drăguș // Principalele însușiri agrochimice ale solului din câmpul experimental Drăguș

No.	Sampling depth	pH (H_2O)	Al	Ah	SB	V_{ah} (%)	HUMUS %	NITROGEN INDEX	P-AL	K-AL
		me per 100 g soil							ppm	ppm
1	0...10 cm	4,8		10,5	4,8	31,3	11,84	3,70	10,8	90,0
2	10...20 cm	4,9		10,0	4,0	28,5	7,87	2,24	7,4	36,0

From the data presented in table 1, it results that the soil on which the experimental field is located, in the 0...10 cm profile, is strongly acidic, poorly supplied in P and medium supplied in K, and in the 10...20 cm zone it is strongly acid, very poorly supplied in P and poorly supplied in K.

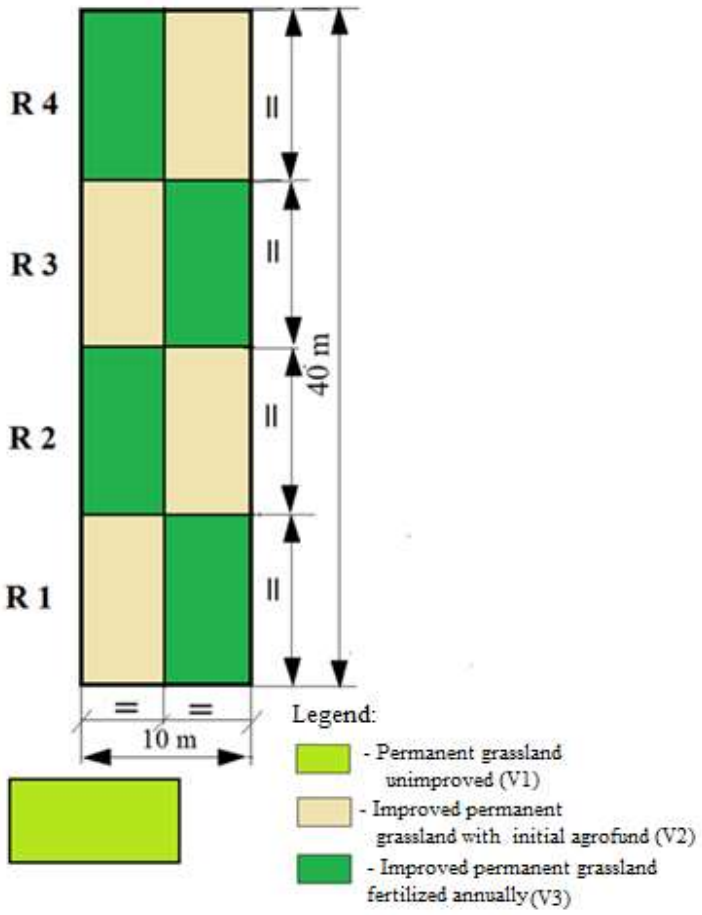


Fig.2 Schematic diagram of the experimental field Drăguş – Făgăraş Depression// Schema amplasării câmpului experimental Drăguş - Depresiunea Făgăraş

The experiment was established in the spring of 2016, on a surface with a slope between 0...2%, with three variants (Fig. 2), in four repetitions:

V 1- Unimproved permanent grassland;

V 2- Permanent grassland improved by total renovation, limed with 3.5 t/ha and fertilized with 30...35 t/ha of manure in the previous year;

V 3- Permanent grassland improved by total renovation, limed with 3.5t/ha and fertilized with 30...35 t/ha of manure in the previous year; fertilized in the years of operation with NPK 50 complex chemical fertilizers; 50;50;50 kg/ha in spring).

For the Dragus experimental device, a suitable mixture for a mixed hay-pasture mode of exploitation was established, composed of:

Grasses: *Festuca pratensis 19%;*
Festuca arundinacea 23%;
Dactylis glomerata 19%;
Phleum pratense 12%.

Legumes: *Trifolium pratense 16%;*
Trifolium repens 5%;
Lotus corniculatus 6%.

A gross seeding rate of 32 kg/ha was used, representing an average of 28 kg/ha of the mixture with a cultural value of 100%.

The technological sequences carried out (Fig. 3) for the total renovation consisted of:

- autumn plowing **(a);**
- harrow plowing with disc harrow (**b**);

- liming with 4.5t/ha of CaCO3 amendment (**c**);
- preparing the seed bed with a disc harrow, 2 passes (**d**);
- rolled before sowing (**e**);
- sown with the grain seeder (**f**);
- rolled after sowing (**g**);
- annual fertilization, in the spring, with NPK 50 complex chemical fertilizers; 50; 50;50 kg/ha (**h**).



Fig. 3 Aspects with the technological sequences from Drăguș demonstrative- experimental field // Aspecte cu secvențele tehnologice din dispozitivul experimental Drăguș



Fig. 4 Detail view of the grassy carpet in the demonstration field Drăguș, used in haymaking system // Vedere a covorului ierbos din lotul demonstrativ Drăguș, valorificat în regim de fâneață

In Fig. 4 is shown an aspect with the grassy carpet from the Drăguș demonstrative plot, used as haymaking.

RESULTS AND DISCUSSION

To determine the climatic characteristics of the Drăguș area, Brasov county, the data from the Făgăraș meteorological station (table 2), belonging to the National Meteorological Administration, the only one serving this territory, were used.

Table 2. Geographical location of Făgăraș meteorological station // Localizarea geografică a stației meteorologice Făgăraș

Station name	Latitude	Longitude	Altitude
Făgăraș	$45^0 50^i$ N	$24^0 56^i$ E	428 m

The analysis of the climatic parameters was carried out on the basis of those parameters that could have consequences, either positive or negative, on the socio-economic activities in the region or the productivity of the lands. The data were compared with multi-year averages for each parameter.

In the first part of the 2015...2016 agricultural year, conditions were less favorable from the point of view of the water regime, against the backdrop of a winter with quantitatively reduced solid precipitation and a snow cover that was maintained only two months, a fact reflected in the process phenologically, but especially the productivity of both in grasslands and agricultural crops. Compared to the previous year, the vegetation period is characterized by a moderate thermal regime and low amounts of precipitation, which contributed to the development of the plants in hostility conditions.

For the Dragus area, it can say that the 2016...2017 agricultural year was good from a thermal point of view, allowing the successful practice of agricultural activities, both on pastures and other crops, especially since the thermal values in the cold season were moderate, except for the months of December and January, with negative minimums below -20^0C. However, they influence productivity according to another climatic parameter, namely precipitation.

Referring to precipitation, it can be noted that, compared to 2015...2016, an agricultural year with a water deficit, the 2016...2017 agricultural year has a moderate water regime and in some months a surplus (for example May), a fact that contributed to the development of crop plants in good conditions and the increase of average productivity per ha compared to the previous year.

The agricultural year 2017...2018 was good from a thermal point of view, allowing the successful practice of grassland cultivation and other agricultural crops, especially since the thermal values in the cold season were moderate.

From the point of view of precipitation, the agricultural year 2017...2018 was a pluviometric surplus, the lowest amounts of precipitation were recorded in August in Făgăraș (10.2 l/m^2), and in April in Bâlea Lake (35.3l/m^2); the highest amounts of precipitation were reported in the months of June and July. Thus, it registered record amounts of precipitation (Făgăraș station in June 334.9 l/m^2; at Bâlea Lake, the amounts exceeded 800 l/m^2 in the same interval).

We can practically talk about a first normal pluviometric period of the 2017-2018 agricultural year and a second pluviometric surplus.

The production of dry matter obtained in the Drăguș experimental field, during the period 2016...2018, on the three variants, is presented in table 3. It recorded values between 4.18 (variant V1 in 2018) and 14.68 t/ ha SU (variant V3 in 2017).

In 2016, variants V2 and V3 achieved production increases in dry matter of 33% and 35%, respectively, compared to the control plot V1.

Table 3. Dry matter yield realized in Drăguș experimental field in 2016-2018 period, t/ha DM // Producția de substanță uscată obținută în câmpul experimental Drăguș în perioada 2016-2018, t/ha SU

Variant	Year		
	2016	2017	2018
V1	6,72	6,22	4,18
V2	8,95	9,56	8,02
V3	9,10	14,68	10,05

In 2017, V2 and V3 had increases in dry matter production of 53% and 136%, respectively, compared to V1, the control plot, unimproved permanent grassland. Comparing the two improved grassland variants, V2 and V3, V3, the annually fertilized variant, shows a 54% increase in production. In 2018, variants V2 and V3 achieved increases in dry matter production of 92% and 142%, respectively, compared to V1, the control variant. Comparing the two improved grassland variants, V2 and V3, on V3, the annually fertilized variant, a 26% increase in production was achieved.

Table 4 shows the production of dry matter and nutritional parameters, for the years 2016, 2017 and 2018, at variants V1, V2 and V3 from the experimental field Drăguș - Făgăraș geographical plateau. It is observed that both variants, V2 and V3, compared to the control variant V1, show statistically assured differences for the crude protein content, as follows: in 2016, the differences are very significant, and in 2017, the differences are significant.

Table 4. Quality parameters of forages from the Drăguș experimental field // Parametrii nutritivi (% din SU) ai furajelor din Câmpul experimental Drăguș

Year	Variant	DM [t/ha]	CP [%]	CA [%]	CF [%]	ADF [%]	ADL [%]	NDF [%]	DMD [%]	OMD [%]
2016	V1-cp	6.72	8.8	6.9	38.8	42.3	5.7	58.3	44.2	40.4
	V2	8.95**	15.2***	9.6***	33.4^{000}	38^{00}	4.9	50.7^{00}	61.8***	58.1***
	V3	9.10***	16.3***	9.7***	32.6^{000}	38.8^{0}	5.2	52.4^{0}	63.2***	58.0***
2017	V1-cp	6.22	14.0	10.5	34.0	38.9	5.6	59.6	52.8	53.0
	V2	9.56***	16.1*	10.6	30.7^{00}	34.9^{0}	6.3	50.5^{00}	55.7	49.5
	V3	14.68***	15.8*	10.1	31.9	36.5	6.4	54.3^{0}	54.1	48.0
2018	V1-cp	4.18	13.0	9.2	34.2	35.4	5.5	55.0	61.5	50.6
	V2	8.02***	14.1	9.7	32.3	35.1	4.9	56.3	61.6	54.4
	V3	10.05***	14.0	10.1*	31.3^{0}	38.0	5.5	60.2^{0}	59.4	52.5
LD 5 %		1.26	1.52	0.86	2.19	3.01	1.02	4.69	4.47	5.22
LD 1 %		1.72	2.13	1.20	3.07	4.22	1.43	6.57	6.27	7.33
LD 0.1 %		2.34	3.01	1.70	4.34	5.97	2.02	9.29	8.87	10.35

LD 5 % = 5.22 LD 1 % = 7.33 LD 0.1% = 10.35

DM- Dry Matter; CP- Crude Protein; CA- Crude Ash; CF- Crude Fiber; ADF-acid detergent fiber; ADL-acid detergent lignin; NDF-neutral detergent fiber; DMD-dry matter digestibility; OMD-organic matter digestibility.

Table 5 shows the results of the Student test (factor A-variant, factor B-year), carried out on the productions obtained in the Drăguș experimental field.

Table 5. Student test results from experimental field Drăguș, Făgăraș Country// Rezultatele testului Student din câmpul experimental Drăguș

Factor B – Year	Factor A – variant	Dry Matter, DM		Differences [t/ha]		Significations
		t/ha	%			
b1 – 2016	a1 – V1	6.72	100	-		
	a2 – V2	8.95	133.2	2.23		**
	a3 – V3	9.10	135.4	2.38		***
b2 – 2017	a1 – V1	6.22	100	-		
	a2 – V2	9.56	153.7	3.34		***
	a3 – V3	14.68	236.0	8.46		***
b3 – 2018	a1 – V1	4.18	100	-		
	a2 – V2	8.02	191.9	3.84		***
	a3 – V3	10.05	240.4	5.87		***

LD 5 % = 1.26 t/ha; LD 1 % = 1.72 t/ha; LD 0.1 % = 2.34 t/ha

From the data presented in table 5, it can be seen that both the V2 and V3 variants show significantly higher production increases than the control variant, V1, regardless of the experimental year, as follows: V2 variant - each experimental year shows distinctly significant increases (between 33 .2% and 91.9%), and for Variant V3, regardless of the experimental year, the increase is very significant (between 35.4% and 140.4%).

CONCLUSIONS

1. The degraded grasslands, located in the Făgăraș Depression, can be improved by total renovation by carrying out some improvement operations that will diminish or annihilate the effect of the

limiting factors of the production and quality of fodder, using mixtures of grasses and legumes of perennial fodder, suitable for the type of exploitation, promoting biodiversity conservation and environmental protection.

2. In the Drăguș experimental field, the differences in dry matter production and forage quality, in these three years 2016, 2017 and 2018, between the variants improved by reseeding (V2 and V3) and the control variant (V1) are distinct or very significant, a fact that justifies the full application of these technological sequences.
3. The location of the experimental assembly had as a criterion that the obtained results could be extrapolated over an area as large as possible, characterized by similar stationary area conditions.

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RESEARCH ON THE BEHAVIOR OF SOME TRITICALE VARIETIES UNDER THE CONDITIONS OF A.R.D.S. SECUIENI BETWEEN 2017-2022

CERCETĂRI PRIVIND COMPORTAREA UNOR SOIURI DE TRITICALE ÎN CONDIȚIILE DE LA S.C.D.A. SECUIENI ÎN PERIOADA 2017-2022

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Abstract

The triticale species is recognized for its utilization of soils less suitable for wheat cultivation, high adaptability to climate and soil conditions, high yields and resistance to diseases and pests.

At A.R.D.S. Secuieni the Romanian triticale varieties and lines were monitored to establish every year their adaptability. The new triticale varieties tend towards high yield, making very good use of less favorable areas for cultivation.

The varieties Zaraza, Tulnic, Utrifun and Haiduc were distinguished by the high yields in optimal growing conditions, while the varieties Oda FD and Tulnic were distinguished by the stability of yields in the pedoclimatc conditions of Central Moldova.

Key words: triticale, climate change, yields, stability, adaptability

Rezumat

Specia triticale este recunoscută datorită valorificării solurilor mai puțin prielnice culturii grâului, a adaptabilității mari la condițiile de climă și sol, a producțiilor mari și a rezistenței la boli și dăunători.

La S.C.D.A. Secuieni se testează an de an cultura națională cu soiuri și linii de triticale românești în vederea stabilirii adaptabilității acestora. Noile soiuri de triticale tind spre producții ridicate, valorificând foarte bine și zonele mai puțin favorabile de cultură.

Soiurile Zaraza, Tulnic, Utrifun și Haiduc s-au remarcat prin producții mari în condiții optime de cultură, în timp ce soiurile Oda FD și Tulnic s-au remarcat prin stabilitatea producțiilor în condițiile pedoclimatice din Centrul Moldovei.

Cuvinte cheie: triticale, schimbări climatice, producții, stabilitate, adaptabilitate

INTRODUCTION

Yield stability is a problem that interests all agricultural specialists and must be an objective of agricultural research, paying special attention to areas with less favorable climatic conditions (Săulescu, 1984).

There has always been a concern for the renewal of cereal varieties, they are becoming more and more productive, being more adapted to the climatic conditions. According to data from the specialized literature, their yields have increased linearly since the 1960s, especially in areas with favorable conditions where the availability of water in soil was not limited and where the cultivation technology was adapted according to the demands of the variety (Zhou and et al., 2007; Fischer and Edmeades, 2010; Matus et al., 2012). Also, the cereal yield has increased a lot in the last decades and in areas where precipitation has been limited (Mediterranean areas), a fact that is due to the increased performance under water stress of the newly created varieties (Nouri et al., 2011; Sánch - García et al., 2013; Hawkesford et al., 2013).

Climate changes in the last period of time have accentuated these extreme variations, with serious consequences on agricultural yield (Săulescu et al., 2006). The physical and chemical properties of the soil (high content in clay, low permeability, low pH, high content of free aluminum ions), associated with the climatic factors that are so different from year to year, cause significant fluctuations in the cereals yields. In this context varieties react differently depending on the ability to adapt to the pedoclimatic condition of the area (Voica, 2009).

The triticale species, through the characteristics inherited from rye: crop good establishment, fast growth and development even at lower temperatures, both in autumn and early spring, allow it to develop a fairly deep early root system, which facilitate them to use the water from the deeper layers of the soil much more efficiently, compared to other cereals (Ittu et al., 2007).

Among cereal crops, triticale is one of the great accomplishments by research in the field of theoretical and applied genetics and which has wide potential possibilities for increasing the yield per surface unit (of products for human nutrition and animal feed), especially in areas with conditions of culture less favorable for other cereals (Ittu et al., 1986, 2001; Ittu and Săulescu, 1988, 2000).

The strong interactions between genotype and environment, in the breeding process, contribut to the varieties creation with specific adaptability to favorable and unfavorable climatic conditions (Tessemma et al., 1998).

The nutritional value of the triticale grain is mostly given by the increased content in protein substances that exceeds the wheat content, as well as the structure of essential aminoacids and especially by the richer content in lysine. Nutritional value, the high digestibility of carbohydrates and protein substances give priority to triticale seeds in feeding non-ruminant animals, pigs and birds. Research carried out in this regard has shown that the protein-energy ratio is generally higher in triticale feeds than in traditional concentrates (Brouwer, 1977). The strong growth rate in the first development phases and the rich vegetative mass of the plants compete with the weed growth that are suppress, thus contributing to clearing the land of weeds (Gaşpar and Butnaru, 1985).

The identification of more valuable varieties than the existing ones is a major objective in the breeding of agricultural species, it being known that the variety participates directly in increasing yield (Leş and Oproiu, 1987).

This paper presents results regarding the yield obtained by some triticale varieties based on multi-year testing in comparative cultures in order to expand the most adapted genotypes for the area of Central Moldova.

MATERIAL AND METHOD

The research was carried out between 2017-2022 at A.R.D.S. Secuieni and followed the behavior of ten varieties of winter triticals, created by N.A.R.D.I. Fundulea (Haiduc, Cascador F, Negoiu, Pisc, Zori, Zvelt, Oda FD, Utrifun, Tulnic and Zaraza), in the pedoclimatic conditions of Central Moldova. The comparative culture was placed according to the method of randomized blocks, in three repetitions, without repeating the basic scheme. The experimental plot had a harvestable surface of 8 m^2.

The placement of the experiments was carried out on a cambic chernoziom soil type, with a pH in water of 6.26; the humus content of 1.46; nitrogen parameter – 17.8 ppm; mobile phosphorus - 36 ppm; potassium – 185.9 ppm. Basic fertilization was done in autumn with NPK were 36 kg N/ha and 92 kg P_2O_5/ ha and in the spring were administered 67 kg N/ha.

The ten varieties studied were analyzed in each of the five years, both from the point of view of yields capacity and morphophysiological characters.

In the experiments, the crop technology specific to the area was applied. The data obtained were processed and statistically interpreted according to the variance analysis method, and the coefficient of variability was determined to assess the stability of the yield (Ceapoiu, 1968).

The unit's area of influence has recently been experiencing an increase in average monthly temperatures due to the decrease in precipitation, and studies on the adaptability of crops to the new environmental conditions are needed.

In the last five years we are facing a continuous increase in average monthly temperatures compared to the multi-year average.

The 2019/2020 year was characterized as the warmest, of the last five years studied, and the warmest months were January and February, months that record deviations of +3.2°C and +3.8°C, respectively, compared to the multi-year average (Figure 1) .

Regarding the rainfall regime, the area of influence of the unit faces a continuous lack of rainfall, starting from 2017/2018 year, when the rainfall that fell in June (140.4 mm) and July (137.7 mm) restored the deficit accumulated up to that date, but starting from August 2018, rainfall has considerably reduced, registering at the end of the year a deviation from the multi-annual average of 6.0 mm. The rainfall deficit increased from one year to the next, so in 2021/2022 year, the amount of rainfall registered from September 1, 2021 to August 31; 2022 was of 260.8 mm, approximately half of the multi-year average of 531.9 mm (Figure 2).

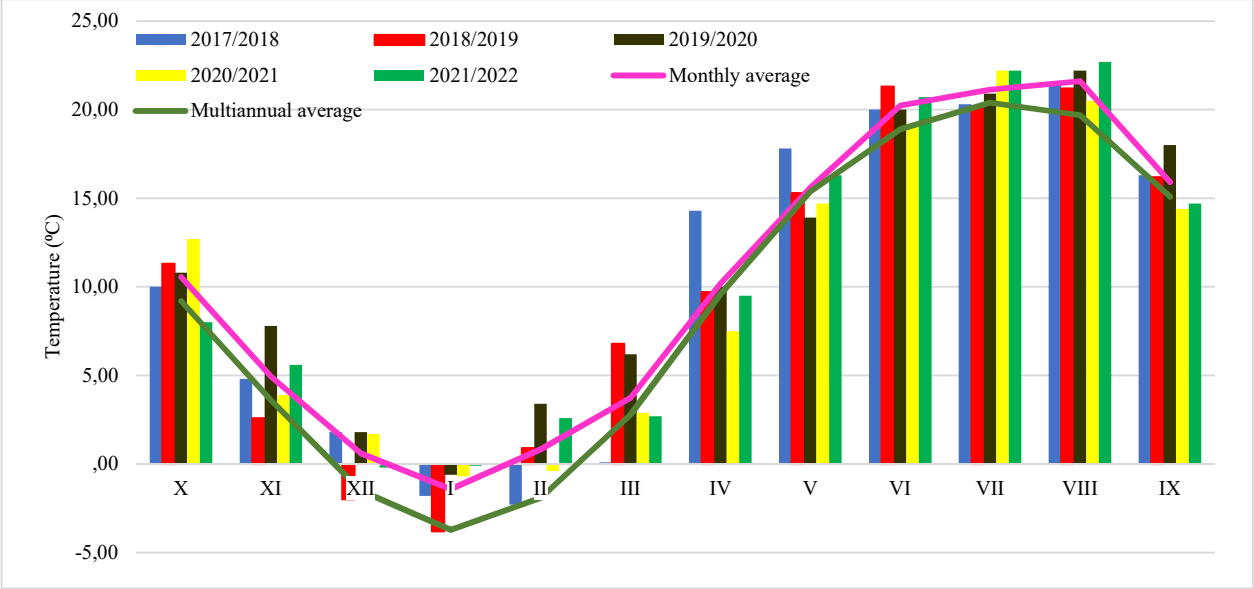


Figure 1. Average temperatures recorded between 2017-2022 at A.R.D.S. Secuieni and the multi-year average (1962-2022) // Temperaturile medii înregistrate în perioada 2017-2022 la S.C.D.A. Secuieni și media multianuală (1962-2022)

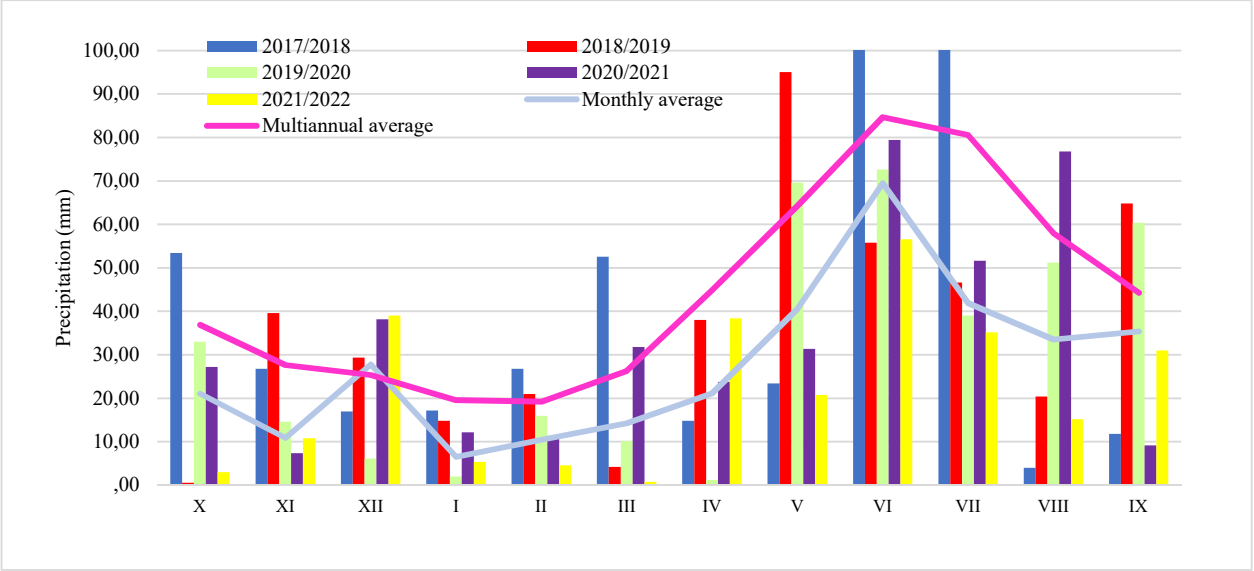


Figure 2. Average rainfall recorded between 2017-2022 at A.R.D.S. Secuieni and the multi-year average (1962-2022) // Precipitațiile medii înregistrate în perioada 2017-2022 la S.C.D.A. Secuieni și media multianuală (1962-2022)

RESULTS

The yields obtained at the ten varieties of triticale were influenced by the amount of rainfall recorded during the vegetation period and the thermal regime.

On average, in the five years studied, the Haiduc variety stands out for a high number of grains/ear (43.6), being a variety with a large ear, of a matte white color, with a pyramidal aristade and with a nutant position at maturity (Table 1).

The weight of grains/ear is a yield parameter influenced by the variety, the applied technology and also the climatic conditions. Under the conditions of A.R.D.S. Secuieni, the varieties Haiduc and Utrifun recorded the highest weights of grains/ear, of 2.06g and 2.03g, respectively (Table 1).

The mass of one thousand grains recorded average values between 40.29 (Zori) and 47.39 (Negoiu) and the hectoliter mass recorded values between 72.90 (Haiduc) and 78.30 (Zaraza). According to the wheat grading plan from C.N.G.S.C. (2017) the Zaraza variety falls into grade 1, with HM > 77 kg/hl, the Tulnic, Utrifun, Zori and Zvelt varieties fall into grade 2 (HM <77, >75) and the other 5 varieties fall into grade 3 (HM < 5,>72) (Tab. 1).

Table 1. The average values of some yiled indices recorded in the period 2017-2022 at A.R.D.S. Secuieni // Valorile medii ale unor indici de productivitate înregistrați în perioada 2017-2022 la S.C.D.A. Secuieni

Varieties	Number of grains/ear	Grains weight/ear (g)	TGW (g)	HM (kg/hl)
Haiduc (Ct.)	43.6	2.06	44.78	72.90
Negoiu	39.6	1.72	47.39	73.16
Oda FD	39.2	1.75	44.38	73.56
Pisc	39.3	1.78	46.13	73.76
Tulnic	37.5	1.69	44.80	76.00
Cascador F	36.5	1.54	42.32	74.36
Utrifun	40.2	1.69	43.60	75.52
Zori	42.9	1.70	40.29	75.92
Zvelt	41.5	1.87	45.84	76.34
Zaraza	42.7	2.03	47.22	78.30

In the first year of experimentation, triticale varieties recorded yields with values between 6636 kg/ha (Pisc) and 8807 kg/ha (Zaraza). The varieties Tulnic, Utrifun, Zori and Zaraza obtained statistically significant increases in yield and interpreted as very significant.

In 2018/2019 year, the yields obtained were between 5813 kg/ha (Cascador F) and 7117kg/ha (Pisc) (Tab.2).

The 2019/2020 year was characterized as a very hot and dry year. The triticale was sown in a dry soil, and the lack of precipitation in April (only 1.2 mm was recorded in the second decade) led to the drying of the brothers, thus recording crop losses. The control variety - Haiduc recorded the highest production (8683 kg/ha), being characterized as an earlier variety, stable, adapted and productive in the conditions of the Central area of Moldova (Pochișcanu et al. 2016).

The Zaraza variety stood out in 2020/2021 year, registering an increase in yields statistically interpreted as very significant. The Utrifun variety recorded an average yield of 9089 kg/ha, registering an increase in yield statistically interpreted as distinctly significant and the Zvelt variety obtained an average yield of 8296 kg/ha, the increase in yield being statistically interpreted as significantly positive (Table 2).

Of the five years studied, the 2021/2022 year was the driest, with negative precipitation deviations throughout the triticale vegetation period. The increases in yields were statistically interpreted as very significant and were recorded for the varieties Tulnic, Utrifun and Zaraza. The varieties Oda FD, Cascador F, Zori and Zvelt obtained increases in yield that were statistically interpreted as distinctly significant (Tab. 2).

On average, in the five years studied, the climatic conditions recorded were very different and the varieties Tulnic, Utrifun and Zaraza surpassed the control variety-Haiduc (Table 2).

Table 2. The yiled of some triticale varieties registered at A.R.D.S. Secuieni in the period 2018-2022 and their comparison with the control Haiduc // Producțiile unor soiuri de triticale înregistrate la S.C.D.A. Secuieni în perioada 2017-2022 și comparația acestora cu martorul Haiduc

Varieties	Yields (kg/ha)					
	2017/2018	2018/2019	2019/2020	2020/2021	2021/2022	Average
Haiduc (Ct.)	7105	6584	8683	7000	4949	6864
Negoiu	7139	6962	6904°°°	5408°°	4086°	6100
Oda FD	7054	6541	7070°°°	6908	6108**	6736
Pisc	6636	7117	6939°°°	5590°	4763	6209
Tulnic	8172***	6474	6753°°°	7293	6657***	7070
Cascador F	7018	5813°	5572°°°	5012°°	6260**	5935
Utrifun	8373***	6180	4679°°°	9089**	6552***	6975
Zori	8234***	6781	4964°°°	6630	6004**	6523
Zvelt	7304	6461	4706°°°	8296*	6209**	6595
Zaraza	8807***	6175	6689°°°	11221***	7119***	8002

Varieties	Yields (kg/ha)					
	2017/2018	2018/2019	2019/2020	2020/2021	2021/2022	Average
LSD 5%	484	734	680	1077	718	739
LSD 1%	687	1043	967	1531	1021	1050
LSD 0.1%	995	1510	1400	2217	1478	1520

Two of the varieties analyzed (Oda FD and Tulnic) can be characterized as having a small variation in yields (CV<10). High coefficient of variation (>20), determined by large variations in yield from one year to the next, were recorded by the varieties Negoiu, Cascado F, Utrifun and Zaraza, which in optimal growing conditions achieve very high yields, but which in drought conditions do not adapts equally well (Figure 3).

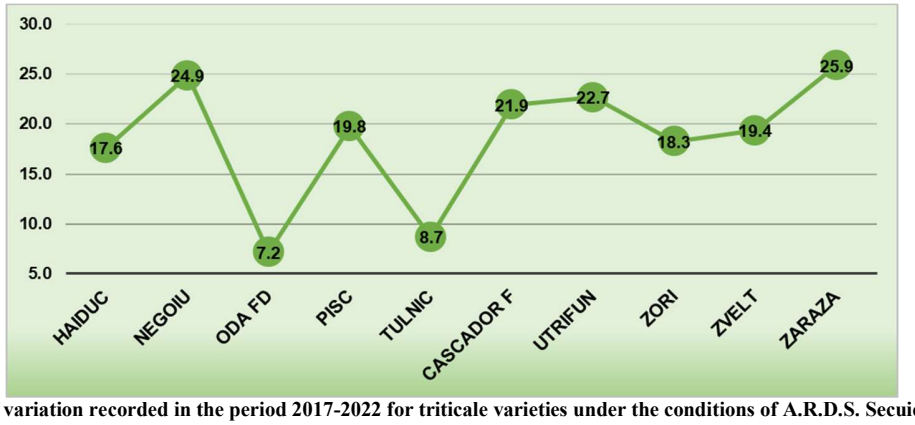


Figure 3. The coefficients of variation recorded in the period 2017-2022 for triticale varieties under the conditions of A.R.D.S. Secuieni // Coeficienții de variație înregistrați în perioada 2017-2022 la soiurile de triticale în condițiile de la S.C.D.A. Secuieni

Classifying the varieties according to the yield performances obtained at A.R.D.S. Secuieni, the first place is occupied by the Zaraza variety (8002 kg/ha) and the last place is occupied by the Cascador F variety (5935 kg/ha). The high fluctuation of yields was determined primarily by the different response of the varieties to the very different climatic conditions during the five years of testing (Figure 4).

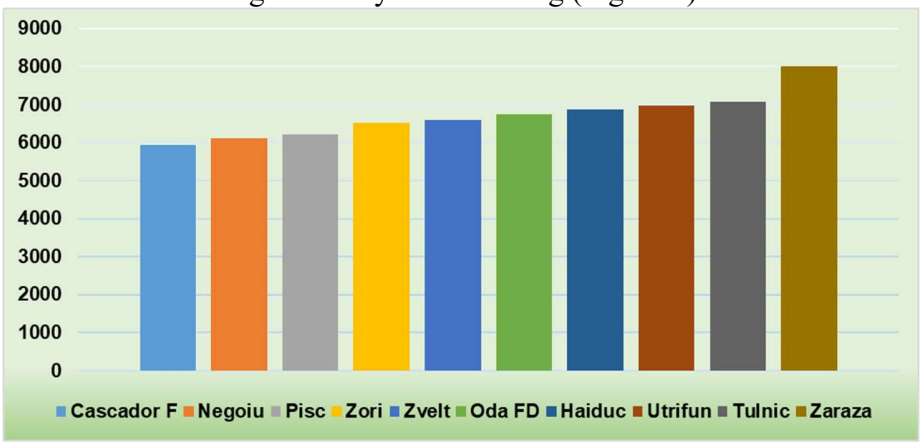


Figure 4. Classification of triticale varieties according to average yield obtained in the period 2017-2022 at A.R.D.S. Secuieni // Clasificarea soiurilor de triticale după producțiile medii obținute în perioada 2017-2022 la S.C.D.A. Secuieni

CONCLUSION

1. The 2017-2022 period was characterized by very large fluctuations in rainfall and temperatures, as a result of which triticale yields varied from one year to another.
2. The average yields obtained at triticale varieties were between 5935 kg/ha, obtained by the Cascador F variety and 8002 kg/ha, obtained by the Zaraza variety.
3. The varieties Oda FD and Tulnic showed the highest adaptability to pedoclimatic conditions, and the varieties Zaraza and Negoiu showed the lowest adaptability.

4. It is recommended to cultivate varieties with wide adaptability to the environmental conditions in order to reduce the risks of decreased yield in unfavorable years.

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RESEARCH ON THE BEHAVIOR OF SOME ROMANIAN VARIETIES OF SOYBEAN, UNDER PEDO-CLIMATIC CONDITIONS AT A.R.D.S. SECUIENI, IN THE PERIOD 2018-2022

CERCETĂRI PRIVIND COMPORTAMENTUL UNOR SOIURI ROMÂNESCĂ DE SOIA, ÎN CONDIȚII PEDO-CLIMATICE LA SCDA SECUIENI, IN PERIOADA 2018-2022

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Abstract
During 2018-2021 in the experimental field of the ARDS Secuieni (Agricultural Research and Development Station) were experienced a number of fifteen soybean varieties (Onix, Eugen, Felix, Darina, Cristina, Mălina, Carla TD, Larisa TD, Caro TD, Ilinca TD, Bia TD, Ada TD, Teo TD, Miruna TD and Felicia TD) creation of the ARDS Turda, in order to establish their adaptability to the pedo-climatic condition of the area. The varieties adapted well to A.R.D.S. Secuieni in the pedo-climatic conditions obtaining, in average yields of 2172 kg/ha. The results showed a hight adaptability to varieties Eugen(2.380 kg/ha) and Larisa TD (2.356 kg/ha).

Key words: soybean, adaptability, yield*.*

Rezumat
În perioada 2018-2021 în domeniul experimental al SCDA (Stația de Cercetare-Dezvoltare Agricolă) au fost experimentate un număr de cincisprezece soiuri de soia (Onix, Eugen, Felix, Darina, Cristina, Mălina, Carla TD, Larisa TD, Caro TD, Ilinca TD, Bia TD, Ada TD, Teo TD, Miruna TD și Felicia TD) crearea SCDA Turda, pentru a le stabili adaptabilitatea la condițiile pedo-climatice ale zonei. Soiurile s-au adaptat bine la S.C.D.A. Secuieni în condițiile pedo-climatice obținându-se producții medii de 2172 kg/ha. Rezultatele au arătat o mare adaptabilitate la soiurile Eugen(2.380 kg/ha) și Larisa TD (2.356 kg/ha).

Cuvinte cheie: soia, adaptabilitate, producție.

INTRODUCTION

Soybean is a cultivated herbaceous plant belonging to the Fabaceae, Fabioideae botanical family, subfamily, *Glycine L*. genus, the most important species of this plant is *Glycine max.* L. synonym *Glycine hispida* (Moench.) Maxim.

Etymologically, the word "soy" comes from Dutch, and is an adaptation of the term "shoyu" (soy sauce), which comes from Japanese, a word that in turn derives from the Chinese language "jiàngyóu".

The plant itself is native to East Asia, where it represents, moreover, an important culture with a rich tradition. Some sources state that around 2853 BC, the legendary Emperor of China, Shennong, proclaimed five plants as sacred: rice, wheat, barley, millet and soybean.

The species is indispensable in the food of Asians who nickname it "meat without bones" or "chinese beef", but which entered Europe only at the beginning of the 1700 AD, and in the United States in 1765 AD.

The true virtues of soy were discovered outside of Asia only at the beginning of the 1900's.

Today, soybean is a valued and demanded crop worldwide due to its ecological plasticity (Cealac and Budac, 2013).

In Romania in the last four years, the cultivated area with this species has an upward rhythm, in 2020, reaching 174 610 ha, this is the direct cause of the soybean breeding programs that aim to create early, productive varieties with stable production and acclimatized to the culture climate (Mureșan et al., 2014).

The present paper presents the results obtained after multi-year experiments in pedo-climatic conditions specific to A.R.D.S. Secuieni of some soybean genotypes patented by A.R.D.S. Turda for the purpose of expansion in the zonal culture of those with high adaptability.

RESEARCH MATERIAL AND METHOD

The experience were carried out during the agricultural period between 2018-2022 and aimed at adapting fifteen Romanian soybean varieties to the pedo-climatic conditions specific to Central Moldova, namely: Onix, Eugen, Felix, Darina, Cristina, Mălina , Carla TD, Larisa TD, Caro TD, Ilinca TD, Bia TD, Ada TD, Teo TD, Miruna TD and Felicia TD.

The placement of the comparative crops in the field was made according to the randomized block method, in 3 repetitions, where the experimental plot had a harvestable area of 10 square meters.

Pedology of the experimental field of A.R.D.S. Secuieni is characterized by the cambic phaeosium soil type, with a medium texture, slightly acidic (pH 6,14), with a low humus content (2,3%), being a poorly fertile soil, poorly supplied with nitrogen (0,134% Nt) , but with a considerable content in phosphorus (74 ppm) and potassium (221 mg/kg) in forms accessible to plants.

In the field, the crop's technology specific to the cultivation area in the Center of Moldova was followed, and the data obtained were processed and statistically interpreted according to the method of variation analysis (Jităreanu, 2016).

From a climatic point of view, the period 2018–2022 was characterized as hot and very dry. During the soybean growing seasons, the deviation from the multiannual average of temperatures was between 0,4 °C (2020/2021) and 1,5 °C (2021/2022), being characterized as normal and warm.

Table 1.Average temperatures recorded at ARDS Secuieni// Temperaturile medii înregistrate la SCDA. Secuieni

Year	Temperature (^{0}C)					The average of the vegetation period	+/-	Charact.
	V	VI	VII	VIII	IX			
2018	17,8	20,0	20,3	21,5	16,3	19,2	1,4	☼
2019	15,3	21,3	20,1	21,2	16,2	18,8	1,0	☼
2020	13,9	20,0	20,9	22,2	18,0	19,0	1,2	☼
2021	14,7	19,2	22,2	20,5	14,4	18,2	0,4	N
2022	16,3	20,7	22,2	22,7	14,7	19,3	1,5	☼
Multianual average	*15,4*	*18,8*	*20,4*	*19,5*	*15,0*	*17,8*	_	_

N = normal; ☼ = warm;

Regarding the rainfall regime, in the period from the sowing of the soybean crop to its physiological maturity, the deviation from the multi-year average of the rainfall recorded values between -21.5 mm (2018/2018) and -180, 1 mm (2021/2022), so that these vegetation periods were characterized as dry (2018/2018), respectively very dry (in the rest of the years experienced).

Table 2. The rainfall regime recorded at ARDS Secuieni// Regimul de precipitații înregistrat la SCDA Secuieni

Year	Rainfall (mm)					Σ_{pp} / vegetation period	+/-	Charact.
	V	VI	VII	VIII	IX			
2018	23,4	140,4	137,8	4,0	11,8	317,4	- 21,5	D**
2019	95,0	55,8	46,6	20,4	64,8	282,6	- 56,3	VD***
2020	69,6	72,6	39,0	51,2	60,4	292,8	- 46,1	VD***
2021	31,4	79,4	51,6	76,8	9,2	248,4	- 90,5	VD***
2022	20,8	56,6	35,2	15,2	31,0	158,8	- 180,1	VD***
Multianual average	65,7	85,0	82,3	60,2	45,7	338,9	-	

*D = Dry; VD*** = Very dry

The important aspect that must be emphasized is that in July 2020, in the Secuieni area, the layer of cumulus-nimbus clouds (Cb) generated the hailstorm that plagued the agricultural sector, soybean production being greatly reduced, being at the level of 31% of the production of 2019, respectively 29% of the production of 2018.

Also, the year 2021 was marked by the storms that occurred from the end of June to the second mid of July, characterized by strong wind gusts and whirlwinds, but which did not lead to a decrease in production of grains to this crop.

OBTAINED RESULTS

Among the productivity elements studied are number of pods/plant, number of grains/plant, weight of grains/plant, hectolitre weight and thousand kernel weight.

Regarding the number of pods/plant, during the experimental period the Felix variety stands out with an average number of 37 pods/plant, at the opposite pole are the Teo TD and Miruna TD varieties with an average number of 27 pods/plant (fig. 1).

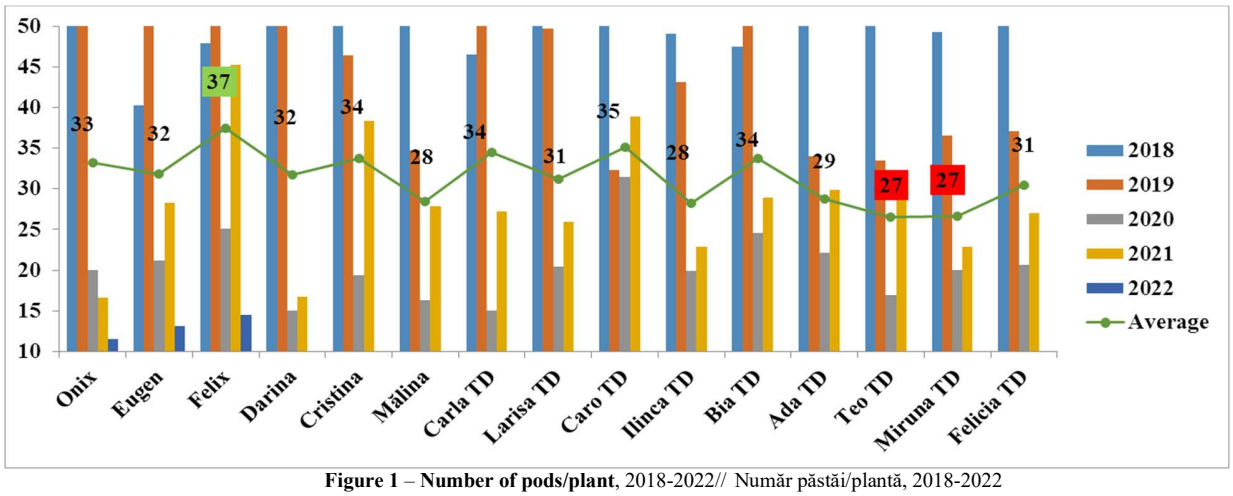


Figure 1 – **Number of pods/plant**, 2018-2022// Număr păstăi/plantă, 2018-2022

The number of grains/plant oscillated within large limits, on average having values between 47 grains (Teo TD variety) and 86 grains (Felix variety) (fig. 2).

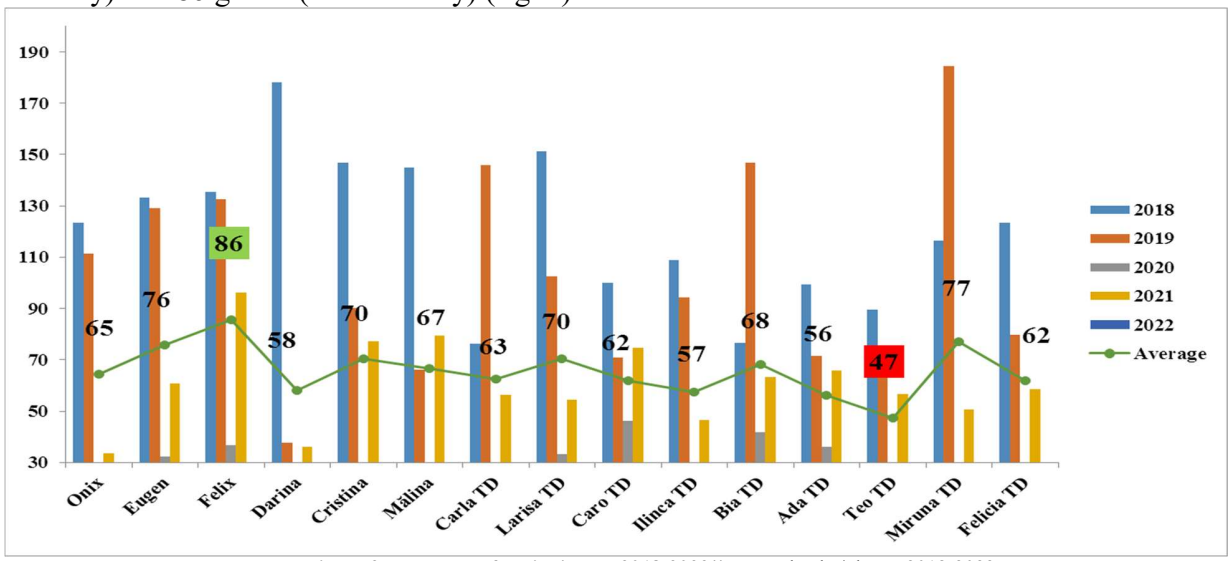


Figure 2 – Number of grains/plant, 2018-2022// Număr boabe/plantă, 2018-2022

The average weight of grains/plant, a quality index directly proportional to the number of pods/plant and the number of grains/plant, also recorded the highest average value (15,4 g) in the Felix variety, twice more than in the case of the average value obtained for the Teod TD variety (7,7 g) (fig. 3).

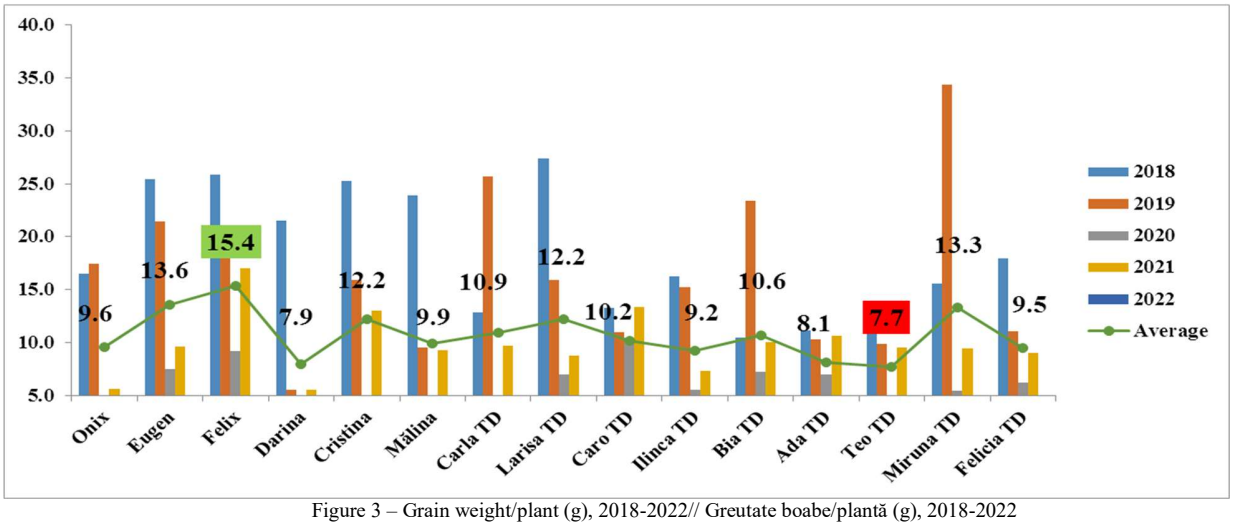


Figure 3 – Grain weight/plant (g), 2018-2022// Greutate boabe/plantă (g), 2018-2022

In the 2018-2022 agricultural period, the Darina variety stands out with the highest average hectolitre weight of 76,4 kg/hl, followed by the Miruna TD and Felicia TD varieties (76,2 kg/ha). For this quality index, the lowest average value was 74,7 kg/hl (Carla TD variety) (fig. 4).

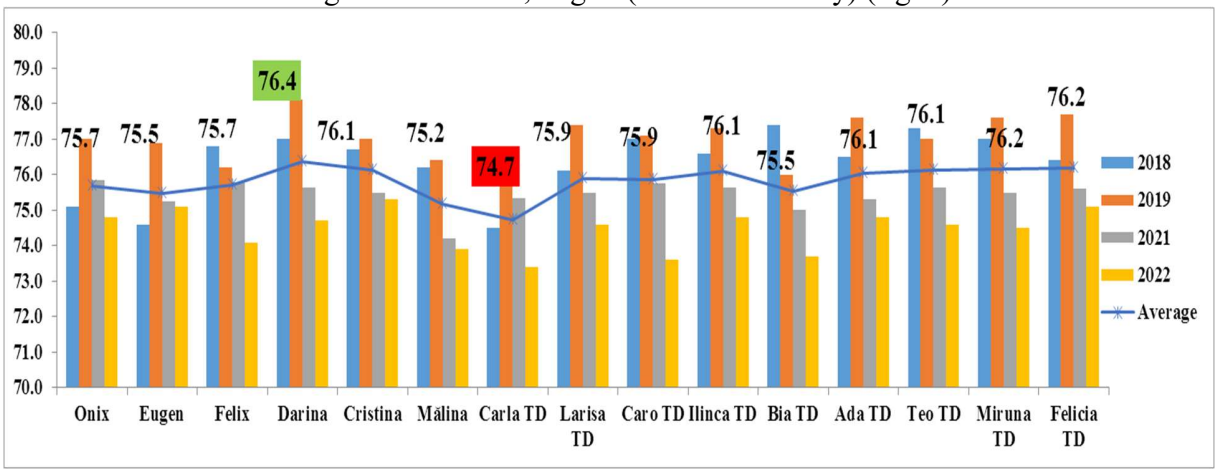


Figure 4 – Hectoliter mass (kg/hl), 2018-2022 // Masa hectolitrică (kg/hl), 2018-2022

The thousand kernel weight had average values between 154,4 g (Onix variety) and 182,2 g (Felix and Miruna TD varieties) (fig. 5).

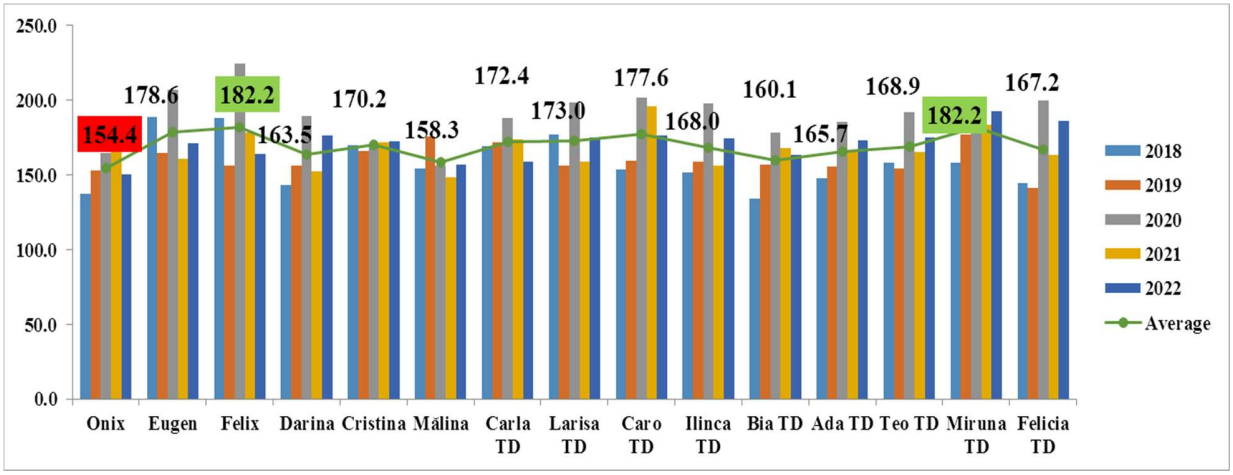


Figure 5 – Mass of a thousand grains (g), 2018-2022// Masa de o mie de boabe (g), 2018-2022

In the year 2018, the yields of the tested varieties varied between 2671 kg/ha (Cristina variety) and 3758 (Ada TD variety). Compared to the control (the average of the experience) there was a statistically significant increase yields (Ada TD variety), and in some varieties the yields obtained were lower, compared to the control, the yields differences were negatively significant (Cristina, Carla TD and Felicia TD).

In the second year of experimentation, 2019, among the genotypes studied, the Bia TD variety stands out, with a yield of 3604 kg/ha, statistically assured and interpreted as very significant, having a yield increase, compared to the control, of 559 kg/ha, and in the Darina variety, there was a yield's decrease by 552 kg/ha, the difference being negative and very significant.

The year 2020, a year marked by the extreme weather phenomenon, namely hail, which plagued the soybean crop and negatively influenced yields, the average value of which did not exceed 900 kg/ha. The average yield of soybeans in 2020 was 933 kg/ha, being at the level of 31% of the 2019's yield, respectively 29% of 2018's yield.

In the year 2021, the average yield of the experimental soybean varieties was 3036 kg/ha, the increases or differences in yield of the experimental varieties compared to the control were not statistically ensured. The highest yield was recorded for the Eugen variety (3660 kg/ha), and the lowest yield was recorded for the Bia TD variety (2576 kg/ha).

In the last year of the experiment, 2022, a very dry and hot year, the average yield had the lowest value of the experiment period, 611 kg/ha. The highest value of grain yield was recorded in the Mălina variety (1134 kg/ha), and the lowest yield value was found in the Teo TD and Felicia TD varieties (293 kg/ha).

Table 3. Average productions recorded in the experimental soybean varieties// Producțiile medii înregistrate la soiurile experimentale de soia

Crt. No.	Variety	Yield (kg/ha)					
		2018	2019	2020	2021	2022	Multianual average
1	Onix	3019	3094	841	3079	627	2132
2	Eugen	3298	3164	866	3660	913	2380
3	Felix	3274	3410**	857	2779	850	2234
4	Darina	3160	2493ººº	789	2902	566	1982
5	Cristina	2671º	3185	757	3045	950	2122
6	Mălina	3386	3083	794	3058	1134*	2291
7	Carla TD	2769º	2688ºº	796	3093	613	1992
8	Larisa TD	3473	3345*	988	3498	474	2356
9	Caro TD	3577	2977	1621***	2699	418	2259
10	Ilinca TD	3279	3114	956	3041	418	2162
11	Bia TD	3438	3604***	978	2576	725	2264
12	Ada TD	3758*	2990	1116	2942	402	2242
13	Teo TD	3010	3102	853	2995	293	2051
14	Miruna TD	3705	2749º	902	2961	491	2162
15	Felicia TD	2701º	2673ºº	877	3217	293	1952
16	Media (Ct)	3235	3045	933	3036	611	2172
DL 5%		489	228	202	640	474	562
DL 1%		660	308	272	862	639	757
DL 0,1%		875	408	362	1144	849	1005

On average, during the five years of experimentation, the yield of the varieties studied varied between 1952 kg/ha (Felicia TD variety) and 2380 kg/ha (Eugen variety), values not statistically guaranteed.

Following the experimentation of the 15 varieties, the Cristina variety stands out as the most stable in terms of grain production, the amplitude being 2428 kg/ha, while the Ada TD variety has the highest value of the amplitude (3354 kg/ha) (fig. 6.)

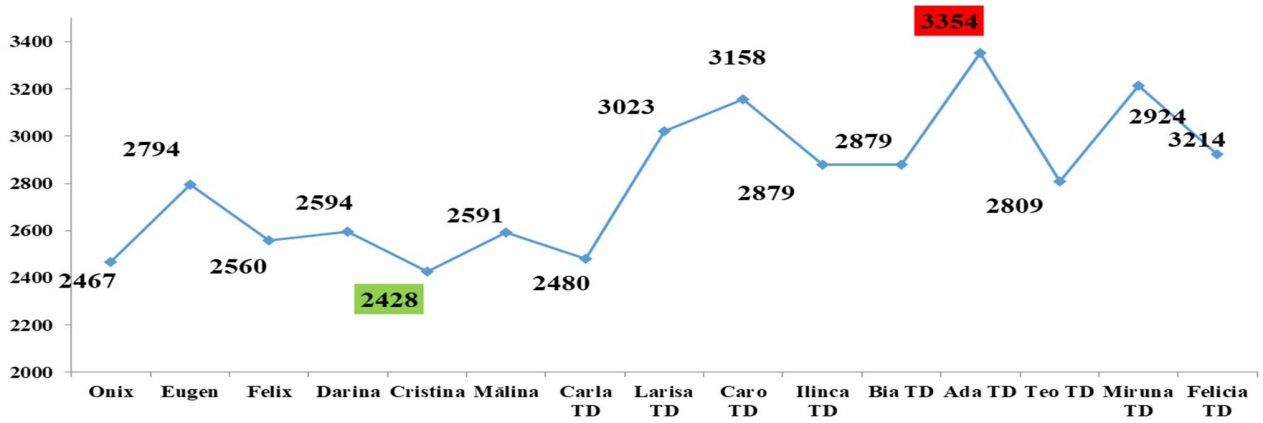


Figure 6 – Amplitude of soybean yields (kg/ha), 2018-2022// Amplitudinea producției de soia (kg/ha), 2018-2022

The quality indices of soybeans refer to the content in protein (%), oil (%) and fiber (%), being also considered the "plant of the future", intended to solve the global protein deficit.

The protein content of the 15 Romanian soybean varieties tested had average values of over 40,0%. The highest protein content of over 42% was recorded in the varieties Miruna TD (42,7%), Bia TD and Larisa TD (42,4%), Eugen (42,3%), Caro TD (42,2%) and Ada TD(42,0%).

Also, in the Darina, Felix, Mălina, Onix, Carla TD, Ilinca TD, Teo TD and Felicia TD varieties, the average protein content was between 40,5% and 41,8% (fig. 7).

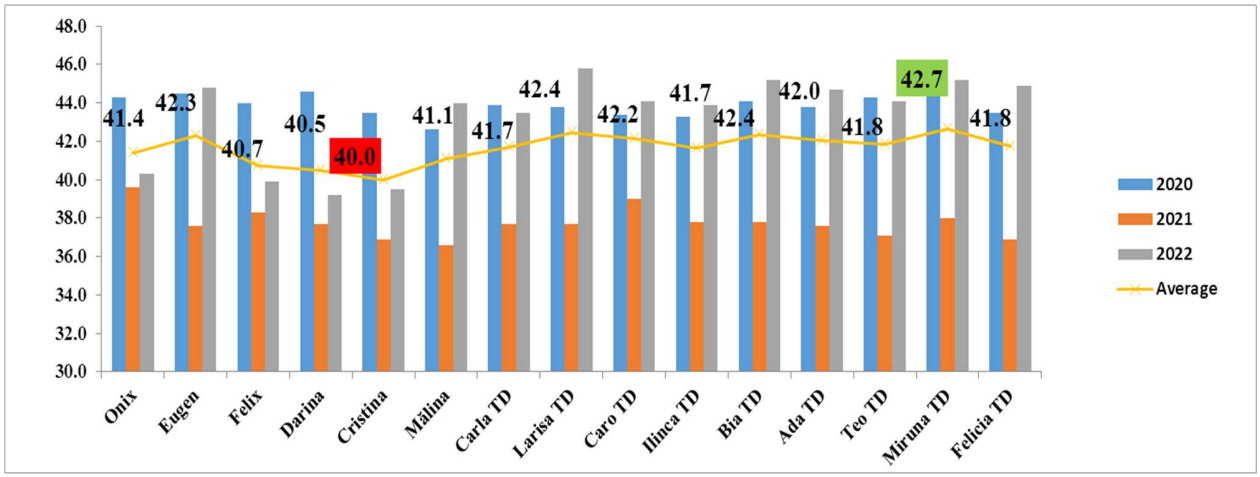


Figure 7 – Protein content of soybeans (%), 2020-2022// Conținutul de proteine din boabele de soia (%), 2020-2022

With the highest oil content, over 22%, the varieties Onix (22,3%) and Felix (22,4%) stand out, at the opposite pole are the varieties Ada TD (20,3%), Ilinca TD (20,4%), Teo TD and Felicia TD (20,5%) and Darina (20,9%), the other varieties had an average oil content between 21,1% and 21,9% (fig 8).

The fiber content of the grains had average values that oscillated within very narrow limits, namely 5,3% (Bia TD and Miruna TD varieties) and 5,6% (Darina and Cristina varieties) (fig. 9).

CONCLUSIONS

1. Regarding the climatic conditions, the experimental period was characterized as hot and dry.
2. Temperatures registered an increase of 1,4 °C, and precipitation an average deficit of 145,7 mm, compared to multi-year averages. During this period, there were also extreme weather phenomena that directly influenced the production of soybeans.

3. During the 5 years, the varieties recorded average yields of over 2000 kg/ha, with the exception of the varieties Felicia TD (1952 kg/ha), Darina (1982,2 kg/ha) and Carla TD (1991,8 kg /ha), the average yield being thus between 1952 kg/ha (Felicia TD variety) and 2380 kg/ha (Eugen variety).

4. During the experimental period, the highest value of yield was recorded for the Ada TD variety (3758 kg/ha - year 2018) and the lowest value was recorded for the Teo TD and Felicia TD varieties (293 kg/ha - year 2022).

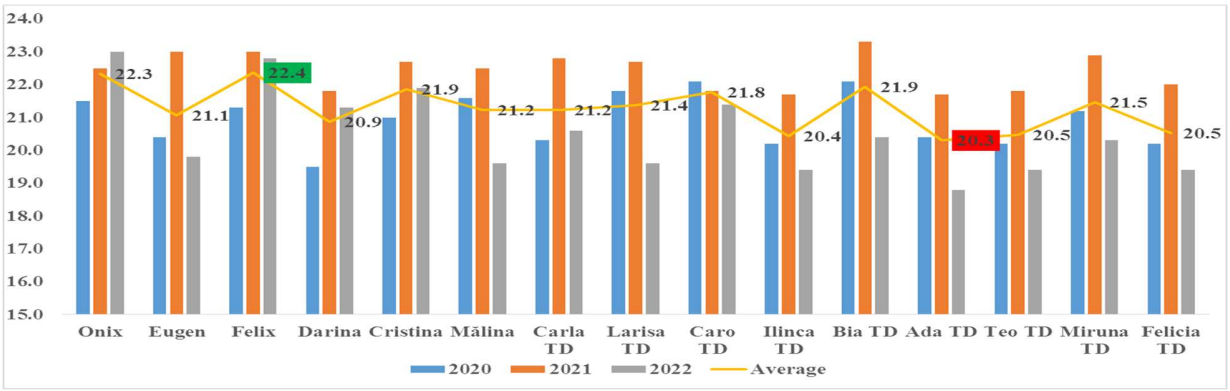


Figure 8 – Soybean oil content (%), 2020-2022// Conținut de ulei de soia (%), 2020-2022

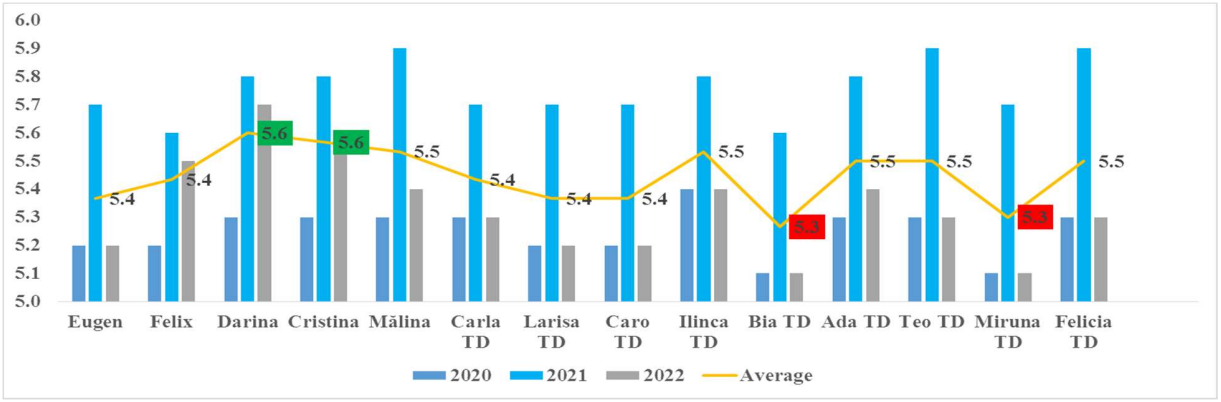


Figure 9 – Fiber content of soybean (%), 2020-2022 // Fiber content of soybean (%), 2020-2022

5. The most pronounced stability of yield was registered with the Cristina variety whose amplitude was 2428 kg/ha.

6. Following the determinations made on the productivity elements, the Felix variety stands out with the highest values.

7. From the point of view of quality elements, all cultivated varieties are high in protein (over 40%) and oil (over 20%).

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THE INFLUENCE OF APPLIED MANAGEMENT ON THE PRODUCTION AND VEGETATION STRUCTURE OF A *Dichanthium ischaemum* (L.) Roberty MEADOW

INFLUENȚA MANAGEMENTULUI APLICAT ASUPRA PRODUCȚIEI ȘI STRUCTURII COVORULUI VEGETAL PE O PAJIȘTE DE *Dichanthium ischaemum* (L.) Roberty

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Abstract:

The researches carried out in the 2021-2022 agricultural year, within the Research and Development Station for Meadows, Vaslui, on a Dichanthium ischaemum (L.) Roberty *meadow, were represented by the analysis of the applied management on the production of dry matter and the vegetation structure changes. The amount of green mass per hectare, the dry matter content and the amount of dry matter per hectare were determined. The study of vegetation was carried out using the geobotanical method. The agricultural year 2021-2022 was a very dry year, affecting the growth and development of permanent grasslands species. The studied meadow had a vegetation structure dominated by species with very poor fodder value and an animal load of 0.55 UVM per hectare. Following some of the measures applied, the grassland potential increased by 10-47%. In the case of the abandoned and mulch variants, the yields obtained were very close to those of the control variant, with no statistically assured differences, and in the case of the $N_{50}P_{50}K_{50}$ and $N_{100}P_{100}K_{100}$ fertilization variants the highest yields were obtained, with very significant increases ranging from 75.2 to 90.3 %. Because of the lack of precipitation the manure has not completely decomposed, remaining at the surface of the soil, the effect of its apiculation being less than that of the applied complex mineral fertilizers.*

Key words: abandonment, mulch, complex mineral fertilizer, manure

Rezumat.

Cercetările efectuate în anul agricol 2021-2022, în cadrul Staţiunii de Cercetare-Dezvoltare pentru Pajişti Vaslui, pe o pajişte de Dichanthium ischaemum (L.) Roberty*, a fost reprezentat de analiza managementului aplicat asupra producţiei de substanţă uscată şi a schimbărilor din structura covorului vegetal. Au fost determinate cantitatea de masă verde la hectar, conţinutul în substanţă uscată, cantitatea de substanţă uscată la hectar. Studiul asupra vegetaţiei s-a realizat prin metoda geobotanică. Anul agricol 2021-2022 a fost un an foarte secetos, fiind afectate creşterea şi dezvoltarea plantelor de pe pajiştile permanente. Pajiştea studiată a avut un covor vegetal dominat de specii cu valoare furajeră foarte slabă şi o încărcătură de animale de 0,55 UVM/ha. Din acest punct de vedere, în urma unora dintre măsurile aplicate, potenţialul pajiştii a crescut cu 10-47 %. În cazul variantelor abandonate şi mulcite producţiile obţinute au fost foarte apropiate de cele ale variantei martor, neexistând diferenţe asigurate statistic, iar în cazul variantelor de fertilizare $N_{50}P_{50}K_{50}$ şi $N_{100}P_{100}K_{100}$ au fost obţinute producţiile cele mai mari, cu sporuri foarte seminificative cuprinse între 75,2-90,3 %. Gunoiul de grajd nu s-a decompus complet, rămânând la suprafaţa solului, efectul aplicării acestuia fiind mai mic decât cel al îngrăşămintelor minerale complexe aplicate.*

Cuvinte cheie: abandon, mulci, îngrăşământ mineral complex, gunoi de grajd

INTRODUCTION

Meadows have played and are playing an important role in providing people with livelihoods for millennia as animal feed producing areas. Grassland areas described as green oceans are important for the ecosystem services they provide, with a key role in the balance of the global ecosystem. Grassland ecosystem services are the subject of numerous publications, being classified and abstracted in various ways. Currently, four ecosystem services are distinguished that meadows provide, such as: Support, supply, regulation and culture. In addition to the production of animal feed (supply service), the current focus is the relation of grasslands to climate and erosion control (regulation services), cultural services (tourism), etc. (Bărbulescu C. et al, 1991; Dragomir N., 2005; Ma W.G. et al, 2010; O'Mara F.P., 2012; Luescher A. et al, 2014; Shen H.H. et al, 2016).

Meadows should be given a higher priority in the future, as they play important roles and provide many benefits to society. Their importance is evident from the new concept of multifunctionality of the meadows, which includes the following functions: biological, social, economic, ecological, aesthetic and recreation. Thus, according to research, worldwide grasslands are essential for about 800 million people, a population that is directly connected to grassland grass for their livelihood and virtually everyone consumes or uses products obtained from grassland grass (e.g.: milk, meat and wool, fiber, fuel etc.) for their daily existence (White R. et al, 2000).

MATERIAL AND METHOD

The experience that is the subject of this paper research was organized in the Research and Development Station for Meadows, Vaslui area, on a *Dichanthium ischaemum* (L.) Roberty meadow. In the studied area there are large areas of permanent meadows, at different stages of degradation, due to the positioning on surfaces with different degrees of inclination, eroded or subject to erosion process, due to abandonment or non-rational use, with an inappropriate load of animals and failure to apply maintenance or improvement measures. Due to these aspects, the production was small and the floral composition was dominated by species with medium and low fodder value. In order to increase the production of permanent grassland in the area and the quality of the feed obtained, it was considered necessary to apply organic or mineral fertilizers, and their effect would be compared to that of abandoned and mulching or simple use, without any variants further improvement measures.

The purpose of the research carried on in the first year of implementation of management measures was the analysis of the management applied on the production of dry matter and the vegetation structure changes.

The objectives and activities of the research were represented by: 1. analysis of management applied on dry matter production (a. determination of green mass production; b. determination of dry matter content; c. calculation of dry matter production) and 2. analysis of management applied on the vegetation structure changes (a. analysis of changes in the floral composition;
b. calculation of pastoral value and grazing capacity for each variant).

To achieve the proposed goal and objectives, in the experimental field of Solesti location (Solesti commune) of Meadows Research and Development Station, Vaslui an experience was placed, according to the method of randomized blocks, with 11 variants, in 3 repetitions (Jităreanu G. and Onisie T., 1998). The area of each variant was 12 m^2 (3 m × 4 m) and the harvested area was 8.75 m^2 (2.5 m × 3.5 m). The total area of the experience was 396 m^2 (33 m × 12 m).

The experience had the following experimental variants: v_1 - unfertilized (control); v_2 - abandonment; v_3 - mulch; v_4 - $N_{50}P_{50}K_{50}$; v_5 - $N_{100}P_{100}K_{100}$; v_6 - 10 Mg·ha^{-1} cattle manure, applied annually; v_7 - 10 Mg·ha^{-1} cattle manure, applied every 2 years; v_8 - 20 Mg·ha^{-1} cattle manure, applied annually; v_9 - 20 Mg·ha^{-1} cattle manure, applied every 2 years; v_{10} - 30 Mg·ha^{-1} cattle manure, applied annually; v_{11} - 30 Mg·ha^{-1} cattle manure, applied every 2 years.

Established doses of mineral and organic fertilizers were applied early in the spring, at the beginning of vegetation growth. The manure used came from cattle and had the following composition: N-0.458 %, P_2O_5-0.237 % şi K_2O-0.674 %.

In the 2021-2022 agricultural year, only one harvest was obtained at the studied meadow. The harvest was carried out on 28.06.2022, it was done manually, at the full flowering of the dominant grasses.

The amount of green mass per hectare (Q_{GM} - Mg·ha^{-1}) was determined by weighing the yield of green mass obtained on the 8.75 m^2 harvested area of an experimental plot and then reported per hectare. Dry matter content (DM - %) was determined by drying at 103^0 C according to SR ISO 6496/2001 standard. The amount of dry matter per hectare (Q_{DM} - Mg·ha^{-1}) - was calculated with the relationship: $Q_{DM} = Q_{GM} \times DM$.

The study on vegetation was carried on by geobotanic method, recording the degree of vegetation coverage on, the presence and classification of existing species by economic groups, as well as geobotanic indices (abundance and dominance). For the vegetation structure determination was used the geobotanical method, expeditious method for determining the floristic composition of the meadows based on the floral description (floristic or phytocenological) and the stational description of representative surfaces for each phytocenosis.

The floral survey highlights the list of the component species of phytocenosis studied, to which are added a series of data on relief (altitude, exposure, slope) and general data on vegetation (general soil coverage, in percentage).

The plant species on each variant were classified, according to their importance as fodder plants, into the following groups: *Gramineae*, *Leguminosae* and species from other botanical families (various).

The pastoral value (VP) was calculated with the relationship (Motcă G. et al, 1994):

$$Vp = \frac{\sum A \times Is}{100}$$, where: A - the average coverage of each species in the floral structure; is it specific index on fodder value, with values between 0 and 5.

With the help of pastoral value, grazing capacity was determined (Cp):

$Cp = Vp \times c$ (UVM·ha^{-1}), where: Vp - pastoral value; c (coefficient) = 0.4-0.6 (the coefficient is minimum for grasslands with poor management and maximum for those with a correct mode of exploitation); UVM - one standard 500 kg cow. Depending on the pastoral value and the grazing capacity, the production and quality of permanent grassland is assessed according to the data from tabele 1..

Tabele 1. Appreciation of the productivity and quality of permanent grasslands with the pastoral value help (Motcă G. et al, 1994)// Aprecierea productivității și calității pajiștilor permanente cu ajutorul valorii pastorale (Motcă G. et al, 1994)

Pastoral value determined by specific coverage	Grazing capacity (UVM/ha)	Meadow characterization
3.75-5.00	> 2-3	Very good
2.50-3.75	1-2	Good
1.25-2.50	0.5-1	Medium
0.25-1.25	0.2-0.5	Weak
< 0.25	< 0.2	Degraded

The validation of the results obtained in the experience was carried out according to the methodology of using the experiences executed in one place in several years (Jităreanu G. and Onisie T., 1998). The results were statistically interpreted by analyzing the variance and calculating the least significant differences.

The studied area is characterized by climatic conditions specific to the silvosteppe area. The weather data was taken from the meteorological station of Research and Development Station for Meadows, Vaslui (figure 1.).

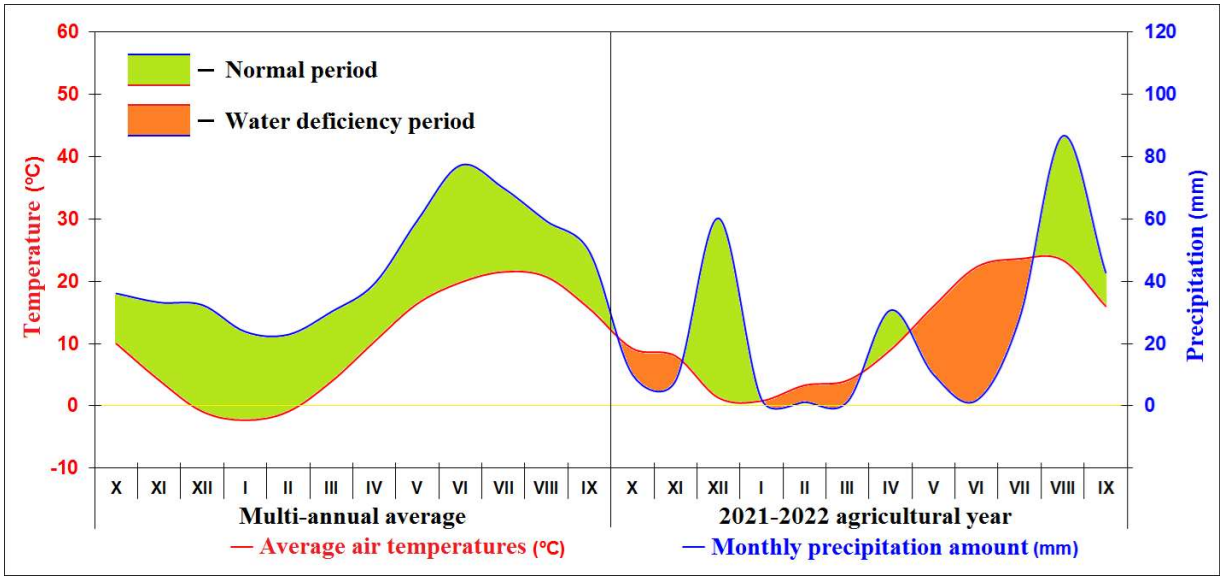


Figure 1. Climate chart of the 2021-2022 agricultural year // Diagrama climatică al anului agricol 2021-2022

In 2021-2022 the air temperature was higher than the multiannual average showing a deviation of 1.5 ºC, and in April-September, during the vegetation period, the deviation was 1.0 ºC. July and August were the warmest months, with average temperatures of 22.8-23.0°C, compared to the multi-year average, with deviations of 1.7-

2.2°C. The lowest temperatures were recorded in December and January (with average temperatures of 0.7-0.8°C).

The amount of precipitation was 250.2 mm lower than the annual average (154.5 mm lower than the multiannual average during the growing season). With the exception of December 2021 and August 2022, negative deviations from the multiannual average were recorded in each of the other months. Between May and July, the negative deviations from the multiannual average were 41.7-75.4 mm. The agricultural year 2021-2022 was a very dry year, affecting the growth and development of permanent meadows.

RESULTS AND DISCUSSION

At the Solesti location (figure 2.), in the area where the experience was located (coordinates: 46º44´59′ N 27º47´34′ E; average altitude: 189 m), the type of meadow identified was *Dichanthium ischaemum* (L.) Roberty (yellow bluestem), a type of secondary meadow, derived from a *Festuca valesiaca* Schleich. Ex Gaudin meadow, irrationally exploited for a long time (Vîntu V. et al, 2004).

The vegetation structure of this type of meadow, with a degree of vegetation coverage of 80%, is represented by *Gramineae* species - 75%, *Leguminosae* species - <1% and species from other botanical families (various group) - 5%. From the point of view of the floristic composition, the dominant species is *Dichanthium ischaemum* (L.) Roberty, with an average coverage of 60 % and the co-dominant species being (depending on the micro-conditions of each zone/grassland plot) *Festuca valesiaca* Schleich. ex Gaudin, *Poa pratensis* L. *Elymus repens* (L.) Gould (synonym: *Agropyron repens* (L.) P. Beauv.) or *Bromus inermis* Leyss. The pastoral value and the grazing capacity was 1.09, respectively 0.55 UVM·ha^{-1}, the meadow being part of the group of the weak meadows.



Figure 3. Location of experience (earth.google.com, 2022)// Locația experienței (earth.google.com, 2022)

In the Solesti location, in the area where the experience was located, on most surfaces occupied with permanent meadows, is the mixed one (the first regeneration of the vegetation season is mowed, and the following regenerations, if any, are grazed). Also, due to the low number of animals, there are also abandoned permanent meadows.

The exposure of the area on which the experience was placed is North-East, and the land has an average inclination of 15% (13.5 °). In the studied area there are slopes of more than 30% (27 °), which are subject to surface erosion (being present stryrations and rigoli) and depth (being present ravines created by rainstorm accumulations from periods with more consistent rainfall). There are also landslides at different stages.

Eroded soil, on which most grassland surfaces are located, is an important element of degradation! The presence of gaps in a large percentage, as well as the large share of the species *Dichanthium ischaemum* (L.) Roberty (with very low fodder value) in the vegetation structure, are very important elements of degradation!

In Table 2 the fodder value of each species is presented, with notes from 2 to 5, according to the scale of Csüros Ş., 1970 (Vîntu V. et al, 2004) and the changes in vegetation structure at each of the 10 working variants, compared to the initial variant (control variant).

Compared to the pastoral value and grazing capacity of the control variant, respectively 1.09 and 0.55 UVM·ha^{-1}, in all the variants where fertilizers, minerals or organic have been applied, the values have increased to 1.21-1.62 and 0.61-0.81 UVM·ha^{-1} (10-47 %) respectively.

The sustainable management of grassland ecosystems can be done only by knowing and linking the vegetation present on them and the stational conditions with the measures of improvement and the way of use (Elsaesser M. et al, 2008; Tonn B. and Briemle G., 2010). The differentiated application of measures to improve permanent grasslands, while maintaining the structural and functional balances established at the level of the pratical ecosystems, cannot be done without a prior study on the influence of fertilization on biomass production, but also their biodiversity (Cŏp T. et al, 2001; Jankowski K. et al, 2003; Samuil C. et al, 2010).

Table 2.Analysis of changes in vegetation structure in 2022, in the studied variants// Analiza modificărilor structurii vegetației în anul 2022, în variantele studiate

Experimental variants	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9	V_{10}	V_{11}
Vegetation coverage degree (%)	*70.0*	*67.1*	*68.6*	*75.6*	*70.8*	*66.2*	*68.5*	*66.7*	*71.8*	*74.0*	*72.0*
Gramineae (%)	64.7	61.3	62.3	64.9	59.1	55.3	60.2	58.5	62	59	57.6
Agropyron pectiniforme Roem. & Schult. (3)										0.2	
Bromus inermis Leyss. (4)	1	1	1	3.3	4	2.3	2	2	2	2.7	2
Dichanthium ischaemum (L.) Roberty (1)	56.7	54.7	54.3	51.7	45	41.7	52.3	46.7	52.7	45.7	43.3
Festuca valesiaca Schleich. ex Gaudin (3)	5.7	4	5.7	3.7	4	5.7	3.3	3.3	3	3	4
Elymus repens (L.) Gould (2)	1.3	1.3	1.3	5	4.7	4.3	2.3	5.7	3	4	7
Dactylis glomerata L. (5)				0.3	0.7	0.3	0.3	0.3	0.3	1	
Koeleria gracilis Pers. (0)								0.3		0.7	0
Poa pratensis L. (5)				0.7	0.7	0.7			0.7	0.7	1
Festuca arundinacea Schreb. (4)		0.3		0.2		0.3		0.2	0.3	1	0.3
Leguminosae (%)	0.6	0.3	1.4	2.8	3.1	2.4	1.7	1.4	1.8	4.1	4.5
Coronilla varia L. (-2)					0.3						
Lathyrus tuberosus L. (3)				0.3		0.7		0.2		0.7	0.7
Lotus corniculatus L. (4)	0.2	0.3		1				0.2		1	
Medicago falcata L. (5)						1	1.3	0		0.5	1
Medicago lupulina L. (5)	0.2	0	0.7	1	1.7		0.2	0	0.2	0.2	0.7
Ononis arvensis L. (1)						0.3					
Trifolium pratense L. (5)					0.3			0.3	0.3		0.3
Vicia cracca L. (3)						0.2		0.7	0.3		
Vicia hirsuta (L.) Gray (3)	0.2		0.7				0.2		1	1	0.8
Vicia sativa L. (1)				0.5	0.8	0.2				0.7	1
Various (%)	4.7	5.5	4.9	7.9	8.6	8.5	6.6	6.8	8	10.9	9.9
Achillea millefolium L. (3)	1.2	1.7	1	1	2.5	2.7	1	1.7	1.3	2	1.3
Achillea setacea Waldst. & Kit. (2)	0.5	0.5		1	1	1	0.2	0.2	0.2	0.3	
Artemisia austriaca Jacq. (-1)						0.2			0.2	0	0.2
Asperula cynanchica L. (0)				0.3							
Carduus acanthoides L. (-1)								0.5		0.5	0.3
Convolvulus arvensis L. (3)		0.2							0.2	1.7	
Centaurea scabiosa L. (0)			0.2					0.3	0.3		0.3
Chenopodium album L. (2)										0.2	
Cichorium intybus L. (3)	0.3	0.3		0.7	0.5	0.7	0.2	0.2	0.3	1	0.3
Cirsium arvense (L.) Scop. (1)			0.2							0.7	1
Echium vulgare L. (0)				0.2							
Eryngium campestre L. (-1)	0.2	0.2	0.7		0.5	0.5	0.2	0.2	0.7	0.7	0.5
Galium humifusum M. Bieb. (0)							0.2		0.2		
Galium verum L. (0)					0.3						
Knautia arvensis (L.) Coult (1)							0.2				
Lappula squarrossa (Retz.) Dumort. (0)			0.3	0.3			0.3	0.3		0.2	
Plantago lanceolata L. (3)	0.3	0.2		1.3	0.5	0.7			0.2		0.3
Plantago media L. (3)							0.3	0.3	0.7		0
Plantago major L. (3)			0.2								0.3
Potentilla cinerea Chaix ex Vill. (0)	1	1.3	1	1	1.3	1.7	1.7	1.7	1	1	1.3
Sambucus ebulus L. (-2)							0.3	0.2			
Salvia nemorosa L. (3)	0.2	0.3	0.3		0.3		1		0.7		0.8
Sonchus arvensis L. (2)				0.3				0.2		0.3	0.3
Thymus glabrescens Willd. (3)	1	0.8	1	1.8	1.7	1	1	1	2	2.3	3
Gaps (%)	30.0	32.9	31.4	24.4	29.2	33.8	31.5	33.3	28.2	26.0	28.0
Number of species	15	16	15	21	19	20	20	25	24	28	27
Vp	1.09	1.18	1.12	1.61	1.62	1.21	1.27	1.21	1.28	1.36	1.35
Cp (UVM·ha^{-1})	0.55	0.59	0.56	0.81	0.81	0.61	0.64	0.61	0.64	0.68	0.68

Research carried on during the first year of use revealed that the application of mineral or organic fertilizers caused important changes in the level of green mass production. Thus, the following aspects have emerged regarding the quantities of feed harvested by variants (table 3.):
- in the case of the V_2 - abandonment and V_3 - mulch variants the obtained productions were very close to those of the control variant, with no statistically assured differences. This is due to the fact that the management measures are in the first year of application;
- the highest yields were obtained in the case of V_4 - $N_{50}P_{50}K_{50}$ and V_5 - $N_{100}P_{100}K_{100}$ variants, with very significant positive differences ranging from 75.2-90.3%. This is due to the fact that nutrients in mineral fertilizers are easier to access by plants, but in the case of higher doses of fertilizers and a lack of precipitation, their effect on production is less;
- in the case of the V_6 - 10 Mg·ha^{-1} cattle manure, applied annually and V_7 - 10 Mg·ha^{-1} cattle manure, applied every 2 years variants productions were obtained with insignificant increases between 7.3-10.8%. This is due to the fact that the nutrients in organic fertilizers are more difficult to access by plants, especially when there is a lack of precipitation, their effect on production being less significant;
- in the case of the V_8 - 20 Mg·ha^{-1} cattle manure, applied annually and V_9 - 20 Mg·ha^{-1} cattle manure, applied every 2 years variants production with distinctly significant differences of between 42.5 and 43.1 % were obtained. This is due to the fact that at higher doses of manure applied, their effect on production is greater;
- in the case of the V_{10} - 30 Mg·ha^{-1} cattle manure, applied annually and V_{11} - 30 Mg·ha^{-1} cattle manure, applied every 2 years variants productions with very significant increases ranging from 56.6-74.4% were obtained. This is due to the fact that, at higher doses of manure applied, their effect on production being higher, but due to the lack of precipitation, the use of nutrients in them by plants was diminished. Manure has not completely decomposed, remaining on the surface of the soil, the effect of its application, even at doses of 30 t·ha^{-1}, being less than that of complex mineral fertilizers applied.

Table 3. The influence of applied management on biomass production obtained on the *Dichanthium ischaemum* (L.) Roberty meadow, in 2022// Influența managementului aplicat asupra producției de biomasă obținută pe lunca Dichanthium ischaemum (L.) Roberty, în 2022

Experimental variants	Q_{GM} (Mg·ha^{-1})	Difference		Signifi-cance	DM (%)	Q_{DM} (Mg·ha^{-1})	Difference		Signifi-cance
		Mg·ha^{-1}	%				Mg·ha^{-1}	%	
v_1 (control)	2.20	control	100	control	35.11	0.77	control	100	control
v_2	2.13[i]	-0.08	96.5		35.11	0.74	-0.03	96.1	
v_3	2.21	0.01	100.4		34.98	0.77	0.00	99.6	
v_4	3.86	1.66	175.2	***	34.83	1.22	0.44	157.4	***
v_5	4.19	1.99	190.3	***	31.56	1.32	0.55	171.3	***
v_6	2.36	0.16	107.3		31.61	0.83	0.06	107.9	
v_7	2.44	0.24	110.8		35.31	0.87	0.09	112.0	
v_8	3.14	0.94	142.5	**	35.49	1.06	0.29	137.1	**
v_9	3.15	0.95	143.1	**	33.79	1.08	0.30	139.3	**
v_{10}	3.84	1.64	174.4	***	34.18	1.29	0.52	167.0	***
v_{11}	3.45	1.25	156.6	***	33.61	1.16	0.38	149.7	**
	LSD 0.5	*0.64*				LSD 0.5	*0.22*		
	LSD 0.1	*0.87*				LSD 0.1	*0.29*		
	LSD 0.01	*1.18*				LSD 0.01	*0.40*		

[i] - for the determination of the production from the abandoned variant, the area of 1 m^2 of each repetition was harvested, and this will no longer be taken into account in the following years.

The research carried on, in terms of dry matter content and the level of dry matter production, highlighted the following aspects (table 3.):
- biomass from harvested plants had an average humidity of 66 %, lower than the normal range of 70-75 %. This was due to the lack of rainfall and high temperatures recorded, which led to the drying of the foliage from the lower floors of the plants;
- the dry matter content was between 34.83-35.11% in unfertilized variants, between 31.56-31.61% in mineral fertilized variants and between 34.83-35.11% in non-fetilized variants, between 33.56-35.49% in manure fertilized variants. Fertilization with complex mineral fertilizers and with 20-30 Mg·ha^{-1} cattle manure resulted in the obtaining of green mass with a higher humidity.

Dry matter yields followed the same trend as green mass yields and ranged from 0.77 to 1.32 Mg·ha^{-1}.

CONCLUSIONS

1. In the area where the experience was located (coordinates: 46º44´59′ N 27º47´34′ E; average altitude: 189 m), the type of meadow identified was *Dichanthium ischaemum* (L.) Roberty (yellow bluestem), a type of secondary meadow, derived from a *Festuca valesiaca* Schleich. Ex Gaudin meadow.
2. The main elements of degradation identified on the meadows in the area where the experience was located are represented by the non-rational use, the presence of erosion (associated with a soil with fertility problems), the presence of gaps in a large percentage, as well as the large share of species with very low fodder value in the vegetation structure.
3. The agricultural year 2021-2022 was a very dry one. The amount of precipitation was 250.2 mm lower than the annual average (by 154.5 mm lower than the multiannual average during the growing season) and the growth and development of the permanent meadows plants was affected.
4. Research conducted during the first year of meadow use revealed that the application of mineral or organic fertilizers caused important changes in the level of green mass production.
5. In the case of the abandonment and mulch variants, the productions was very close to those of the control variant, with no statistically assured differences, which is due to the fact that the management measures are in the first year of application.
6. In the case of variants fertilized with $N_{50}P_{50}K_{50}$ and $N_{100}P_{100}K_{100}$ were obtained the highest yields, of 3.86-4.19 Mg·ha^{-1} green mass, with very significant positive differences between 75.2-90.3% compared to the non-fertilized control variant, due to the fact that the nutrients in mineral fertilizers are easier to access by plants, but in case of higher doses of fertilizers and a lack of precipitation, their effect on production is less.
7. In the case of variants fertilized with 10 Mg·ha^{-1} cattle manure, yields with insignificant increases between 7.3 and 10.8 % have been obtained, and in the case of variants fertilized with 20-30 Mg·ha^{-1} cattle manure, green mass production with distinct and very significant positive differences has been obtained, due to the fact that, at higher doses of manure applied, their effect on production is greater, but due to the lack of precipitation, the use of nutrients in them by plants has been diminished.
8. Manure has not completely decomposed, remaining on the surface of the soil, the effect of their application, even at doses of 30 Mg·ha^{-1}, being less than that of complex mineral fertilizers applied.
9. Dry matter yields followed the same trend as green mass yields and ranged from 0.77 to 1.32 Mg·ha^{-1}.
10. Compared to the pastoral value and the grazing capacity of the control variant, of 1.09 and 0.55 UVM·ha^{-1} respectively, for all the variants where fertilizers, minerals or organic have been applied, the values have increased to 1.21-1.62 and 0.61-0.81 UVM·ha^{-1} respectively.

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RESULTS OF THE MAIZE BREEDING PROGRAM FROM A.R.D.S. TURDA IN THE CREATION OF SEMI EARLY MAIZE HYBRIDS

REZULTATE ALE PROGRAMULUI DE AMELIORAREA PORUMBULUI DE LA S.C.D.A. TURDA ÎN CREAREA HIBRIZILOR SEMITIMPURII DE PORUMB

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Abstract : *Maize (Zea mays) represents one of the most important agricultural crops, both worldwide and in Romania, where it is the plant cultivated on the largest areas. Agricultural research must respond to market demand with productive hybrids, adapted to the cultivation area, so at the Agricultural Research and Development Station Turda the creation of such hybrids is a priority objective. In the last decade, 5 semi-early maize hybrids with high yields were registered and introduced into production: Turda 332, Turda 344, Turda 335, Turda 2020 and Turda 380. These maize hybrids were tested both at Agricultural Research and Development Station Turda, as well as in the network of research stations belonging to Academy of Agricultural and Forestry Sciences. Their testing was also carried out in the State Institute for Variety Testing and Registration Network, in order to be registered in the Official Catalogue of cultivated plant varieties in Romania. All five hybrids outperformed the controls used in the experiment, having superior yields. At A.R.D.S. Turda, the average yield in 3 years of experimentation exceeded 12.7 t/ha for all 5 hybrids tested, but Turda 344, Turda 335 and Turda 2020 stand out. In the case of experimentation in the A.A.F.S. network the hybrids Turda 335, Turda 2020 and Turda 380 stood out, with the highest yields, but it should be noted that for all 5 hybrids yield of over 9.3 t/ha were recorded. In the S.I.V.T.R. network the hybrids Turda 335 and Turda 2020 stood out, with average productions of approximately 13 t/ha obtained in three and two years of testing, respectively.*

Keywords: maize, hybrids, yield, moisture dynamics

Rezumat:

Porumbul (Zea mays) reprezintă una dintre cele mai importante culturi agricole, atât la nivel mondial, cât și în România, unde este planta cultivată pe cele mai mari suprafețe. Cercetarea agricolă trebuie să răspundă cererii pieței cu hibrizi productivi, adaptați zonei de cultură, așa că, la Stația de Cercetare Dezvoltare Agricolă Turda crearea unor astfel de hibrizi este un obiectiv prioritar. În ultimul deceniu au fost înregistrați și introduși în producție 5 hibrizi semitimpurii de porumb cu randamente mari: Turda 332, Turda 344, Turda 335, Turda 2020 și Turda 380. Acești hibrizi de porumb au fost testați atât la Stația de Cercetare-Dezvoltare Agricolă Turda, precum și în rețeaua de stații de cercetare aparținând Academiei de Științe Agricole și Silvice. Testarea acestora a fost efectuată și în rețeaua Institutului de Stat pentru Testarea și Înregistrarea Soiurilor, pentru a fi înregistrate în Catalogul Oficial al soiurilor de plante cultivate din România. Toți cei cinci hibrizi au depășit martorii utilizați în experiment, având randamente superioare. La A.R.D.S. Turda, randamentul mediu în 3 ani de experimentare a depășit 12,7 t/ha pentru toți cei 5 hibrizi testați, dar se remarcă Turda 344, Turda 335 și Turda 2020. În cazul experimentării în cadrul A.A.F.S. rețeaua s-au remarcat hibrizii Turda 335, Turda 2020 și Turda 380, cu cele mai mari producții, dar de remarcat că pentru toți cei 5 hibrizi s-au înregistrat randamente de peste 9,3 t/ha. În cadrul S.I.V.T.R. rețeaua s-au remarcat hibrizii Turda 335 și Turda 2020, cu producții medii de aproximativ 13 t/ha obținute în trei, respectiv doi ani de testare.

Cuvinte cheie: porumb, hibrizi, randament, dinamica umidității

INTRODUCTION

Maize (*Zea mays*) is one of the main crops in the world. The areas cultivated with this plant are impressive; worldwide there are around 202 million hectares cultivated in recent years, thus occupying the second place from this point of view. Maize is overtaken in areas cultivated only by wheat and is followed by rice. From the point of view of the total productions obtained, however, maize is ahead of wheat. It can also be seen from figure 1 the impressive growth of the areas cultivated with maize in the last 60 years, they have doubled.

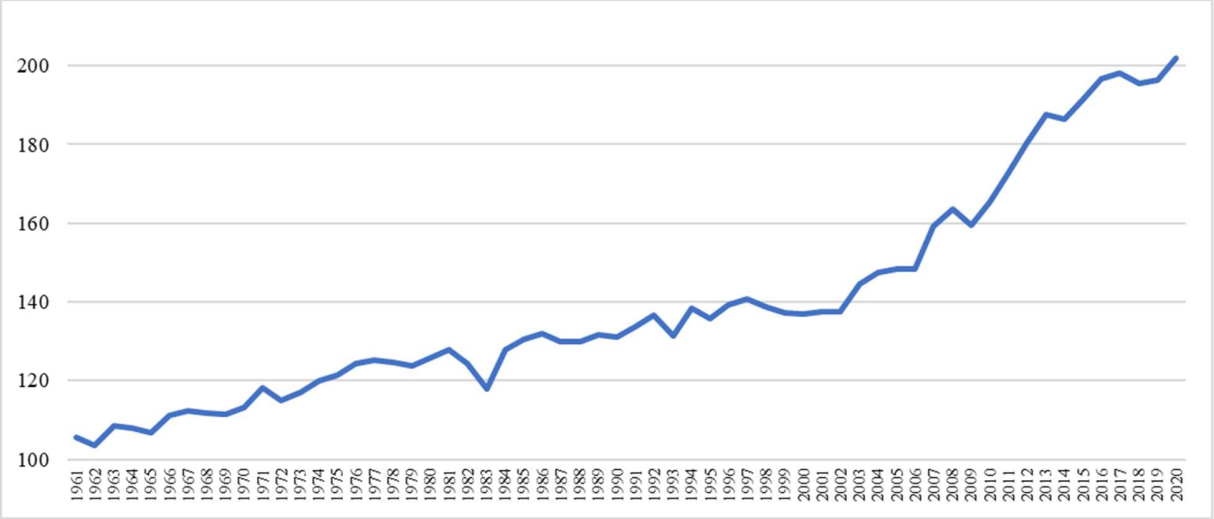


Figure 1. Areas cultivated with maize, worldwide, between 1961-2020 (FAOSTAT, 2023)// Suprafețe cultivate cu porumb, la nivel mondial, între 1961-2020

The introduction of maize hybrids into culture (initially between varieties, and later as inbred lines) led to higher yields, thus increasing the interest in this plant. The increases in production are not exclusively due to the introduction of hybrids, the improvement of culture technology, the ability of hybrids to respond better to higher densities and high amounts of fertilizers also contribute to these high productions (Haș, 2004).

Maize is one of the most important crops in Romania, occupying the largest cultivated area in the country, approximately 2.5 million hectares annually. Also, at the level of the European Union, Romania is ranked first having the largest area cultivated with maize (FAOSTAT, 2023).

Maize breeding in Turda began with the establishment of the Turda Maize Cultivation Experimental Station in 1957 and was initially oriented towards the promotion of hybrid maize in the Transylvania area, then towards the creation of extra-early and early genotypes adapted to the climatic conditions of the area. In the last decades, due to the climate changes that are also felt in the Transylvania maize growing areas, it has become possible to successfully cultivate semi-early hybrids, genotypes that are characterized by superior yield. The maize breeding activity at the Agricultural Research and Development Station Turda was therefore reoriented towards another type of hybrids: with a slightly longer vegetation period, higher production capacity, better adaptability to crop areas, increased tolerance to stress factors.

From the beginning of the maize breeding activity until now, at A.R.D.S. Turda, 45 maize hybrids were registered. Among them, 17 hybrids are simple, 20 trilinear and 8 double, and if we refer to the vegetation period, we can say that 5 are extra early, 21 early, and 19 semi-early. Among the creations of the last decade, the hybrids Turda 332, Turda 344, Turda 335, Turda 2020 and Turda 380 stand out, hybrids also found in the Official Catalogue of Cultivated Plant Varieties in Romania. All the 5 mentioned hybrids stood out due to their superior yields compared to the controls, superior quality and superior ability to capitalize on technological factors (Haș et al., 2014; Haș et al., 2018; Haș et al., 2021; Varga et al., 2022).

In this paper, we proposed to present of the five maize hybrids registered in the last decade, for a better knowledge of their agro-biological characteristics.

MATERIAL AND METHODS

All five hybrids are achievements of the A.R.D.S. Turda Maize Breeding Laboratory, their parental forms being own creations. The sources used as starting material for the creation of inbred lines were synthetics obtained in the reciprocal selection programs or commercial hybrids. In order to obtain the specific heterosis of maize hybrids, it was considered the inclusion of the parental forms different from germplasm groups.

The hybrids were tested in the Academy of Agricultural and Forestry Sciences (A.A.F.S.) network, under the specific conditions of the research stations: A.R.D.S. Turda, A.R.D.S. Secuieni, A.R.D.S. Livada, A.R.D.S. Lovrin and R.D.S.C.B. Târgu Mureș.

Experimental data also come from nine test centres within the ecological network of the State Institute for Variety Testing and Registration (S.I.V.T.R.), under non-irrigated conditions, at different times, as follows:

Turda 332 – 2011-2013 (registered in 2014)

1. Turda 344 – 2014-2016 (registered in 2017)
2. Turda 335 – 2018-2020 (registered in 2021)
3. Turda 2020 – 2019-2020 (registered in 2021)
4. Turda 380 – 2019-2021 (registered in 2022)

The experiments were carried out in comparative cultures, according to the experimental protocols of A.A.F.S. and S.I.V.T.R., in three repetitions and two rows/plot. Each variant was sown at a plot of 70,000 plants/ha, in two rows of 8.7 m length and 0.7 m between rows, respectively, 12.2 m^2 harvestable area.

Characterization of the new creations

Turda 332 (FAO 380) is a simple hybrid tested in the ecological network of S.I.V.T.R. in the period 2011-2013 and registered in 2014. The characteristics of this hybrid are: plant height of 241±20 cm, insertion of the main ear at a height of 90±5 cm, erect leaves, cylindrical ears, with a weight of 216±10 g, 18-22 kernel rows and red cob. The kernels are semi-dent, dark yellow, and the thousand kernel weight (TKW) has average values of 270-300 g and a shelling percentage of 80-82%. This hybrid stands out for its good resistance to low temperatures in the first part of the vegetation period, as well as a very good resistance to plant falling, drought and heat, as well as to the attack of *Ostrinia nubilalis*. Among the specific characteristics, it is possible to produce seeds completely without castration, based on cytoplasmic male-sterility.

Turda 344 (FAO 380) is a trilinear maize hybrid registered by A.R.D.S. Turda in 2017. The pronounced degree of diversification of parental genotypes gives it possibilities of adaptation to a wide the range of ecological conditions. This hybrid is characterized by the following features: plant height of 270±20 cm, the insertion of the main ear is at 107±10 cm, semi-erect leaves, cylindrical ear, 18-20 kernel rows, red cob, dent, light yellow kernels, with a TKW of 280-300 g and a shelling percentage of 82-88%. The hybrid shows very good resistance to low temperatures in the first part of the vegetation period, good resistance to plant falling, drought and heat, as well as good to medium resistance to diseases and pests. The Turda 344 hybrid also has the advantage of a hybridization formula with male sterile and completely pollen fertility restoring parental genotypes, as well as a good coincidence between the flowering of the male flowers and the appearance of the stigmas.

The hybrid **Turda 335** (FAO 380) was registered in 2021, after being experimented for three years (2018-2020) in the S.I.V.T.R. network. The plants of this hybrid are tall (293±7 cm), the insertion of the main ear at a height of 101±9 cm, semi-erect leaves. The ears of this hybrid have a cylindrical shape, 16-20 kernel rows and white cob. The dent, yellow-orange kernels have a TKW of 350-370g and a shelling percentage of 84-86%. The Turda 335 hybrid has a very good resistance to low temperatures in the first part of the growing season, good resistance to plant falling, and good tolerance to drought and heat. The hybrid is characterized by an increased rate of water loss from the grain, at maturity.

The hybrid **Turda 2020** (FAO 380) was tested in the ecological network of S.I.V.T.R. in 2019-2020, being registered in 2021 after only 2 years of testing, as it outperformed the controls in both years. The height of this hybrid is 300±10 cm, the insertion of the main ear is at 139±4 cm, and the leaves are semi-erect. The ears of this hybrid have a cylindrical-conical shape, 18-22 kernel rows and dark red cob. The kernels, dent and dark yellow coloured, have a TKW of 260-280 g and a shelling percentage of 82-84%. The Turda 2020 hybrid has very good resistance to low temperatures in the first part of the growing season, good resistance to plant falling, and good tolerance to drought and heat. An advantage of this hybrid is the possibility of being produced based on a hybridization formula with male-sterile and completely pollen fertility restoring parental forms.

Turda 380 (FAO 380) is the newest creation of A.R.D.S. Turda, being registered in the Official Catalogue of Cultivated Plant Varieties in Romania in 2022. The plant is vigorous, tall (310±10 cm), high and uniform ear insertion, erect leaves. The ear is cylindrical, having medium length (18-20 cm), 14-16 kernel rows, dent, and

yellow-normal kernels. The hybrid stands out for: very good resistance to low temperatures in the first part of the growing season, good resistance to plant falling, good tolerance to drought, heat and an increased rate of water loss from the grain, at maturity.

RESULTS AND DISCUSSIONS

1. The testing of the hybrids at A.R.D.S. Turda - 2019-2021

The 5 hybrids created at A.R.D.S. Turda were tested according to the prevision protocol. The experimental field was placed in a three-year rotation: soybean, wheat, maize. The soil works consisted of autumn plowing and two works of preparing the seed bed, the first being carried out already in the autumn. The experimental field was fertilized by applying 400 kg/ha of complex fertilizer of type NPK 27:13.5:0, together with the preparation of the germination bed. Herbicide was applied at pre-emergence with 1.2 l/ha, using dimetanamid-p as the active substance (720 g/l) and post-emergence with 2 l/ha using tembotrione (44 g/l) and isoxadiphen-ethyl (22 g/l) as active substances.

From a climatic point of view, these years can be characterized as follows: the year 2019 was normal in terms of precipitation, while the year 2020 was very rainy; the two experimental years were warm, thus being favourable for maize cultivation. In 2021, a normal year from the point of view of precipitation, there are an excessively dry June, followed by a rainy July, while temperatures were excessively above the multi-year average, the year being characterized as one of heat, offering favourable conditions for maize.

The hybrids Turda 335 and Turda 380 were noted with average yield of over 13 t/ha, and Turda 332, Turda 344 and Turda 2020 exceeded 12.5 t/ha. The superior yield of the hybrids registered in the last decade at Turda is also correlated with a slight increase in the vegetation period. Higher yield can be observed in 2021, when the new Turda 380 hybrid achieved a production of 16.3 t/ha, and the yield of the Turda 332, Turda 335 and Turda 2020 hybrids exceeded 15 t/ha, while for the trilinear hybrid Turda 344, it was 14.7 t/ha. The average yields in the three experimental years exceeded 12.7 t/ha in all 5 analysed hybrids (figure 2).

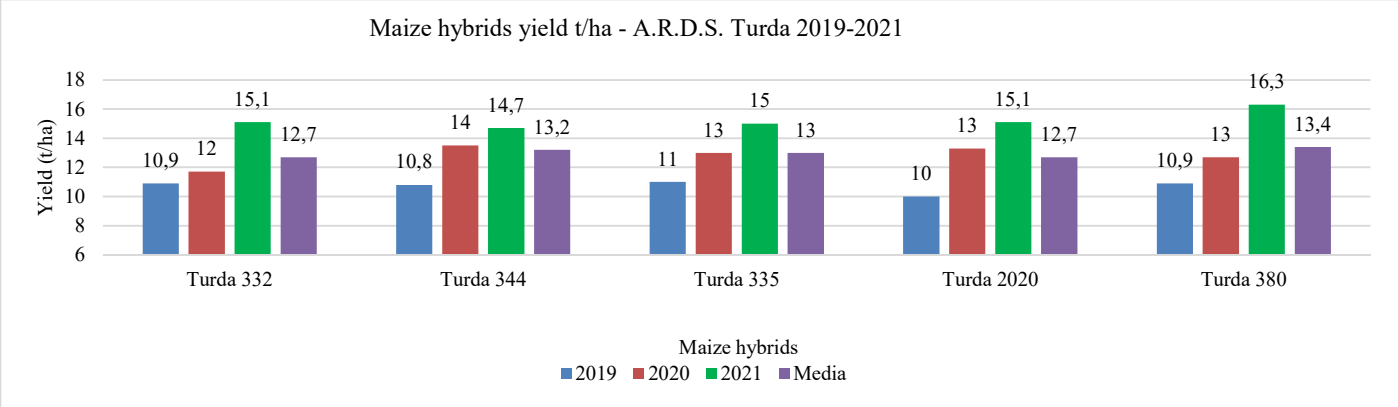


Figure 2. The yield (t/ha) obtained at A.R.D.S. Turda – 2019-2021// Producția (t/ha) obtinută la SCDA Turda – 2019-2021

The rate of grain water loss is a characteristic of hybrids that is highly dependent on environmental conditions, particularly on rainfall after the maturity period. Some of the hybrids produced at Turda were also studied from the perspective of grain moisture loss from physiological maturity to harvest. When climatic conditions are favourable (sun and wind), as they were in 2019 and 2020, maize hybrids tend to have a superior rate of grain water loss. When these conditions become unfavourable (rains and low temperatures), as it was in 2021, the characteristic of hybrids to have a good rate of moisture loss from the grains becomes very important. The hybrid Turda 335 stands out, with the best loss of moisture from the grains, in all three experimental years. A good rate of grain moisture loss was also observed at the other hybrids (table 1).

Table 1. Grain moisture loss between physiological maturity and harvest// Pierderea de umiditate a boabelor între maturitatea fiziologică și recoltare

No.	Hybrid	2019		2020		2021	
		U% at harvest	Moisture loss U%/day	U% at harvest	Moisture loss U%/day	U% at harvest	Moisture loss U%/day
1.	**Turda 332**	23.0	-0.53	21.4	-0.61	27.1	-0.21
2.	**Turda 344**	22.2	-0.63	22.2	-0.64	24.3	-0.41
3.	**Turda 335**	25.5	-0.65	20.2	-0.92	25.8	-0.48
4.	**Turda 2020**	24.5	-0.52	24.6	-0.59	24.7	-0.35
5.	**Turda 380**	27.9	-0.43	23.4	-0.84	26.1	-0.42

The trait of faster grain water loss was sometimes associated with the expression of several characters influencing it (table 2). In the case of the research carried out on the hybrids created at Turda, the relationships that stood out were between the higher rate of water loss from the grains and the some traits: the large number of rows of dent kernels, the reduced rate of the husk on the ears, found in hybrids Turda 344 and Turda 2020, the reduced thickness of the cob (Turda 344), the nutant position of the ear at maturity (Turda 332, Turda 344), the high insertion of the ear (Turda 380, Turda 344, Turda 335).

Table 2. Ear traits that influence the grain and cob moisture loss// Caracteristicile știuletelui care influențează pierderea de umiditate a boabelor și a știuleților

No	Trait / Hybrid	Ear height insertion (cm)	Kernel rows (no)	Cob thickness (cm)	Husk rate (%)	Ear position at maturity
1.	**Turda 332**	132	18-20	2.6	8	Nutant
2.	**Turda 344**	145	18-20	2.4	6	Nutant
3.	**Turda 2020**	141	18-20	2.5	6	Semi-erect
4.	**Turda 335**	140	14-16	2.6	8	Erect
5.	**Turda 380**	150	14-16	2.5	7	Erect

2. The testing of the hybrids on the A.A.F.S. network – 2020-2021

In 2020 and 2021, all 5 hybrids were tested in the A.A.F.S. network, in 5 locations. The productions obtained showed some oscillations depending on the experimental year and the location, but the average productions in both years and the five research stations exceeded 9.2 t/ha for all hybrids, two of them, Turda 335 and Turda 2020, even exceeding 10.5 t/ha. In both years, at A.R.D.S. Turda, all five hybrids had yields above 10 t/ha. High yields were also obtained at R.D.S.C.B. Târgu Mureș, especially for Turda 335, Turda 2020 and Turda 380. The hybrids studied stood out for their high yields in 2021 at A.R.D.S. Secuieni, and in 2020 at A.R.D.S. Livada (table 3). It can therefore be stated that hybrids can be cultivated with good results both in Transylvania and in Moldova.

Table 3. The yield (t/ha) of the newest 5 maize hybrids, A.A.F.S. ecological network, 2020-2021// Producția (t/ha) al celor mai noi 5 hibrizi de porumb, in reteaua ecologică a SCDA, 2020-2021

Nr. crt.	Hibrizi testați	Ani	Yield (t/ha) at (U=14%)					
			Turda	Târgu Mureș	Secuieni	Livada	Lovrin	Media
1.	**Turda 332**	2020	10,7	9,5	6,6	11,8	7,8	9,3
		2021	10,7	9,3	12,1	9,6	4,8	
2.	**Turda 344**	2020	12,2	10,1	6,7	14,4	8,5	9,7
		2021	10,7	8,5	10,0	9,4	6,8	
3.	**Turda 335**	2020	11,6	8,9	7,7	14,7	10,9	11,1
		2021	11,1	13,8	12,6	9,4	10,7	
4.	**Turda 2020**	2020	11,7	10,3	6,0	12,6	10,3	10,5
		2021	10,5	15,0	11,4	8,9	8,5	
5.	**Turda 380**	2020	11,7	11,0	6,4	13,1	8,1	9,9
		2021	11,1	10,8	10,7	8,8	7,8	

3. The testing of the hybrids in the S.I.V.T.R. network

The Turda 332 hybrid was tested for registration in the period 2011-2013, in 9 localities in the country (table 4). The hybrid was distinguished by superior yields as compared to the controls, in all test years and at all

locations, with an average yield of over 9.4 t/ha. The year 2012 was very unfavourable for maize crop due to the drought, productions being generally low, but in 2011 and 2013, the average productions in the 9 localities exceeded 10.8 t/ha (table 4). The maximum yield obtained in the S.I.V.T.R. network was 16.1 t/ha at Rădăuţi (2011) and of 15.9 t/ha at Dej (2013). The highest average yield in the three years of experimentation were obtained in Dej (12 t/ha), Satu Mare (11.7 t/ha) and Rădăuți (11.3 t/ha).

Table 4. The yield (t/ha) of Turda 332 from S.I.V.T.R. network/ Producția (t/ha) hibridului Turda 332 in reţeaua ISTIS

Locality / Year	Yield (t/ha) – Turda 332									
	Târgoviște	**Șimleul Silvaniei**	**Sibiu**	**Satu Mare**	**Rădăuți**	**Negrești**	**Inand**	**Dej**	**Bacău**	**Average of localities**
2011	6,9	9,5	12,6	15,4	16,1	9,6	8,3	13,3	12,3	11,6
2012	5,3	7,2	5,0	7,3	5,5	3,9	7,2	6,8	5,7	6,0
2013	6,9	10,7	11,5	12,5	12,5	7,5	6,1	15,9	13,3	10,8
3 years average	6,4	9,1	9,7	11,7	11,3	7,0	7,2	12,0	10,5	9,4
Average of controls	**6,1**	**8,3**	**7,8**	**9,2**	**9,3**	**6,5**	**6,9**	**10,2**	**8,7**	**8,1**

The Turda 344 hybrid was tested in 6 localities, between 2014-2016, being approved in 2017 and then patented in 2018. The maximum production during the testing period at S.I.V.T.R. (table 5) exceeded 13 t/ha at Luduş (2016) and over 12 t/ha at Dej (2014). The average yield in the three years, over 9.6 t/ha, exceeded the controls with 1.5 t/ha. Average yield of over 10 t/ha were obtained in Tecuci, Satu Mare, Luduș and Dej.

Table 5. The yield (t/ha) of Turda 344 from S.I.V.T.R. network// Producția (t/ha) hibridului Turda 344 în reţeaua ISTIS

Locality / Year	Yield (t/ha)– Turda 344						
	Tecuci	**Satu Mare**	**Negrești**	**Luduș**	**Inand**	**Dej**	**Average of localities**
2014	9,1	11,5	10,7	11,3	5,9	12,3	10,1
2015	11,9	9,4	7,2	6,9	5,9	10,6	8,7
2016	10,7	9,5	7,1	13,0	8,8	10,5	9,3
3 years average	10,6	10,1	8,3	10,4	6,9	11,1	9,6
Average of controls	**8,6**	**8,5**	**7,4**	**8,9**	**6,0**	**9,3**	**8,1**

Turda 335 was tested in the period 2018-2020, being registered in the official catalogue of varieties in 2021 and patented in 2022. The average production of the hybrid Turda 335 in the three experimental years, in the nine localities (table 6) was approximately 13 t/ha, exceeding 12.6 t/ha every year. Some high production values are noted in the three years in Dej (17.4 t/ha), Rădăuți (15.3 t/ha), Sibiu (14.1 t/ha) and Luduș (13.6 t/ha). Highest yields were noted in Dej, in 2019 (21.8 t/ha) and in Rădăuți in 2020 (17.3 t/ha).

Table 6. The yield (t/ha) of Turda 335 from S.I.V.T.R. network// Producția (t/ha) hibridului Turda 335 în reţeaua ISTIS

Locality / Years	Yield (t/ha) – Turda 335									
	Târgoviște	Șimleul Silvaniei	Sibiu	Satu Mare	Rădăuți	Negrești	Luduș	Inand	Dej	Average of localities
2018	11,3	11,0	13,8	11,4	13,8	14,2	14,2	9,5	13,8	12,6
2019	13,6	8,6	13,9	11,8	14,9	15,3	13,5	9,2	21,8	13,6
2020	9,4	12,4	14,5	16,5	17,3	8,8	13,1	6,7	16,5	12,8
3 years average	11,4	10,7	14,1	13,2	15,3	12,7	13,6	8,4	17,4	13,0
Average of controls	10,7	10,5	12,6	11,9	14,2	11,1	11,9	7,7	13,4	11,6

Turda 2020 stood out during the testing period by yield superior to all witnesses, in all testing centres, being proposed for registration after only two years, 2019 and 2020, so that in 2021 it was registered and in 2022 patented. The average yield in the two years of experimentation in nine localities exceeded 13 t/ha (table 7). Some localities were noted where the average yield over the two years was: 18.6 t/ha in Dej, 17.0 t/ha in Rădăuți, 14.3 t/ha in Satu Mare and 14.0 t/ha ha in Sibiu. The highest productions were obtained in 2019 at Dej (21.1 t/ha) and Rădăuți (17.1 t/ha), as well as in 2020 in the same test centres, when yield had values of 16, 1 t/ha, respectively 16.9 t/ha.

Table 7. The yield (t/ha) of Turda 2020 from S.I.V.T.R. network// Producția (t/ha) hibridului Turda 2020 în rețeaua ISTIS

Locality / Years	Yield (t/ha)– Turda 2020									
	Târgoviște	Șimleul Silvaniei	Sibiu	Satu Mare	Rădăuți	Negrești	Luduș	Inand	Dej	Average of localities
2019	15,4	8,5	13,3	12,4	17,1	12,2	11,9	9,2	21,1	13,5
2020	8,9	13,3	14,7	16,2	16,9	8,2	12,2	6,7	16,1	12,6
2 years average	12,1	10,9	14,0	14,3	17,0	10,2	12,1	8,0	18,6	13,0
Average of controls	11,1	10,2	12,4	12,4	14,5	9,9	11,0	7,1	16,6	11,7

The newest creation of A.R.D.S. Turda, the semi-early hybrid Turda 380 was registered in 2022 after 3 years of testing (2019-2021) and is patent pending. The average yield in the three years of experimentation, in nine localities, was 11.6 t/ha. Maximum yield was achieved at Dej in 2019, 18.8 t/ha, and 16.6 t/ha were obtained in Rădăuți in 2019 and 2020. The highest average yield obtained in the three years of testing were noted in Rădăuți, Satu Mare and Dej.

Table 8. The yield (t/ha) of Turda 380 from S.I.V.T.R. network// Producția (t/ha) hibridului Turda 380 în rețeaua ISTIS

Locality / Years	Yield (t/ha) – Turda 380									
	Târgoviște	Șimleul Silvaniei	Sibiu	Satu Mare	Rădăuți	Negrești	Luduș	Inand	Dej	Average of localities
2019	14,0	8,4	14,0	12,8	16,6	13,3	12,5	8,1	18,8	13,2
2020	8,8	12,9	14,7	15,1	16,6	9,2	12,6	6,5	13,9	12,3
2021	5,4	11,7	11,7	10,0	12,6	6,8	11,2	3,8	11,0	9,4
3 years average	9,4	11,0	13,5	12,6	15,3	9,8	12,1	6,1	12,6	11,6
Average of controls	9,1	10,0	12,1	11,6	13,8	9,1	10,1	5,9	15,1	10,8

CONCLUSIONS

1. Climate change, the competition with hybrids on the market, as well as the demand from farmers have determined the change in the orientations of breeding programs towards the creation of maize hybrids with a slightly longer vegetation period, but with higher productivity, the creations of the last decade standing out in this regard.
2. The yields obtained by the maize hybrids Turda 332, Turda 344, Turda 335, Turda 2020 and Turda 380, in the ecological network of the research stations within A.A.F.S., as well as from the testing at S.I.V.T.R. determines the recommendation to cultivate these hybrids with good results in various regions of the country, especially in favourability zones I and II in Transylvania and Moldova, as well as in the hilly areas in the west of the country.

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BEHAVIOR OF SOME WINTER TRITICALE VARIETIES UNDER PEDOCLIMATIC CONDITIONS OF ARDS TELEORMAN, DURING 2020-2022

COMPORTAREA UNOR VARIETĂȚI DE TRITICALE DE TOAMNĂ IN CONDITILE PEDOCLIMATICE DE LA SCDA TELEORMAN ÎN PERIOADA 2020-2022

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Abstract

The paper presents yield performances of 16 triticale cultivars (Zori, Zvelt, Zaraza, Tulnic, Utrifun, FDL Ascendent, Haiduc, Negoiu, Oda, Pisc, FDL Cordial, 14225T2-1, 16026T4-1, Vultur, 14187T1 și TF2), under pedoclimatic conditions of ARDS TELEORMAN, during 2020-2022, under two technologies; optimum nitrogen and phosphorus (N96:P40) and optimum phosphorus reduced dose of nitrogen(N40:P40) fertilization. The tested cultivars differently reacted to environment during research. There were identified cultivars well adapted to unfavorable environmental conditions, such as Vifor, FDL Cordial, 14225T1-02, 16026T4-1 și 14187T1, and cultivars rather more adapted to favorable environmental conditions, Oda, Pisc, Utrifun, Zvelt, Zaraza și FDL Ascendent. The new cultivars Utrifun și FDL Ascendent expressed a high yield potential in quite contrasting environmental conditions.

Introduction of cultivars with large adaptability to contrasting environmental conditions, could contribute to the yield stability in triticale, irrespective the availability of favorable crop conditions in some years. The superior yield capacity of the both, the new triticale genotypes under registration and the perspective lines as compared to the former ones proved in different environmental conditions recommend them to be cultivated in the area in order to obtain sustainable yields.

***Key words: triticale, environmental conditions, cultivar reaction, yield potential*.**

Rezumat

Lucrarea prezintă rezultatele de producție obținute la 16 soiuri de triticale (Zori, Zvelt, Zaraza, Tulnic, Utrifun, FDL Ascendent, Haiduc, Negoiu, Oda, Pisc, FDL Cordial, 14225T2-1, 16026T4-1, Vultur, 14187T1 și TF2), în condițiile pedoclimatice de la SCDA TELEORMAN, în perioada 2020-2022, cultivate în două condiții de fertilizare cu azot și fosfor în optim (96 kg azot s.a./ha și 40 kg fosfor s.a./ha) și în condiții de fertilizare cu doza optimă de fosfor și redusă cu azot, (40 kg azot s.a /ha și 40 kg fosfor s.a./ha). Soiurile testate au reacționat diferit la mediu și la tehnologia de fertilizare. Au fost identificate soiuri bine adaptate atât la condiții nefavorabile de mediu, cum ar fi: Vifor, FDL Cordial, 14225T1-02, 16026T4-1 și 14187T1, cât și la condiții favorabile de mediu, Oda, Pisc, Utrifun, Zvelt, Zaraza și FDL Ascendent. Noile soiuri Utrifun și FDL Ascendent sunt bine adaptate la condițiile de mediu din zona de sud a țării, având un potențial mare de producție. Prin cultivarea soiurilor cu adaptabilitate mare la condițiile de mediu contrastante, reducerea randamentului de producție înregistrată în anii nefavorabili ar putea fi redusă. Noile genotipuri în curs de omologare și de perspectivă sunt bine adaptate la condițiile de mediu din zonă, având un potențial de producție superior, comparativ cu soiurile mai vechi și recent omologate, ceea ce le recomandă pentru cultivarea cu succes (asigurarea unui nivel optim de producție) în anii nefavorabili de cultură.

Cuvinte cheie: triticale, condiţii de mediu, reacţie soiuri, nivel de producţie. Cuvinte cheie: triticale, condiţii de mediu, reacţie soiuri, producţie.

INTRODUCTION

Among cereal crops, triticale is one of the great achievements of the man in the field of theoretical and applied genetics and which has wide potential possibilities for increasing the yield per surface unit (Ittu et al., 1986, 1990, 2001; Ittu and Săulescu , 1988, 2000).

The major contribution to the improvement of grain production was made by the genetic advances obtained to increase the number of grains per ear, grain weight and hectoliter mass. Also, an important contribution was the reduction of the plant's height with favorable effects on the improvement of resistance to falling (Ittu et al., 2007). Of particular importance was the transfer from wheat of the semi-dominant gene RhtB1b for reducing plant

height. This gene played a major role in the creation of intensive triticale genotypes with high resistance to fall and at the same time with a greater efficiency of use of assimilates in increasing grain production. A new important step in genetic diversification was the introduction into the Romanian triticale germplasm of the dominant height reduction gene, Hl, transferred from rye, the mutant EM 1, obtained by K o b i l i a n s k i, in the former U.S.S.R. (Ittu et al. , 2007)

The genetic recombinations carried out further in the breeding program carried out at I.N.C.D.A. Fundulea, for improving the resistance to sprouting in the ear, allowed the creation (I t t u et al., 2006 b) (a) of a new germplasm with very low amylolytic activity in the grain (fall index 250-325 sec.) and with a duration of significantly higher dormancy than the best varieties for this characteristic. This germplasm is the basis for the future creation of new triticale varieties with improved resistance to sprouting in the ear.

Considering that the triticale species is mainly intended for use as fodder, silage or especially as grains, the increase in production potential was achieved without decreasing the protein content. The nutritional value of products obtained from triticale is mostly given by the increased content of protein substances that exceeds that of wheat, as well as by the amino acid structure of the protein complex and especially by the richer content in lysine. The nutritional value, the high digestibility of carbohydrates and protein substances give priority to triticale especially in feeding non-ruminant animals, pigs and birds.

The research carried out in this regard demonstrated that the protein-energy ratio is generally higher in the case of fodder obtained from triticale, than that of traditional concentrated fodder (BROUWER; 1977). The strong growth rate in the first phases of growth and the rich vegetative mass of the plants compete with the growth of weeds, which it can practically eliminate. (GAŞPAR; BUTNARU; 1985). Appreciating the essential properties of the triticale species compared to the basic cereals, corn and wheat ZILLINSKI and BORLAUG (1971), NASCIMENTO et al. (2004). , as well as in human nutrition in bakery, pastry, spirits industry, and in the perspective of being used on a large scale in the production of biofuels (bioethanol, biogas) (HILLS et al., 2007).

The genetic diversification of the autochthonous triticale germplasm was achieved, mainly, through the intensive use of Romanian wheat and (Romanian) triticale germplasm in hybridizations, which allowed important progress to be made in improving grain filling, increasing hectoliter mass and, as a result, of production potential. In order to continue to maintain in the breeding program an upward genetic progress in triticale, it is necessary to achieve the improvement and sustained genetic diversification of the germplasm, by carrying out exchanges with other breeding programs in the world and especially by introducing new genetic variability of to wheat and rye for the main agronomic characters (I t t u et al., 2007).

The extremely large variation, both in the total amount of precipitation from one year to another, and in their distribution throughout the year, causes, in some years, important water deficits during the vegetation of grassy cereals, while in other years there are an excess of moisture. The climate changes of the last period of time have accentuated these extreme variations, with serious consequences on agricultural production, triticale genotypes reacting differently depending on the adaptation capacity (V o i c a , 2009). The use of genetic diversity at the territorial level, by cultivating several different species in each area, represents the simplest and most accessible way of reducing crop fluctuations. In this paper, we aimed to analyze the influence of environmental conditions on the production of some triticale varieties, cultivated and some lines of perspective, at SCDA Teleorman, considering the fact that in this area, an element of stress frequently encountered, it is constituted by, both atmospheric and ground drought. The aim of the current work is to analyze the behavior of some triticale genotypes under approval alongside the newest approved varieties, based on multi-year tests in comparative crops, in order to identify, approve and recommend for the expansion of the crop, the best adapted from each other.

MATERIALS AND METHODS

The data presented in this paper refer to the behavior of 16 genotypes of autumn triticale, of which 10 are varieties approved in the period 2012-2022 (Zori, Zvelt, Zaraza, Tulnic, Utrifun, FDL Ascendent, Haiduc, Negoiu, Oda, Pisc, varieties that can be found in the Official Catalog for the year 2022, 5 perspective lines: FDL Cordial, 14225T2-1, 16026T4-1, Vultur, 14187T1 and TF2, the first variety created in Romania in 1984. The experimentation period was three years (2019-2022), characterized by different climatic conditions in terms of

precipitation and temperature regimes, under optimal nitrogen and phosphorus fertilization conditions (96 kg nitrogen s.a./ha and 40 kg phosphorus s.a./ha), and in conditions of fertilization with the optimal dose of phosphorus and reduced with nitrogen, (40 kg nitrogen s.a./ha and 40 kg phosphorus s.a./ha)

The experiment was carried out at S.C.D.A. Teleorman, on a cambic/vertic pseudogleyed chernozem soil, with the following characteristics: humus content: 3.0-3.6%; clay content (in the 0-45 cm soil layer): 45-48%; total nitrogen: 224 ppm; phosphorus: 684 ppm; potassium: 388 ppm; pH (in water): 6.3.The main hydro-physical indices of the soil on the 0-80 cm horizon have the following average values: apparent density 1.43 t/m3, field capacity 27.3% (310.4 mm), wilting coefficient 15.0% (171 .0 mm), minimum ceiling 21.1% (240.7 mm).

The preceding plants was in the years 2019-2020 and 2020-2021were peas and chickpeas, and in the year 2021-2022, sunflower, and the sowing rate was 500 germinable grains/m2. The harvestable plot was 5 m2. The following scheme was used for weed control: Mustang (florasulam 6.25% + Acid 2,4D EHE (2-ethylhexyl-ester) 30%) – 0.5 l/ha + Nuance 10 g/ha.

The varieties studied were characterized, in each of the three years, both from the point of view of production capacity and some morphological characters.

The obtained experimental results were processed by analysis of variance (Ce a p o i u, 1968). Also, the production data were processed statistically, as an experience with 16 varieties in three years in the same locality, and the analysis of the links between the characters was estimated by the method of simple correlations. The reaction of each variety to the environmental conditions was determined by analyzing the regression of each variety, in the three environmental conditions, against the production of all the varieties in the three environmental conditions (Brukner and Frohberg , 1987).

The stability of the production was assessed based on the coefficients of variation. Keim and Kronstand (1979), using the regression analysis method, suggested that a variety is adapted to unfavorable environmental conditions when b<1 (subunit regression slope) and a" (regression constant, intercept) has positive values; adapted to favorable environmental conditions when b>1 (slope of the above-unit regression); widely adapted to different environmental conditions when b>1 and "a" has large values.

The determination of the resistance to sprouting in the ear was done on 10 ears of each variant, harvested at full maturity, which were then submerged in water for three hours to soak well with water. The spikes of each variety were tied into a bouquet, with an identification tag, then these bouquets were placed in a stand in a vertical position with the base in a tray of water. In order to ensure and maintain high humidity, the bunches of ears thus prepared were then covered with a bell made of plastic foil attached to a metal frame. To maintain humidity, the ears were sprayed daily with water by using a hand pump. The resistance of the varieties was assessed after 10 days, by visually assessing the degree of sprouting and scoring on a scale from 1 to 9, where 1 = very resistant to sprouting in the ear (very little sprouted), and 9 = very sensitive to sprouting in the ear (very sprouted). The scoring was done on each ear, and the recorded score represents the average of 10 ears.

Characterization of the behavior of the main pathogens*: Septoria, Helminthosporium, Puccinia striiformis* and *Puccinia recondite* was done in the field, during natural infection, by grades from 1 to 9 for *Septoria and Helminthosporium* and by the frequency and degree of rust attack on a scale from 0 to 100, and to establish the behavior of triticale genotypes to *Fusarium graminearum*, artificial infections were made. The method of artificial infection consisted of applying the inoculum by injecting directly into the herringbone or sprinkling the plants at the anthesis. The inoculum was produced according to the method described by Ittu et al., 1997.The reaction of genotypes to this pathogen was expressed according to the severity of the attack (% spicules attacked at 20 post-inoculation days) and the spread of infection in the ear, based on the calculation of the AUDPC index (area under the disease progress curve (Wilcoxson RD, Skovmand B, Atif AA (1975)

RESULTS AND DISCUSSION

The rainfall regime of the three years of experimentation was very different (table no. 1), the year 2019-2020 with drought in autumn, the year 2020-2021 with excess water in autumn and winter, and the year 2021-2022 with excess of water in autumn and drought in winter and at grain filling. These very diverse rainfall regime conditions allowed a good assessment, under field conditions, of the behavior of the tested triticale genotypes.

Table 1 Rainfall (mm) registered under different vegetation stages at ARDS Teleorman during 2019-2022
Precipitaţiile (mm) înregistrate la S.C.D.A. Teleorman în diferite fenofaze de creştere şi dezvoltare în perioada 2019-2022

Years	Sowing and sprouting of plants X-XI	Winther reserve XII-III	Intense growth-blooming - IV-V	Grain filing VI
2019-2020	30.9º	216.1	99	84
2020-2021	129*	313.5*	119	99
2021-2022	115.9*	135.5º	95	36.5ºº
Multi-year-average	82.67	185.77	103.03	72.43

o drought; o very dry; *rainy; **very rainy

During the entire vegetation period of autumn triticale, the average monthly temperatures exceeded the multiannual average. High temperatures in winter had a negative influence on the phenomenon of vernalization. In the winter months there was no snow cover.

In April, the autumn grassy cereals were affected by the differences between the average daytime and nighttime temperatures, with an effect on the number of grains in the ear. There was a lack of grain along the length of the ear, and the base and tip of the ear had sterile spikelets. Due to high temperatures and insufficient water in the 2019-2020 crop year, fall triticale had a weaker twinning based on the genotypic expression of each cultivated variety.

The productions obtained by the grassy cereal genotypes during the experimental period generally reflect the amount of precipitation that fell during the sowing-emergence period, the winter reserve and intense growth at flowering, (tables 2 and 3), while the number of ears and the number of grains in the spike generally reflects the amount of precipitation that fell during the period of sowing - emergence and intense growth - flowering. The weight of the grains is influenced by the amount of precipitation that fell during the period of grain filling, intense growth-flowering, but also the winter reserve, and the height of the plants (reflected) by the amount of precipitation that fell during the period of winter and intense growth-flowering.

Table 2 Correlation coefficients between yield and some productivity traits and rainfall registered under different vegetation stages (N 96:P40)
Coeficienţii de corelaţie dintre producţie şi alte caractere de productivitate şi precipitaţiile din diferite faze de vegetaţie (N 96:P40)

Specification	Sowing and sprouting of plants X-XI	Winther reserve XII-III	Intense growth-blooming - IV-V	Grain filing VI
yield	0.70*	0.81**	0.96**	0.60*
Number of plants/m2	0.99***	0.4	0.45	0.2
Number of ears/m2	0.93**	0.49	0.74*	0.17
Number of grains/ear	0.83**	0.67*	0.88**	0.40
Greutatea boabelor /spic	0.49	0.92***	0.99***	0.77*
height	0.57	0.92	0.96***	0.69*

Table 3 Correlation coefficients between yield and some productivity traits and rainfall registered under different vegetation stages (N40:P40)
Coeficienţii de corelaţie dintre producţie şi alte caractere de productivitate şi precipitaţiile din diferite faze de vegetaţie (N 40:P40)

Specification	Sowing and sprouting of plants X-XI	Winther reserve XII-III	Intense growth-blooming - IV-V	Grain filing VI
yield	**0.88****	0.59	**0.82****	0.30
Number of plants/m2	**0.99*****	0.14	0.45	0.20
Number of ears/m2	**0.93*****	0.17	0.14	0.49
Number of grains/ear	**0.79***	**0.71***	**0.90****	0.46
Greutatea boabelor /spic	0.22	**0.97*****	**0.96*****	**0.93****
height	0.36	**0.94*****	**0.99*****	0.85**

Table 4 Yield of winter triticale cultivars tested at ARDS Teleorman, during 2019-2022(N96:P40) . // Producţia de boabe obţinută la genotipurile de triticale testate la SCDA Teleorman, in perioada 2019-2022, (N96:P40)

No crt	GENOTYPES	Yield Kg/ha 2020	Diff. witness face	yield Kg/ha 2021	Diff. witness face	yield Kg/ha 2022	Diff. witness face	average
1	HAIDUC(martor)	4839	0	7329	0	4810	0	5659
2	NEGOIU	5315	476	7570	241	5205	395*	6030
3	ODA FD	3983	-856	7753	424	5032	222	5589
4	PISC	4388	-451	8034	705**	5164	354	5862
5	TULNIC	3670	-1169^{o}	6610	-719^{oo}	5397	587**	5226
6	UTRIFUN	4963	124	8085	756**	5651	841***	6233
7	VIFOR	6370	1531**	8224	895***	5995	1185***	6863
8	ZORI	4808	-31	7670	341	5695	885***	6058
9	ZVELT	3508	-1331^{oo}	7910	581*	6022	1212***	5813
10	ZARAZA	4483	-356	7679	350	5501	691**	5888
11	FDL ASCENDENT	4996	157	8169	840***	5946	1136***	6370
12	FDL CORDIAL	6625	1786***	7906	577*	5421	611**	6651
13	14225T1-02	7055	2216***	8666	1337***	5781	971***	7167
14	16026T4-1	6110	1271**	8500	1171***	6126	1316***	6912
15	14187T1-1	6598	1759***	8664	1335***	5883	1073***	7048
16	TF2	5546	707	6355	-974^{ooo}	5016	206	5639
average		5204	365	7820	7820	5540	730	6188
	5%		877		415		383	
	1%		1224		597		555	
DL	0.1%		1573		834		777	

Table 5 Yield of winter triticale cultivars tested at ARDS Teleorman, during 2019-2022 (N 40:P40)
Producţia de boabe obţinută la genotipurile de triticale testate la SCDA Teleorman, in perioada 2019-2022 (N40:P40)

No crt	GENOTYPES	yield Kg/ha 2020	Diff. witness face	yield Kg/ha 2021	Diff. witness face	yield Kg/ha 2022	Diff. witness face	average
1	HAIDUC(martor)	5486	0	7067	0	5666	0	6073
2	NEGOIU	6035	549	7252	185	6025	359	6437
3	ODA FD	4926	-560	6736	-331	6037	371	5900
4	PISC	4378	-1108$^{oo.}$	7012	-55	6228	562*	5873
5	TULNIC	3078	-2408^{ooo}	6363	-704^{o}	6230	564*	5224
6	UTRIFUN	5001	-485	7872	805**	6354	688**	6409
7	VIFOR	6490	1004*	7611	544*	6790	1124	6964
8	ZORI	4472	-1014^{o}	7324	257	6250	584*	6015
9	ZVELT	4373	-1113^{oo}	7215	148	6691	1025***	6093
10	ZARAZA	5235	-251	7100	33	6393	727**	6243
11	FDL ASCENDENT	4953	-533	7391	324	6685	1019***	6343
12	FDL CORDIAL	6780	1294**	7742	675*	6655	989***	7059
13	14225T1-02	7140	1654***	7920	853**	6384	718**	7148
14	16026T4-1	6320	834*	7810	743**	6794	1128***	6975
15	14187T1-1	7793	2307***	7895	828**	5976	310	7221
16	TF2	5993	507	6251	0	5372	-294	5872
average		5528		7285		6283		6366
	5%		763.96		494.86		460.92	
	1%		1070.80		706.11		660.12	
DL	0.1%		1467.62		979.33		917.75	

As a result of the fluctuations of the environmental factors, there was a great variability of the production from one year to another (Table 4). In the fertilization variant N96: P40, the average productions varied from 3508 kg/ha (Zvelt, 2020) to 8666 kg/ha. (14225T2-1, in 2021). The lowest productions were obtained in 2020, a year characterized by drought during the sowing-emergence period and low temperatures in April. The highest average production, respectively 7820 kg/ha, was obtained in 2021, a normal year in terms of the amount of precipitation, which in each vegetation phase was above the multiannual average. Among the varieties studied, the genotypes: Utrifun, Vifor, FDL Cordial, 14225T1-02, 16026T4-1, 14187T1-1 performed very well, exceeding the production of the control variety Haiduc with distinctly significant and very significant increases. Distinctly significant and very significant production losses compared to the control were recorded in the genotypes: Zvelt (2020), Tulnic and TF2 (2021).

In the version fertilized with N40:P40, the average productions varied from 3078 kg/ha (Tulnic, 2020) to 7920 kg/ha (14225T2-1, 2021). The lowest productions were obtained in 2020. The highest average production was obtained in 2021 (7285 kg/ha). In the period 2019-2020 and 2021-2022, in which the drought phenomenon manifested itself during the vegetation period, a higher production was recorded in the triticale crop in the N40:P40 fertilization variant, compared to that of N96:P40. The genotypes: Utrifun, FDL Cordial, 14225T1-02, 16026T4-1, 14187T1-1 were distinguished by distinctly significant and very significant increases in production, compared to the control variety Haiduc. Distinctly significant and very significant production losses compared to the control, were recorded in the genotypes: Pisc, Tulnic and Zvelt (2020) (Table 5).

Table 6 Maximum, minimum and average yields and its amplitude at 16 triticale cultivars and their response parameters to the variation of environmental conditions, at Teleorman, during 2019-2022 (N 96:P40)// Producţiile medii, minime şi maxime şi amplitudinea producţiei la genotipurile de triticale şi parametrii de răspuns ai acestora la variaţia condiţiilor de mediu din perioada 2019-2022 (N 96:P40)

No crt	Genotypes	Yield kg/ha			Amplitude	a	b	R	CV %
		average	minimum	maximum					
1	HAIDUC	5659	**4810**	**7329**	2519	-576	1.01	0.99	25.55
2	NEGOIU	6030	**5205**	**7570**	2365	-222	0.99	0.96	22.14
3	ODA FD	5589	**3983**	**7753**	**3770**	-2769	1.35	0.99	34.82
4	PISC	5862	**4388**	**8034**	**3646**	-2458	1.34	1	32.76
5	TULNIC	5226	**3670**	**6610**	2940	-393	0.91	0.87	28.27
6	UTRIFUN	6233	**4963**	**8085**	**3122**	-868	1.14	1	26.32
7	VIFOR	6863	**5995**	**8224**	2229	**1871**	**0.81**	**0.96**	**17.39**
8	ZORI	6058	**4808**	**7670**	2862	-198	1.01	0.98	24.18
9	ZVELT	5813	**3508**	**7910**	**4402**	-2670	1.37	0.88	37.99
10	ZARAZA	5888	**4483**	**7679**	**3196**	-1071	1.12	0.98	27.73
11	FDL ASCENDENT	6370	**4996**	**8169**	**3173**	-598	1.12	0.98	25.57
12	**FDL CORDIAL**	6651	**5421**	**7906**	2485	**2266**	**0.71**	**0.81**	**18.68**
13	**14225T1-02**	7167	**5781**	**8666**	2885	**1891**	**0.85**	**0.84**	**20.17**
14	**16026T4-1**	6912	**6110**	**8500**	2390	**971**	**0.96**	**0.99**	**19.90**
15	**14187T1**	7048	**5883**	**8664**	2781	**1191**	**0.94**	**0.93**	**20.49**
16	TF2	5639	**5016**	**6355**	1339	3098	0.41	0.87	**11.96**
	average	6188		**7820**	2616				

In the three years of experimentation, the biggest difference between the minimum and maximum production of the same variety, was registered with the Zvelt variety (4402 kg/ha). in the version fertilized with N96:P40 (Table 6). Differences in production of over 3000 kg/ha were recorded in contrasting environmental conditions and in the varieties: Oda FD, Pisc, Zaraza and FDL Ascendent, while minimal values regarding the differences in production were recorded, in the same conditions of medium in the genotypes: TF2, Negoiu, 16026T4-1 and Vifor.

The evaluation of the coefficient of variation (CV%) of the triticale genotypes studied, highlights minimum values of the TF2 variety, in the N96:P40 fertilized version, which attests to the high level of production stability of this variety (11.96). The higher values of the coefficients of variation (> 30%) recorded for the genotypes: Vifor, FDL Cordial, 14225T1-02, 16026T4-1, 14187T1-1, Negoiu. The Oda, Pisc and Zvelt suggesting their lower level of stability. . Depending on the regression coefficient values, the analyzed genotypes can be classified as adapted to unfavorable environmental conditions (b<1): Negoiu, Tulnic, Vifor, FDL Cordial, 14225T1-02, 16026T4-1, 14187T1 and/or adapted to favorable environment (b>1), such as: Oda, Pisc, Utrifun, Zvelt, Zaraza and FDL Ascendent. It should be noted that a characterization of varieties based exclusively on the value of the regression coefficient does not lead to the identification of varieties with wide adaptability to varied environmental conditions. An optimal characterization can be obtained by analyzing both the value of the regression coefficient and the regression constant. According to the two parameters of the regression, the triticale genotypes can be classified into the following categories:

- good adaptability to unfavorable environmental conditions (b<1, a positive values): Vifor, FDL Cordial, 14225T1-02, 16026T4-1, 14187T1 and TF2;
- good adaptability to favorable environmental conditions (b>1, a negative values): such as) Oda, Pisc, Utrifun, Zvelt, Zaraza and FDL Ascendent.

Table 7 Maximum, minimum and average yields and its amplitude at 16 triticale cultivars and their response parameters to the variation of environmental conditions, at Teleorman, during 2019-2022 (N 40:P40)// Producţiile medii, minime şi maxime şi amplitudinea producţiei la genotipurile de triticale şi parametrii de răspuns ai acestora la variaţia condiţiilor de mediu din perioada 2019-2022 (N 40:P40)

No. crt	Genotypes	Yield kg/ha			Amplit.	a	b	R	CV %
		average	Minim.	maxim.					
1	HAIDUC	6073	5486	7067	1581	177	0.92	0.94	14.25
2	NEGOIU	6437	6035	7252	1217	1848	0.72	0.90	10.96
3	ODA FD	5900	4926	6736	1810	-545	1.01	0.98	15.47
4	PISC	5873	4378	7012	2634	-3427	1.46	0.95	23.03
5	TULNIC	5224	3078	6363	3285	-6090	1.77	0.84	35.59
6	UTRIFUN	6409	5001	7872	2871	-3951	1.63	1	22.41
7	VIFOR	6964	6490	7611	1121	2841	0.65	0.98	8.33
8	ZORI	6015	4472	7324	2852	-4131	1.59	0.98	23.95
9	ZVELT	6093	4373	7215	2842	-3833	1.56	0.91	24.82
10	ZARAZA	6243	5235	7100	1865	-394	1.04	0.98	15.08
11	FDL ASCENDENT	6343	4953	7391	2438	-2259	1.35	0.95	21.36
12	FDL CORDIAL	7059	6655	7742	1087	3392	0.57	0.85	15.01
13	14225T1-02	7148	6384	7920	1536	3954	0.50	0.58	28.99
14	16026T4-1	6975	6320	7810	1490	1521	0.86	0.99	26.38
15	14187T1	7221	5976	7895	1919	6224	0.15	0.13	51.18
16	TF2	5872	5372	6251	879	4690	0.18	0.36	12.45
	average	6366							

In the N40:P40 fertilized variant (Table 7), the biggest difference between the minimum and maximum production of the same variety, in the three experiments, was registered with the Tulnic variety (3285 kg/ha). Large differences in production (>2800 kg/ha) were also recorded in the Utrifun and Zvelt varieties. The smallest differences in production in different environmental conditions were recorded in the varieties TF2, FDL Cordial, Vifor and Negoiu.

The lowest coefficient of variation was registered with the genotype Vifor (CV%=8.33), which suggests a higher level of production stability, followed by the genotypes: Negoiu, TF2, Haiduc, Oda, Zaraza and FDL Cordial. The high values of the coefficient of variation recorded in the genotypes 14187T1-1 (CV%=51.18) and Tulnic (CV% =35.59), indicate their lower stability.

According to the value of the regression coefficient, the studied genotypes are divided into the following categories: adapted to unfavorable environmental conditions (b<1): Haiduc, Negoiu, Vifor, FDL Cordial, 14225T1-02, 16026T4-1, 14187T1 and TF2, adapted to favorable environmental conditions (b>1): Oda, Pisc,

Tulnic, Utrifun, Zori, Zvelt, Zaraza and FDL Ascendent. Taking into account the two parameters of the regression, the genotypes Haiduc, Negoiu, Vifor, FDL Cordial, 14225T1-02, 16026T4-1, 14187T1 and TF2, showed a higher level of adaptability to unfavorable environmental conditions (b<1, a positive values), compared to Oda, Pisc, Tulnic, Utrifun, Zori, Zvelt, Zaraza and FDL Ascendent, well adapted to favorable environmental conditions (b>1, a negative values)

Table 8 Correlation between some productivity traits and yield (N 96:P40)
Corelația dintre unele elemente de productivitate şi corelația dintre acestea şi producție (N 96:P40)

Specification	Yield	Number of plants/m2	Number of ears/m2	Number of grains/ear	Grain weight/ear	height
Producțion	1					
Number of plants/m2	0.67	1				
Number of ears/m2	**0.90****	**0.93****	1			
Number of grains/ear	**0.97*****	**0.82****	**0.97*****	1		
Grain weight/ear	**0.97*****	0.47	0.76*	**0.83****	1	
height	**0.91****	0.52	0.77*	**0.88****	**0.95*****	1

From the study of the correlations between the analyzed characters, significant and/or distinctly significant values of the correlation coefficient between production and the number of ears were identified (r=0.90**); the number of grains in the ear (r=0.97***), the weight of the grains per ear (r=0.97***) and the height of the plants (r=0.91**); between the number of plants and the number of ears (r=0.93**); between the number of ears and the number of grains in the ear (r=0.97***); between the number of grains in the ear and the weight of the grains in the ear (r=0.83**) and the height of the plants (r=0.88**); between the weight of the grains in the ear and the height of the plants (r=0.95***) (variant fertilized with N96:P40) (Table 8).

Table 9 Correlation between some productivity traits and yield(N 40:P40)
Corelația dintre unele elemente de productivitate şi corelația dintre acestea şi producție (N 40:P40)

Specification	Producțion	Number of plants/m2	Number of ears/m2	Number of grains/ear	Grain weight/ear	height
Producțion	1					
Number of plants/m2	**0.87****	1				
Number of ears/m2	0.68*	**0.95*****	1			
Number of grains/ear	**0.98*****	0.78*	**0.95*****	1		
Grain weight/ear	0.64	0.20	0.14	0.77*	1	
height	0.76*	0.35	0.015	**0.85****	0.98***	1

In the version fertilized with N40:P40 (table 9), the correlations between production and the number of plants/m2 (r=0.87**) and the number of grains in the ear (r=0.98***), between the number of plants/ m2 and the number of ears/m2 (r=0.95***), between the number of ears and the number of grains in the ear (r=0.95***), between the number of grains in the ear, the weight grains in the ear (r=0.77* and waist (r=0.85**) and between the weight of the grains in the ear and waist. (r=0.98***)

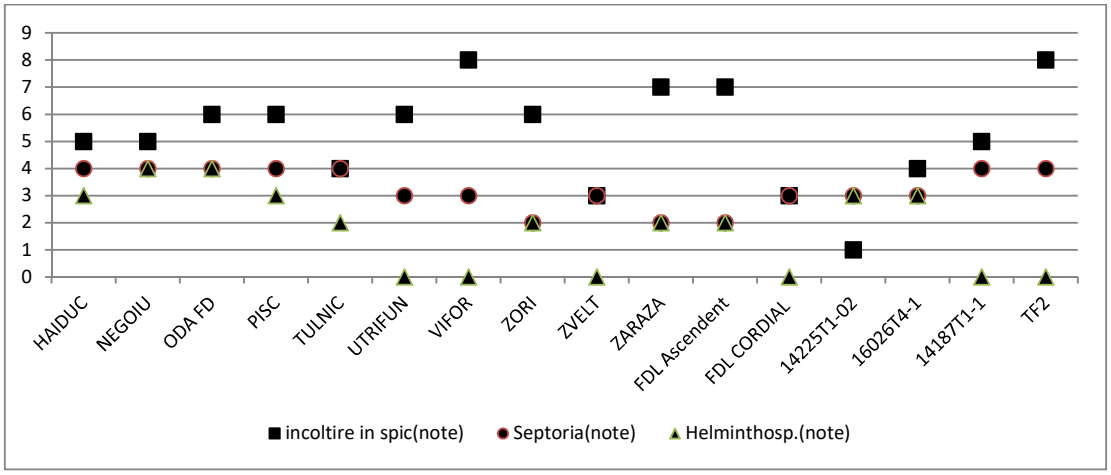


Fig. 1 Behavior of genotypes to Septoria triticii, Helminthosporium sp. (natural infection) and germination of grains in the ear (artificial conditions), (SCDA Teleorman, 2021 and 2022)
Reacția genotipurilor de triticale la *Septoria triticii*, *Helminthosporium* sp. (infecție naturală) și încoltirea boabelor în spic (condiții artificiale), (SCDA Teleorman, 2021 și 2022)

Regarding disease resistance, a level of variability was found (Fig. 1). The least affected by Helminthosporium were the genotypes: Utrifun, Vifor, Zvelt, FDL Cordial, and by septoriosis Zori, Zaraza and FDL Ascendent (grade 1-2).

The phenomenon of sprouting in the ear manifests itself when at the full maturity of the field of triticale, there is a period of rain accompanied by a drop in temperature. In such conditions, the water reaches the grain and the grain sprouts. Since we did not have the necessary conditions for the manifestation of this phenomenon, we resorted to testing under artificial conditions. The genotypes: 14225T1-02 (grade 1), Zvelt, FDL Cordial (grade 3) and 16026T4-01 (grade 1-4) had very good resistance.

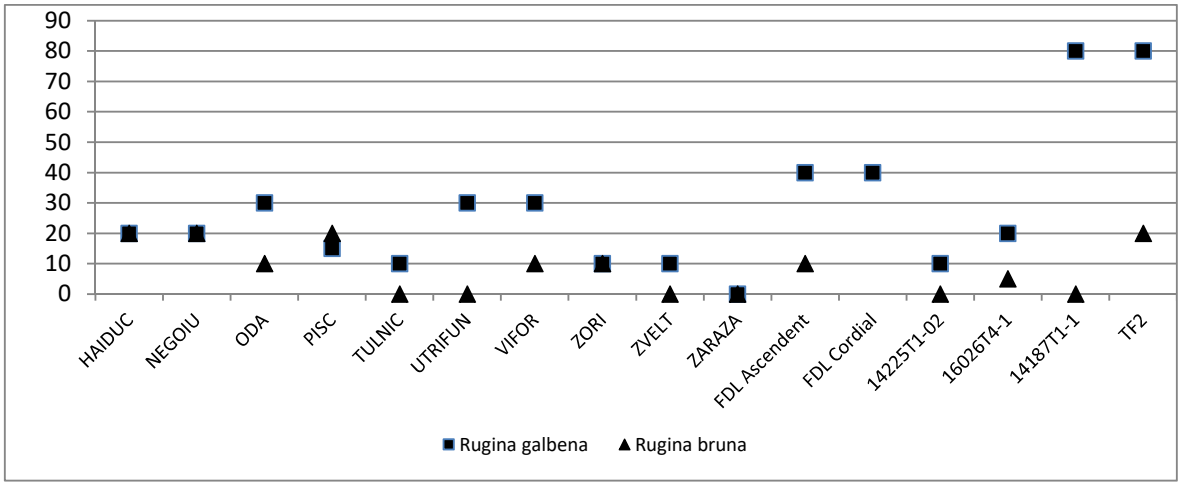


Fig. 2 The behavior of triticale genotypes to yellow rust and brown rust (SCDA Teleorman, natural infection, 2021)
Reacția genotipurilor de triticale la rugina galbenă și rugina brună (SCDA Teleorman, infecție naturală, 2021)

The yellow and brown rusts appeared only in the year 2021 (Fig. 2) and only in the first phase, because there followed a period with more abundant precipitation that washed away the uredia (pustules), so the attack could no longer be noted. Attack frequency was between 0-80% (RB) and between 0-20% (RY). The genotypes least affected by these diseases were: Zori, FDL Cordial, Tulnic, Utrifun, Zvelt, Zaraza, 14225T2-1, Vifor and Oda

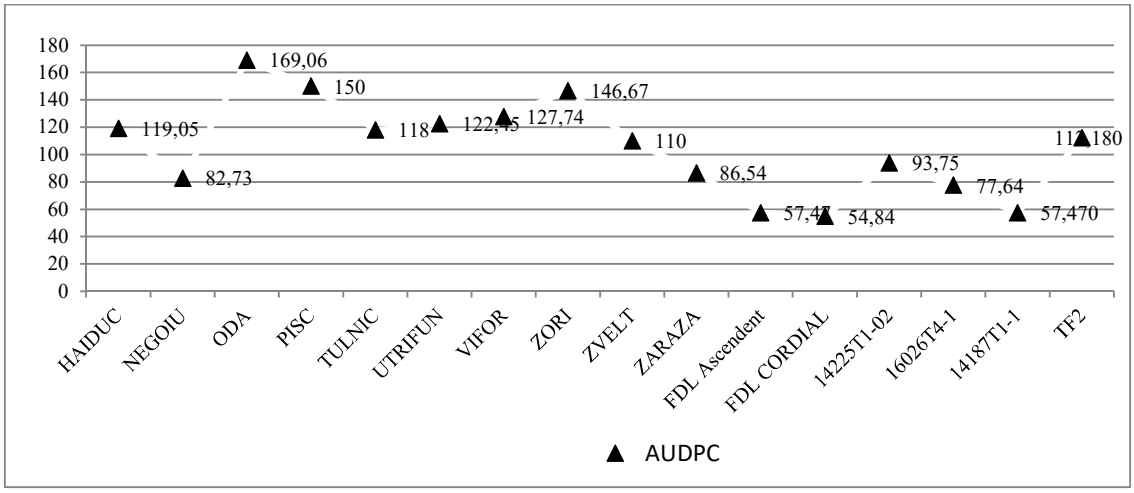


Fig. 3 The behavior of triticale genotypes to fusariosis of the ear (SCDA Teleorman, artificial infection, year 2022)
Reacția genotipurilor de triticale la fuzarioza spicului (SCDA Teleorman, infectie artificiala, 2022)

Fusarium head blight is a widespread damaging disease of cereals in all growing areas of the world. Besides the significant reduction in production, the accumulation of specific toxins (trichotecenes) in the seeds, associated with the attack of the Fusarium pathogen, irreversibly depreciates their quality. The genotypes: FDL Ascendent, 14187T1-1, FDL Cordial, 14225T2-1 and 16026T4-1 were distinguished by a slower evolution of the disease (AUDPC= 54.84-57.47)

The results obtained from the study of an assortment of triticale genotypes, carried out at SCDA Teleorman, in the period 2019-2022, highlighted the genetic variability for the analyzed characters and some lines were identified in the process of homologation and/or perspective, with production potential and resistance to diseases and sprouting in the ear, adapted to less favorable culture conditions. In a period marked by unpredictable climate changes in terms of intensity and evolution, ensuring the diversity of agricultural crops within new varieties adapted for cultivation and exploitation, in less favorable conditions, is a major desire.

CONCLUSIONS

Following this study, the following conclusions were drawn:

1. On average over three years, in the fertilization variant N96:P40, the genotypes: Vifor, FDL Ascendent, FDL Cordial, 14225T1-02, 16026T4-1 and Utrifun generated the highest yield 8000-8666 kg/ha, while the varieties Vifor, FDL Ascendent, FDL Cordial, 14225T1-02, 16026T4-1, Negoiu and Utrifun made the best use of the N40:P40 fertilization conditions, the recorded yields being between 7600-7900 kg/ha;
2. In an experimental cycle of three years, only the year 2021 was favorable for the triticale culture, when the average yield obtained were over 7800 kg/ha, compared to the yield between only 5200-5500 kg/ha, in the years 2020 and 2022. , characterized by long periods of drought
3. The highest yield was recorded in 2021 with the 14225T1-02 variety (8666 kg/ha), and the lowest with the Tulnic variety (3508 kg/ha) within the N96P:40 fertilization variant and 7920 kg/ha. (14225T2 -1 in 2021) and 3078 kg/ha (Tulnic 2020) in the N40:P40 fertilization variant.
4. Triticale genotypes well adapted to unfavorable environmental conditions were Vifor, FDL Cordial, 14225T1-02, 16026T4-1 and 14187T1 and to favorable environmental conditions Oda, Pisc, Utrifun, Zvelt, Zaraza and FDL Ascendent;
5. It should be noted that the new varieties introduced into culture (Utrifun and FDL Ascendent) make very good use of the soils in the southern part of the country.

6. The new genotypes under approval and of perspective are well adapted to the environmental conditions in the area, having a higher production potential, compared to the older and recently approved varieties, which can also ensure an optimal level of production in unfavorable crop years.

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HARMFUL VIRUSES OF GRAPEVINE: IMPACT, SPREAD AND BIOTECHNOLOGIES FOR HEALTHY PLANTS REGENERATION

VIRUSURILE DĂUNĂTOARE LA VIȚA-DE-VIE: IMPACT, RĂSPÂNDIRE ȘI BIOTEHNOLOGII DE REGENERARE DE PLANTE SĂNĂTOASE

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Abstract

Grapevine is a horticultural crop with major economic influence worldwide. At the same time, to grapevine is known the largest number of viruses of a cultivated plant. The diseases produced by viruses to grapevine reduce the vigour and longevity of the plants, the production quantity and quality. Therefore, the timely monitoring of the factors that can have a negative effect on plant growth and products quality, primarily the control of the causative agents of grapevine diseases, is of critical importance for grape production. The widespread of viruses, the fact that the viruses can be controlled best using healthy propagating material has led to increased research regarding the virus elimination methods and the application of their results. The paper reviewed the research carried out at NRDIBH Stefanesti on virus-free plants obtaining by meristem culture, thermotherapy, in vitro chemotherapy, electrotherapy.

Key words: Vitis, GFLV, GLRaV-1, GLRaV-3, GFkV, GPGV, control

Rezumat

Vița-de-vie reprezintă o cultură horticolă cu influență economică majoră la nivel mondial. În același timp, la vita-de-vie este cunoscut cel mai mare număr de virusuri al unei plante de cultură. Maladiile produse la vița de-vie de virusuri reduc vigoarea și longevitatea plantelor, cantitatea şi calitatea producţiei. Prin urmare, monitorizarea la timp a factorilor care pot avea un efect negativ asupra creșterii plantelor și calitatea produselor, în primul rând controlul agenților cauzali ai maladiilor viței-de-vie, are o importanță critică pentru producția de struguri. Larga răspândire a virusurilor, faptul că virusurile pot fi controlate cel mai bine prin utilizarea unui material de înmulțire sănătos au condus la intensificarea cercetărilor privind metodele de eliminare a virusurilor și aplicarea rezultatelor acestora. În lucrare au fost trecute în revistă cercetările efectuate la INCDBH Ștefănești privind obținerea de plante libere de virusuri prin cultură de meristem, termoterapie, chimioterapie in vitro, electroterapie.

Cuvinte cheie: Vitis, GFLV, GLRaV-1, GLRaV-3, GFkV, GPGV, control

INTRODUCTION

Grapevine is an agricultural crop with major economic influence worldwide. The viticultural market is considered to be one of the most dynamic and fastest agricultural markets in recent years. Therefore, the timely monitoring of factors that can have a negative effect on plant growth and products quality, primarily the control of the causative agent of grapevine diseases, is of critical importance for grape production.

The genus *Vitis*, with its numerous species, is considered one of the most affected genera by various types of viruses. More than 80 viruses belonging to 17 families and 34 genera have been described so far (Fuchs, 2020), of which 31 are associated with the four main viral disease complexes known as: infectious degeneration (12 Eurasian/European/Mediterranean nepoviruses) and decline (4 American nepoviruses), leafroll (5 viruses), rugose wood (6 viruses) and fleck (4 viruses). In addition,

7 grapevine viroids are known, of which only 2 induce visible symptoms. Vectors include nematodes, mealybugs, soft scale insects, eriophyid mites, cycads (Martelli, 2017).

Diseases produced in grapevines in the presence of viruses reduce the plants vigour and longevity, the production quantity and quality (Martelli and Boudon-Padieu, 2006, Guță and Buciumeanu, 2016).

Continuous research in the grapevine virology field has led to the continuous identification and characterization of new viruses that cause significant damage to grapevines and can spread from one geographic region to another. One such example is *Grapevine Pinot gris virus* (GPGV) which is not included, according to the legislation, in the list of pathogens that must be identified in the viticultural propagation material, but which is increasingly making its presence felt in vineyards in most of the world's grapevine growing countries. Currently, GPGV is considered a major grapevine pathogen in Europe (Cieniewicz et al., 2020).

International publications have reported the presence of viral infections in grapevine varieties originated from Romania (Boscia and Demarinis, 1998; Milkus et al., 2000). Also, the monitoring carried out in the country indicated the presence of viral infections in varieties from the national (Buciumeanu et al., 2009) and international assortment (Pop et al., 1993). Evaluation of phytosanitary status in five vineyards with Romanian grape varieties from The Hills of Vallachia and Oltenia viticultural region revealed the presence of virus infections, with a high level in newly established plantations. This situation can be the consequence of the intensification of circulation of the grapevine propagation material, the sanitary check and the defective traceability of this material (Buciumeanu et al., 2015).

The European Union recommended common rules to the member states regarding the grapevine propagation and selection (EEC recommendation no. 68/193); however, each national legislation recognizes the European recommendations according to the social and environmental conditions of viticulture, so differences can still be found in the way of clonal and sanitary selection achievement in Europe. In our country, Order no. 1267/2005 harmonized with European legislation regulates the quality of viticultural propagation material from the virological point of view. Viruses to be diagnosed and eliminated are: *Grapevine fanleaf virus* and *Arabis mosaic virus* (GFLV+ArMV), *Grapevine leafroll-associated viruses serotypes 1 and 3* (GLRaV-1, GLRaV-3) and *Grapevine fleck virus* (GFkV). Also, Decision no. 164/2015 regarding the approval of the Methodological Norms for the application of the grapevine and wine law in the system of the common organization of the wine market mentions that the initial material is the vegetative propagation material of the grapevine - "it is made up of clones free of virus diseases recognized by the regulations in force, planted on sanitary families".

The identification of viruses and especially of plants infected with viruses becomes important and necessary when the grapevines are used in the programs of propagating material production and creation of new varieties or clones. The circulation of propagating material throughout the world is the main source of spread of viral diseases.

THE IMPACT AND SPREAD OF HARMFUL VIRUSES IN GRAPEVINE

Grapevine fanleaf virus

Several nepoviruses infect grapevines in Europe and the Mediterranean area, causing degenerative diseases whose symptoms are similar or slightly different from those produced by fanleaf, a disease caused by GFLV. The name of this virus comes from the specific malformation of the infected leaves that have wide open petiolar sinus (Fig. 1) and the primary veins abnormally gathered, the leaves having a fan shape.

The GFLV distribution along the green shoots during the active growth period (determined by ELISA) was directly correlated with symptoms of fanleaf disease in St. George variety (Guță and Buciumeanu, 2011a).

GFLV and other several European grapevine nepoviruses have strains that deform the plant, and chromogenic strains (producing yellowing of the leaf), thus classifying the disease symptoms produced by this virus into two groups (Maliogka et al., 2015). These can be found in mixed infections. Tolerant varieties produce good yields, while the sensitive ones are seriously affected, showing a progressive decline, low quantitative and qualitative production, shortening of the production period, low grafting yield, reduced rooting capacity of the propagating material, decreased resistance to adverse climatic factors. Production losses can be as high as 80%, but they dependent on the interaction between genotype, virus strain and environmental factors. Fanleaf is the oldest virus disease known and one of the most important and widespread in grapevines. European literature records over 150 years ago grapevine leaves with typical symptoms that were found in herbaria made before the introduction of American rootstock hybrids. The disease is known all over the world. GFLV is transmitted by *Xiphinema index* nematode (Andret-Link et al., 2004; Martelli and Boudon-Padieu, 2006). ArMV is transmitted by *X. diversicaudatum* nematode, but not by *X. index* (Brown et al., 1995).



Fig. 1. Fanleaf symptoms to Feteasca neagra variety (original) // Simptome de scurtnodare la soiul Fetească neagră (original)

Grapevine leafroll-associated viruses

Leafroll is the most widespread and important viral grapevine disease worldwide. It is associated with a complex of 6 viruses (Fuchs, 2020), from which GLRaV-3 is the most important in this virus complex and the most widespread grapevine pathogenic virus worldwide (Burger et al., 2017). Spreading of leafroll viruses in plantations is by coccidia species (Pop, 2009).

The leafroll is a disease of high economic importance, reducing the yield by 10-70% and reducing the sugar content of the grapes. In the case of some red table varieties, grapes harvested from affected bunches cannot be marketed due to their uneven colouring. The disease reduces the capacity of grafting and rooting of cuttings and reduces the resistance of plants to frost. The disease produces diffuse reddish spots between the main veins of the leaves at the base of the shoot in red varieties (Fig. 2). The spots gradually enlarge and merge, giving to the leaves a uniform red colour, except for some green bands, of few millimetres in wide, located along the main and secondary veins. The leaves curl towards the lower face. In the last stage, necrosis appears at the edge of the blade and in the spaces between veins, and the bands along the veins become pale yellow. In the white varieties, the symptoms are similar to the red ones, but instead of redness, there is a chlorosis of the spaces between veins. The symptoms are very obvious towards the end of the growing season (Pop, 1988; 2009).



Fig. 2. Leafroll symptoms to Feteasca neagra (original) // Simptome de răsucirea frunzei la soiul Fetească neagră (original)

Fleck virus

The disease produced by GFkV (marbrure) is latent in European grapevine species and most American rootstocks. Symptoms are expressed on *Vitis rupestris* and consist of clearing of third and fourth order veins, producing localized translucent spots. The leaves with intense spots are wrinkled, twisted and may curl upward. The virus influences the vigour, the rooting capacity of the rootstocks, the grafting rates. Severe strains induce varying degrees of stunting. There is not known vector for GFkV and the virus is not mechanically transmitted. The disease is ubiquitous, being reported in all grapevine growing countries of the world (Martelli and Boudon-Padieu, 2006).

The studies revealed that the presence of GFkV in different *V. vinifera* genotypes induced a reduced rooting capacity of the cuttings, a small number of shoots and roots as compared to the healthy material. The influence of the virus depends on the infected grapevine genotype (Guţă et al., 2007a).

Grapevine Pinot gris virus

GPGV was first identified in 2012 in Pinot gris variety, in Italy, by next-generation sequencing techniques (Giampetruzzi et al., 2012), although the symptoms of the disease produced by it, known as grapevine leaf mottling and deformation (GLMD) were observed from 2003 (Martelli, 2014). Reports on GPGV revealed a wide spread of the virus in many grapevine growing areas around the world (Fig. 3).

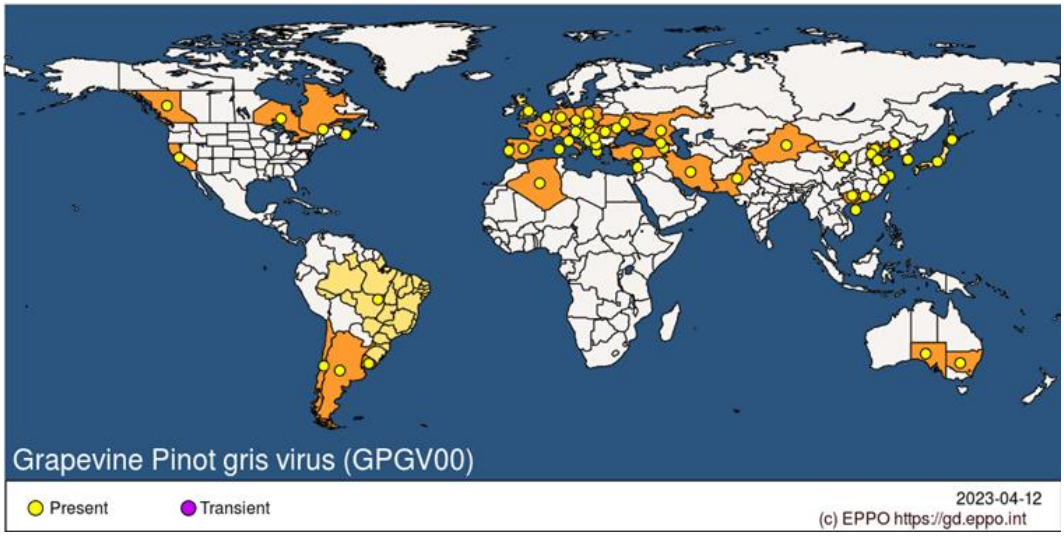


Fig. 3. Distribution of Grapevine Pinot gris virus in the world // Distribuția virusului Pinot gris la vița-de-vie în lume (https://gd.eppo.int/taxon/GPGV00/distribution)

The first information regarding the presence of GPGV in our country was reported in the viticultural material collected after 2010, during the analysis of some genotypes from germplasm collections in several European countries, including Romania (Bertazzon et al., 2015). EPPO Global Database mentions the presence of GPGV in Romania, but without further details, based on the same information (https://gd.eppo.int/taxon/GPGV00/distribution).

An assessment of GPGV incidence grapevines in Romania was realized in 2019-2020 period, when were analysed 199 samples belonging to newly created varieties/clones, clonal selections, old varieties, traditional fruitful varieties originating from grapevine collections from the country, and rootstocks. Symptoms were observed on the leaves (deformations, chlorosis, mosaic) (Fig. 4), poor development of the hub and shoots with short internodes. Serological tests showed an incidence of GPGV infection of 53.76% (107 ELISA positive samples from 199 analysed). Of these, the largest percent (37.38%) it was recorded by old varieties from germplasm collections. As expected, with a close percentage (32.71%) followed the clones, which reinforces the necessity to use virus-free grapevines in breeding programs. In the study, 12 samples collected from 4 rootstock genotypes were analysed, all of them being ELISA positive for GPGV (Guță and Buciumeanu, 2021).

Molecular characterization and phylogeny of different GPGV isolates indicated the existence of symptomatic and latent variants (Saldarelli et al., 2015; Bertazzon et al., 2017). Since the virus is transmitted by grafting, it spreads primarily through the propagating material (Saldarelli et al., 2015). Furthermore, GPGV has been detected also in other host plants: *Silene latifolia, Chenopodium sp., Asclepias syriaca, Rosa sp., Rubus sp.* and *Fraxinus sp*. (Demián et al., 2018). GPGV is transmitted to healthy grapevines by the infested *Colomerus vitis* mite. However, mechanical transmission of GPGV in herbaceous plants has not been found (Malagnini et al., 2016). GPGV was identified in seedlings developed from infected grapevine seeds (Zhang et al., 2022). Currently, GPGV is considered a major grapevine pathogen in Europa (Cieniewicz et al., 2020).



Fig. 4. Symptoms induced by Pinot gris virus to Augusta variety // Simptome produse de virusul Pinot gris la soiul Augusta (original)

The survey carried out in five vineyards with Romanian grapevine varieties in the Hills of Vallachia and Oltenia viticultural region revealed the presence of simple and mixed virus infections in different percentages, without a positive correlation with the presence of symptoms. The percentage of infected plants was higher in newly established plantations (Buciumeanu et al., 2015) (Table 1).

Table 1. Incidence of virus infections in vineyards with Romanian grapevine varieties in the Hills of Vallachia and Oltenia viticultural region (Buciumeanu et al., 2015) // Incidența infecțiilor virale în plantații cu soiuri românești de viță-de-vie în Regiunea viticolă Dealurile Munteniei și Olteniei (Buciumeanu și colab., 2015)

Grapevine genotype/ Year of plantation	Virus-infected samples/ analysed samples (No.)	Virus infection (%)	
		Total	Specification
Feteasca neagra/1986	9/39	23.08	12.82 – GLRaV-1+3; 10.26 – GFkV
Feteasca neagra/1978	6/18	33.33	22.23 – GLRaV-1+3; 5.55 – GFkV; 5.55 – GFkV+ GLRaV-1+3
Feteasca neagra/2010	24/43	55.80	18.60 – GFLV+ArMV; 16.28 – GLRaV-1+3; 9.30 – GFkV; 2.32 – (GFLV+ArMV) + (GLRaV-1+3); 6.98 – (GFLV+ArMV) + GFkV; 2.32 – (GFLV+ArMV) + (GLRaV-1+3) + GFkV
Feteasca neagra/2011	21/50	42.00	26.00 – GFLV+ArMV; 2.00 – GLRaV-1+3 10.00 – GFkV; 2.00 – (GFLV+ArMV) + GFkV; 2.00 – (GLRaV-1+3) + GFkV
Augusta /1993	5/41	12.20	4.88 – GLRaV-1+3; 7.32 – GFkV
Feteasca regala/1989	11/42	26.19	4.76 – GLRaV-1+3; 16.67 – GFkV; 4.76 – GLRaV-1+3 + GFkV
Novac/1994	5/42	11.90	4.76 – GLRaV-1+3; 7.14 – GFkV
TOTAL	81/275	29.45	7.64 – GFLV+ArMV; 8.37 – GLRaV-1+3; 9.82 – GFkV; 1.45 – GLRaV-1+3 + GFkV; 0.36 – GFLV+ArMV + GLRaV-1+3; 1.45 – GFLV+ArMV + GFkV; 0.36 – GFLV+ArMV + GLRaV-1+3 + GFkV

BIOTECHNOLOGIES FOR GRAPEVINE VIRUS ELIMINATION

The first methods of virus-free plant regeneration were meristem/meristematic apex culture with and without thermotherapy

Meristem culture is the oldest method used to eliminate plant viruses (Faccioli and Marani, 1998).

The mechanism of virus elimination through meristematic culture has not been fully elucidated, but various hypotheses are accepted, such as: the absence of plasmodesmata at the level of meristematic cells, the competition between the synthesis of nucleoproteins necessary for cell division and virus replication, or the intervention of inhibitory substances.

Viral replication requires the presence of enzymes normally available in cells close to the meristematic dome. The excision of the meristem causes a trauma that induces a temporary unavailability of these enzymes and, therefore, the viral replication is interrupted and the viral RNA degrades. Thus, if the explant is smaller (0.1-0.3 mm), the chance of obtaining healthy plants is higher. The experimental results confirmed that the elimination of GFLV was possible in a percentage of 54%, and of GLRaV-1 and GLRaV-3 in a percentage of 73-93% (Barba et al., 1992).

Under similar working conditions, Koruza and Jelaska (1993) noted the absence of GLRaV-1 and GLRaV-3 in all plants obtained by meristem culture (0.2-0.4 mm) in 3 clones of the Refošk variety; In the case of GFLV infection, the healthy plants were 60% only.

GFLV was eliminated at a rate of 85% and GLRaV-1 at a rate of 87.5% in Thompson seedless genotype, using a meristem size of 1 mm (Youssef et al., 2009).

Meristem of 0.5 mm led after 6 months of culture to the regeneration of 75% GFLV- free plants and 82.5% GLRaV-1 – free plants of Flame seedless genotype (Fayek et al., 2009).

Grapevine rupestris stem pitting-associated virus (GRSPaV) elimination by meristem of 0.7 mm culture, applied to 6 grapevine genotypes led to unexpected results: only 2 genotypes could regenerate virus-free plants, with very different rates (14.2% to Albarola and 72.7% to Bosco) (Gribaudo et al., 2006).

The research carried out in our country at NRDIBH Stefanesti revealed the possibility of obtaining healthy plants through meristem cultures in a proportion of 60-84.2% for GFLV in complex with GFkV, and 83-95% in the case of leafroll viruses, with the condition that the excised explants should not be larger than 0.2-0.3 mm (Vişoiu and Teodorescu, 2001).

Despite the successes achieved by this method, there are some problems that prevent the use and recommendation of this technique (Altmayer, 1989; Hatjinikolakis and Roubelakis-Angelakis, 1993).

One of the major problems is the difficulty of isolating the meristem as small as possible, followed by the multiple requirements necessary to ensure the viability of the explant and the initiation of regeneration processes: the appearance of adventive buds at the base of the pseudo-petiole of the phylloid formations after 40-45 days of culture, the formation of shoot primordia from the adventive buds after 60 days of culture, the proper multiplication, after approximately 90 days.

A proof of this regard is the experiment carried out by Gribaudo et al. (1997) who obtained a virus elimination percentage of 79.3% for grapevine virus A (GVA), GLRaV-1 and GLRaV-3, pointing out that the rate of elimination of the virus can be influenced by the genotype, the type of the virus and, last but not least, by the difficulty of isolating the meristem.

Thermotherapy and *in vitro* culture are based on decreasing the virus multiplication rate under the influence of high temperature, 35-45°C. Heat-treated plants can produce virus-free tissues, and the lack of vascular elements in the meristem makes difficult to transport viral particles to the meristematic dome. These tissues, if they are collected and grown *in vitro*, can generate new plants identical to mother plant.

Combining tissue culture with thermotherapy led to the virus elimination from different grapevine species (Stellmach, 1980; Barlass et al., 1982; Goussard et al., 1991; Savino et al., 1991; Buciumeanu and Vişoiu, 1996).

In virus elimination experiments by thermotherapy on 4 serotypes associated with leafroll to *V. vinifera*, Black Seedless variety, heat treatment at 38°C for over than 150 days and the use of 3 distal buds for micropropagation led to 100% virus elimination (Stellmach, 1993).

The effect of high temperature (36-38°C), applied for a long period (60-70 days) to infected plant used as donor explants, belonging to *V. vinifera* (Räuschling variety), and *in vitro* cultivation of meristematic apices led to GLRaV-1 total elimination (Staudt and Kassemeyer, 1994).

The results of the research undertaken by Hatzinikolakis and Roubelakis-Angelakis (1993) demonstrated that high percentages of healthy plants can be obtained also through *in vitro* thermotherapy of fragment of a node explants.

In vitro thermotherapy of small explants (apices of 5 mm) led to their necrosis after 8 weeks (Leonhardt et al., 1998). The authors recommended the use of rooted microshoots for virus elimination by *in vitro* thermotherapy. Two months after thermotherapy almost all clones included in the experiment were virus-free.

GVA- elimination of by *in vitro* thermotherapy (57 days at 37°C) was confirmed by RT-PCR in 60% of regenerated plants (Panattoni et al., 2007).

In vitro thermotherapy applied to some rootstock genotypes infected with ArMV, GLRaV-3 and GFLV gave satisfactory results (Křižan, 2008). GFLV elimination in grapevine rootstocks was achieved concomitant by *in vivo* and *in vitro* thermotherapy at 37°C for 45 days. *In vivo* thermotherapy gave better results, although it is more laborious (Křižan, 2009).

Regarding the GPGV elimination have been reported results on the regeneration of virus-free plants by meristem culture with and without thermotherapy. Infected vines belonging to Traminer and Pinot gris genotypes were kept for 2-4 months at 34-38°C before meristem excision. Regenerated plants of Traminer genotype were confirmed virus-free by RT-PCR after an acclimatization period of 3-6 months, during which they did not show characteristic symptoms of the disease. The dependence of the virus elimination method by the genotype was confirmed once again because the plants of Pinot gris did not survive to the heat treatment (Gualandri et al., 2015).

In California, Rieger (2017) reported GPGV elimination by apices micropropagation. The process of virus-free plants obtaining took for 18-24 months before they could be planted, as they were repeatedly analysed for the phytosanitary status safety.

In Romania, Buciumeanu and Vișoiu (2000) reported the elimination of GFLV, GLRaV-3 and GFkV by i*n vitro* culture and/or *in vivo* thermotherapy (Table 2).

Table 2. Elimination of grapevine viruses by thermotherapy and/or *in vitro* culture (Buciumeanu and Vișoiu, 2000) // Eliminarea virusurilor la vița-de-vie prin termoterapie și/sau cultură *in vitro* (Buciumeanu și Vișoiu, 2000)

Thermotherapy (no. of days)	*In vitro* culture (explant)	Virus-free regenerated plants (%)		
		GFLV	GLRaV-3	GFkV
0	Meristem/apex/axillary bud	72/0/0	-/50/16	91/-/-
30	Apex/ axillary bud	60/33	51.7/37.5	-
40	Apex/ axillary bud	100/50	66.5/38.4	84.7/70
60-65	Apex/ axillary bud	100/50	100/100	96/-
80	Apex/ axillary bud	-	-	-/70

A bibliographic study carried out in 1991-2010 period regarding the use of different virus-free plant regeneration methods revealed that thermotherapy was the first and most used method, with a frequency of 56.8% (Panattoni et al., 2013).

Chemotherapy

The deficiencies of the method related to the viability of the explants, the dependence of the virus elimination rate by the genotype and virus, the long time required for the regeneration of new plants and the energy-consuming equipment led to the necessity to develop alternative methods to block viral replication. Thus, the first studies on virus elimination through the use of chemotherapeutics from human medicine and electric current that can cause a degradation of the viral protein at the cellular level appeared.

Among the viricides used to eliminate various types of viruses in horticultural plants, ribavirin was the most frequently used, followed by 5-azacytidine (AZA), acycloguanosine, azidothymidine, 2,4-dioxohexahydro-1,2,5-triazine (DHT), dihydroxy propyladenine (DHPA), oseltamivir, mycophenolic acid (6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methyl-hex-enoic acid) (MPA), selenazole (2-β-D-ribofuranosylselenasole-4-carboxamide) (SE), 3-deazauridine (DZD), cyanoguanidine, etc.

The use of chemotherapeutics in the *in vitro* culture for the purpose of regenerating virus-free grapevine raised many questions regarding their possible phytotoxic effect on explants grown on the medium. Panattoni et al. (2011) reported 100% explant mortality rate starting from 20 mg/L ribavirin, with oseltamivir registering a mortality rate of 11.1% at 40 mg/L concentration.

Starting from 2006, at NRDIBH Stefanesti were carried out studies on grapevine virus elimination by *in vitro* chemotherapy. The methods were applied for GFLV, GFkV, GVA, GLRaV-1, GLRaV-3, both in simple and mixed infections (Fig. 5).

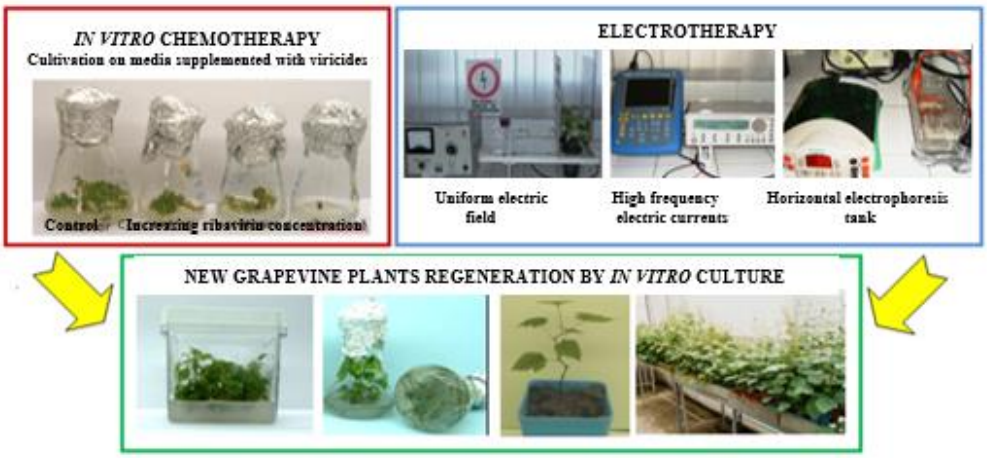


Fig. 5. Alternative methods of grapevine virus elimination developed at NRDIBH Stefanesti // Metode alternative de eliminare a virusurilor la vița-de-vie dezvoltate la INCDBH Ștefănești

In vitro chemotherapy consisted of growing of plant fragments from infected grapevines on medium supplemented with viricides. The chemotherapeutic substances ribavirin and oseltamivir were tested at various concentrations and exposure time, both alone and in combination. Also, in order to expand the list of chemotherapeutics useful for the plant virus elimination, the antiviral substances darunavir and isoprinosine were studied.

The studies shown that ribavirin has a phytotoxic effect expressed by the multiplication rate and mortality of the inoculum more pronounced as compared to oseltamivir (Guţă et al., 2009). At 40 mg/L ribavirin, GFkV-infected Caner genotype behaved by significantly multiplication rate decreasing by 4.3 and 3.03 times, after the first and second subcultures respectively, so that after three treatment subcultures the inocula did not survive. On the other hand, oseltamivir induced the intense proliferation of adventive buds after the first subculture, formations that did not fully evolve in the differentiation and elongation of shoots, and no mortality of the inoculum was recorded at different concentration variant (Guţă, 2010).

The simultaneous use of ribavirin and oseltamivir in order to increase the elimination rate of GFkV from Tamaioasa romaneasca 3-2-2 genotype allowed the initiation of callogenesis processes on all experimental variants, explant mortality being not recorded even after three consecutive treatment subcultures. Visual observations revealed that the differentiation and elongation of shoots, as well as the accumulation of chlorophyll pigments, dependent on the viricides concentration, especially ribavirin, whose phytotoxic effect was more pronounced as compared to oseltamivir. Generally, the multiplication rate decreased as compared to the control in all variants and subcultures. In the first subculture of treatment, the highest concentration of ribavirin caused a higher decrease of multiplication rate (2.2) as compared to the control (3.0), and reducing the viricide concentration by half, completed by the presence of oseltamivir, caused an intense proliferation of adventive buds. Even 40 mg/L ribavirin combined with 40, 80 mg/L oseltamivir caused the multiplication rate decreasing, but does not induce explant mortality after three treatment subcultures (Guţă, 2010). This behaviour is due, on the one hand, to the different phytotoxic effect of the viricides and, on the other hand, to the tolerance to the viricides of the genotypes and their different regenerative potential.

The same treatment with a mixture of viricides applied to Burgund 63 Mn genotype naturally infected with the GFkV and GVA viral complex determined an increase of the multiplication rates after the first subculture, on all viricide concentration variants. In the second subculture, the rates decreased approximately by half for all variants without differences depending on the concentration. This behaviour could be explained by the presence of viricides in the media which produce a stimulation of

adventive buds formation, which in the next subculture did not evolve in the favour of shoot differentiation and elongation.

Grapevine virus elimination by *in vitro* chemotherapy showed the dependence of sanitation rate on viral infection, working parameters and even the grapevine genotype (Table 3).

Table 3. Grapevine viruses elimination by *in vitro* chemotherapy (original) // Eliminarea virusurilor la vița-de-vie prin chimioterapie *in vitro* (original)

Viricide	Concentration (mg /L)	Virus infection	Virus elimination rate(%)	References
Ribavirin	20	GFLV	100	Guță et al., 2007b
		GFkV GLRaV-1+3	100 78.6	Guță et al., 2008
		GVA + GLVaV-1	11	Guță and Buciumeanu, 2010; 2011b
	60	GLRaV-1	26	Guță et al., 2017a
		GFLV GFkV	33.3 37.5	Guță and Buciumeanu, 2012a, b
Oseltamivir	30	GLRaV-1+3	71.42	Guță et al., 2010
	40	GLRaV-1+3 GFkV	62.5 23.3	Guță and Buciumeanu, 2012a, b
Ribavirin +Oseltamivir	20 Ribavirin +40 Oseltamivir	GFkV	100	Guță et al., 2014
	40 Ribavirin +40 Oseltamivir	GLRaV-1	13.3	Guță et al., 2015
	20 Ribavirin +40 Oseltamivir	GFLV	6.8	Guță et al., 2017b
	60 Ribavirin+ 120 Oseltamivir	GFkV	100	Guță et al., 2016a
Darunavir	20-120	GFLV; GLRaV-1+3; GFkV	0	Unpublished
Isoprinosine	20-120	GFLV; GLRaV-1+3; GFkV	0	Unpublished

All these results led to the development and patenting of a virus elimination method in grapevines (Patent no. 123133/2010), currently used for the regeneration of virus-free plants belonging to genotypes destined to the grapevine germplasm collection of INCDH Stefanesti.

Regarding the GPGV-elimination by *in vitro* chemotherapy, the unique available reference indicated the use of ribavirin in two concentrations (10 and 20 mg/L) and 3 treatment times (4, 6 and 8 weeks), in two successive stages. Explants cultivated on ribavirin containing media were transferred after the first phase of treatment to regular media for recovery, for 8 weeks, and then treated again with the same ribavirin concentration and exposure time as in the first treatment. After 8 weeks of treatment in the first phase, the efficiency of the method registered a maximum of 66.7% at 10 mg/L ribavirin and a maximum of 86.7% virus elimination at 20 mg/L ribavirin. Applying the same treatment parameters in a new stage led to the complete elimination in two grapevine genotypes out of three treated at both viricide concentrations. The same treatment scheme led to GFKV- elimination in first stage, after 8 weeks of treatment in one of three genotypes treated with both 10 mg/L and 20 mg/L ribavirin. Repeated application of the treatments with 10 mg/L ribavirin determined the GFkV -elimination after only 4 weeks in one genotype, after 6 weeks in all three treated genotypes, next registering rates of 75, 93.3 and 100% after 8 weeks. Increasing the ribavirin concentration to 20 mg/L eliminated virus in all three genotypes after 4 and 6 weeks of treatment, with low decrease in one genotype to 93.3% at 8 weeks (Komínek et al., 2016).

Electrotherapy as a virus elimination method uses electric current in different forms in order to degrade the viral protein, as a result of temperature increasing at the cellular level. This is not the temperature of the chamber where the tissues are incubated as it happens in traditional thermotherapy. The effect of denaturation is applied to all proteins in the plant tissue, not only to the viral

nucleoprotein, and this is a reversible process when the electric current is stopped (Gonzales et al., 2006).

Grapevine cuttings from GFLV-infected plants were subjected to 34 V/cm for 3 hours. Also, the plants were subjected to 1 V/cm and leaf samples were collected after 1, 5, 10, 20, 30, 40, 50, 60 days. The ELISA evaluation showed that the values of the readings obtained from samples of infected mother plants subjected to 1 V/cm decrease with increasing treatment time, only up to 60 days of application of the electric field, after which they stabilized. However, plants regenerated from cuttings subjected to electric current remained positive for GFLV (Burger, 1989).

The effects of electric current were studied in 30 cm long cuttings of grapevine rootstock placed horizontally and crossed by electric current of 30, 60 V, for 3, 6, 9 hours. The rooting rate, the number and the weight of the roots were studied depending of the treatments. At 30 V, all parameters increased with increasing exposure time, while at 60 V they decreased when the time exceeded 3 hours. The treatment with 60 V for 3 hours showed the highest increasing of rooting rate (122%) and number of roots (100%) as compared to the control. These results indicate the possibility of propagation improving of rootstocks with rooting difficulties (Köse, 2007).

Until now, at NRDIBH Stefanesti, studies have been carried out to eliminate the most important grapevine viruses by electrotherapy. For virus elimination, electric current was used in the form of uniform electric field, direct stimulation with high frequency electric currents and electric treatment in horizontal electrophoresis tank.

The electric field applied to grapevine plants belonging to Cabernet Sauvignon genotype, infected with GLRaV-3 led to obtaining high virus elimination rates (57.1-100%), without direct correlations between the exposure period and the treatment efficiency. Three electric field intensities (10, 20, 40 V/cm) and three exposure time (5, 10, 20 min) were applied. At the stimulation variant of 20 V/cm for 20 min, GLRaV-1+3 was completely eliminated, *in vitro* multiplication and rooting rate increasing significantly as compared to other variants (Guță et al., 2008 a, b).

Electrotherapy applied to eliminate GLRaV-1+3 from another grapevine variety, Ranai Magaraci, led to 33.3-66.6% sanitation rates at 10V/cm, 20 min (Guță et al., 2010), which confirmed once again the dependence of virus elimination by treatment parameters and, not the least, on genotype. Uniform electric field electrotherapy was not effective for GFLV-elimination (Guță and Buciumeanu, 2012a, b).

Direct stimulation with alternative electric current of microshoots collected from infected grapevine, followed *by in vitro* culture has been investigated as a technique for virus elimination of both simple and complex infections. Electric current of 1000 and 10000 kHz was applied to the ends of herbaceous grapevine cuttings; after that, the treated axillary buds went through the stages of micropropagation. ELISA performed on regenerated and acclimatized plants showed encouraging results regarding the elimination of filamentous viruses GLRaV-1 and GLRaV-3 (12.5% elimination at 100 kHz and 4.5% at 10000 kHz, respectively, after 10 min of exposure time). The results were not reproducible in the case of GLRaV-1 - elimination from plants with mixed infections. No satisfactory results were obtained regarding the elimination of isodiametric viruses (GFLV, GFkV) in simple or mixed infections (Guță et al., 2011b; Guță and Buciumeanu, 2012a, b).

Another method of electrical stimulation, electrotherapy in a horizontal electrophoresis tank led to the total elimination of GFkV at 100 mA - 20 min by apices culture. GLRaV-1+3 was eliminated at the best rate (33.3%) at 50 mA - 10 min from axillary buds. GFLV was 50% eliminated treating apices with 40 mA - 20 min. GFkV + GVA virus complex was 50% eliminated by apex culture, at 40 mA – 10 min, and of 49.95% by axillary bud culture, at 50 mA - 20 min (Guță et al., 2016, 2019).

CONCLUSIONS

1. The virological analyses of the grapevine aim to obtain a healthy propagation material, free of the viruses mentioned by the Romanian legislation harmonized with the EU legislation: *Grapevine fanleaf virus* and *Arabis mosaic virus*; *Grapevine leafroll-associated viruses serotypes 1 and 3*; *Grapevine fleck virus*.
2. The widespread distribution of *Grapevine Pinot Gris virus*, its transmission by seed and the natural infection of several herbaceous plant species supports the recommendation to include this virus in the breeding programs and the in schemes of high biological value grapevine propagation material production.
3. Visual observations carried out in the field regarding the symptoms developed by the grapevine in the presence of virus infections and laboratory analyses confirmed the existence of viruses and virus complexes, the level of infection being higher in young plantations, as a result of the sanitary check and the inadequate traceability of the material of multiplication.
4. The control of grapevine virus diseases can be achieved using virus-free propagating material. For this purpose, several methods of virus elimination have been applied and developed: intensely regenerative meristem/apex culture with and without thermotherapy, *in vitro* chemotherapy, electrotherapy and their combinations.
5. The studies revealed the dependence of virus elimination rates on the sanitation method, the virus type and the grapevine genotype.

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BEHAVIOR OF MAMAIA AND COLUMNA VARIETIES IN THE CONDITIONS OF MURFATLAR VINEYARD ECOSYSTEM

COMPORTAREA SOIURILOR MAMAIA ȘI COLUMNA ÎN CONDIȚIILE ECOSISTEMULUI VITICOL MURFATLAR

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Abstract

The objective of the present study was to evaluate the influence of climate variability of the last three years, namely 2020-2022, on grape quality and implicitly of obtained wines from two varieties for white and red wines created at the Murfatlar Research Station, Columna and Mamaia. The increase in average annual temperature values by approximately +2.0°C compared to the reference period (years 2000-2019) in the months of February and March, correlated with the lack of precipitation, contributed to the advance the moment of vine budding and flowering, on average by 5-7 days. It was also observed a tendency for early ripening of the grapes (harvesting taking place 5 days earlier for the Columna variety and 7 days for Mamaia variety). The quality of the grapes highlighted by the content of sugars, total acidity and pH, showed significant differences in the three years under study, the sugar concentrations at the time of harvest reaching over 190 g/L, allowing the wines to be classified in the category of quality wines with controlled designation of origin DOC Murfatlar; the best results were obtained in 2022 with values of 208.7 g/L for the Columna variety and 210.8 g/L for Mamaia variety.

Keywords: *new varieties, climate, phenophases, grape quality, wine quality*

Rezumat

Obiectivul acestui studiu a constat in evaluarea influentei variabilităţii climatice din ultimii trei ani, respectiv 2020-2022, asupra calităţii strugurilor şi implicit a vinurilor obţinute din două soiuri de struguri pentru vinuri albe şi roşii create la SCDVV Murfatlar, Columna şi Mamaia. Creşterea valorilor medii anuale ale temperaturilor cu aproximativ +2,0 oC comparativ cu perioada de referinţă (anii 2000-2019) în lunile februarie şi martie, corelata cu lipsa precipitaţiilor a contribuit la devansarea momentului declanşării dezmuguritului şi înfloritului, în medie cu 5-7 zile. S-a constat deasemenea, o tendinţă de maturare timpurie a strugurilor (recoltarea realizându-se mai devreme cu 5 zile la soiul Columna şi 7 zile la Mamaia). Calitatea strugurilor evidenţiată prin conţinutul în zaharuri, aciditatea totală şi pH, a prezentat diferenţe semnificative în cei trei ani studiaţi, concentraţiile în zaharuri la momentul recoltării fiind de peste 190 g/l permiţând încadrarea vinurilor în categoria vinurilor de calitate cu denumire de origine controlată DOC Murfatlar; cele mai bune rezultate s-au evidenţiat în anul 2022 cu valori de 208,7 g/l la soiul Columna şi 210,8 g/l la Mamaia.

Cuvinte cheie: *soiuri noi, clima, fenofaze, calitate struguri, calitate vinuri*

INTRODUCTION

It is well known that the great wines of the world are produced in regions with a favorable climate for growing grape varieties for quality white and red wines. The Murfatlar viticultural area is of great economic importance in the wine industry, due to its favorable climatic conditions, but also to its tradition in wine grapes cultivation. The natural conditions of this region are particularly suitable for this crop, the entire surface being located in the CII region (cf. Ord. no. 645/2005 MARD). From a climatic point of view, the area benefits from considerable heliothermal resources and low amounts of precipitation. What is worth emphasizing is the uneven distribution of these precipitations, some intervals registering very large deviations from the normal amount, with excess or deficit precipitation, alternating with periods of values above average, followed by periods with pluviometric deficit. The

soil consists mainly of calcareous chernozem, rich in calcium carbonate, an important factor of inluence on wine organoleptic qualities.

Grapevine growth and development in the context of current climate changes represents a challenge for winegrowers/oenologists, in order to adapt to these changes through various strategies (optimization of some technological links and viticultural practices), to compensate these imbalances and obtain quality and balanced wines. The consequences that the frequency of climatic accidents and their intensity have on cultivated plants are aspects that can no longer be ignored, especially in case of perennial plants (1). The temperature and humidity regime are among the basic elements that define the viticultural terroir (2), the average air temperature during the growing season being particularly important. Given the current climatic characteristics, it is expected that there will be changes both in terms of the impact on native ecosystems, but also on crops and, implicitly, in the change of viticultural zoning.

Each grape variety has a unique combination of characteristics, which together with applied agrotechnical measures, geographical location and zonal ecological characteristics, as well as winemaking techniques, influences directly the quality of the grapes and of the resulted wine (3). The impact that climate changes of the last decades have on grapevine are highlighted in a series of scientific works in the field. Among the environmental factors with the greatest influence on the phenological development of the vine, temperature is the climatic parameter with the greatest impact. The increase in temperature leads primarily to the initiation and early onset of phenophases (4, 5, 6), a trend observed in many regions of the world; early phenophases onset can lead to early bud exposure to climatic accidents (7), high temperatures can remove certain varieties from the optimal areas for their cultivation (8), this tendency can lead to excessive accumulations of sugars, the decrease of acidity and the loss of aromas.

Climate change also leads to the exposure of vines to drought, either due to reduced amounts of precipitation or through the evapotranspiration process due to increased temperatures. This leads to low crop yield, particularly through reduced berry size (9,10) and reduced bud fertility (11).

The purpose of this paper was to evaluate the climatic conditions during the years 2020-2022, compared to the multiannual average (2000-2019) and their influence on the quality of the grapes and implicitly of the wine.

MATERIAL AND METHOD

Two varieties created at the Murfatlar Research Station were studied, one for white wines, Columna, and one for red and aromatic rosé wines, Mamaia.

The Columna variety was homologated in 1985; it was obtained by sexual hybridization of the Pinot gris and Grasă de Cotnari varieties; it is a variety that stands out for its straight shoot growth, in the form of a curtain, that allows the airing of the stocks and the display of the grapes, facilitating cultivation in ecological system; from a qualitative point of view the must stands out for its relatively high acidity of 5-6 g/L.

The Mamaia variety was approved in 1991; it was obtained by sexual hybridization between the Merlot x (Babeasc㠠neagră x Muscat Ottonel) varieties; this variety is also suited for cultivation in an ecological system, obtaining quality wines that are easily distinguished by the specific aroma.

The experimental plots were placed on a parcel with N-S exposition, slope of 3-5%, soil of chalky chernozem type with clay texture. The vine is planted on the rootstock *Vitis berlandieri x V. riparia Oppenheim 4 selection*, clone SO_4-4 and trained with semi-high stems of 70 cm and bilateral cordons with cane pruning. The density of the plantation was 4,132 vines/ha, with 2.2 m between rows and 1.1 m between trunks.

The meteorological data presented in this study are part of the climatic database recorded daily using an automatic iMetos 3.3 station of SCDVV Murfatlar, including daily observations of maximum and minimum temperatures, insolation and precipitation.

The data on the development stage of the plants were recorded following observations in the field, noting the budburst on the date when 50% of the existing buds are in the "woolly" stage (cf. Baggiollini); in the case of flowering, the date at which 50% of the inflorescences were opened was considered, and in the case of veraison, the date at which 50% of the berries became pigmented was taken into account.

The must sugar content was determined refractometrically; the total acidity by titrimetry with NaOH solution of 0.1 n concentration; the pH was determined using the WTW inoLab pH 7110 device. Wine physical-chemical composition was evaluated based on the general composition parameters (alcohol concentration, total and volatile acidity, reducing sugar, non-reducing dry extract) which were determined by standardized methods.

The statistical computation was performed using the SPSS Statistics 17.0 program, applying the Duncan test, for DS 5%.

RESULTS AND DISCUSSION

In order to observe the influence of the meteorological parameters during the three years under study (2020-2022) on the production of amaia and Columna varieties, the average monthly temperatures and the amount of precipitation were compared with the average values recorded in the last two decades, correlated with the influence on vegetative phenophases and on the productivity of these varieties from a quantitative and qualitative point of view. The temperature regime, as can be seen in Figure 1, registered a significant increase especially in the months preceding the vegetative season (February and March) compared to the multiannual averages (2000-2019). In February, the average temperatures recorded were approximately 2°C higher, the largest difference of 2.3° being recorded in 2021, and the smallest difference of 1.8 °C in the year 2022. In March the difference in temperature from the average was moderate, with a significant increase of 3.6°C in 2020. These differences in temperature led to the early onset of budding, especially in 2020, and the early start of the vegetation period. Significant temperature differences can be observed also during the ripening period (June and August) during all the years under study, for the month of July the difference being over 3°C, with the biggest difference (3.7°C) in 2020. In August, there were differences between 2.5 and 3.5°C, the smallest difference compared to the multiannual average being recorded in 2021, and the largest in 2022. This phenomenon led to an increased water stress on the plant and a shortening of the ripening period of the grapes.

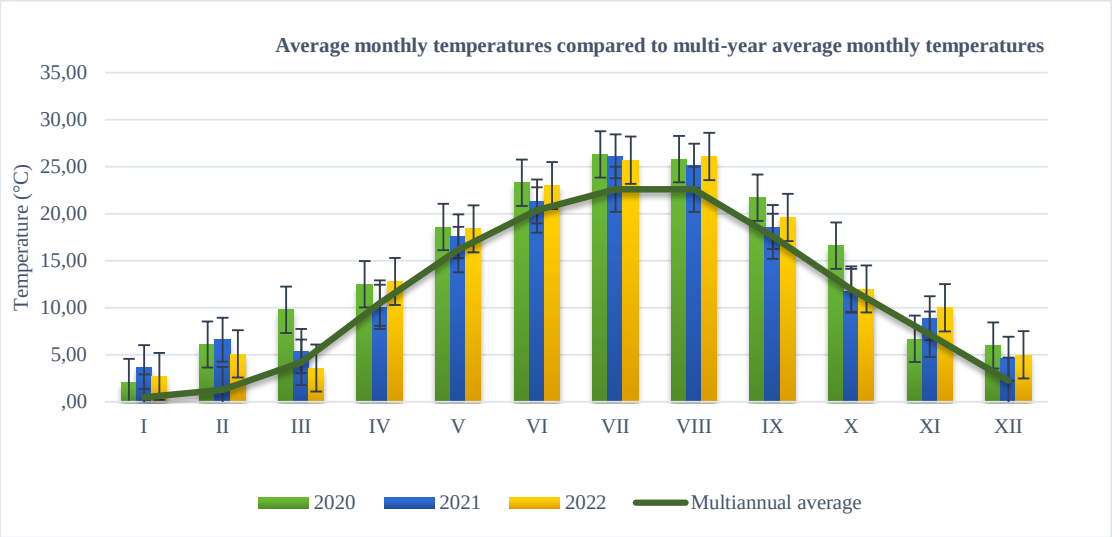


Figure 1. Average monthly temperatures compared to multiannual average monthly temperatures (2000-2019) under the conditions of the Murfatlar wine-growing center // Temperaturile medii lunare comparativ cu temperaturile medii lunare multianuale (2000-2019) în condițiile centrului viticol Murfatlar

Regarding the amount of precipitation, in Figure 2 it can be seen that it presents an uneven distribution, with the highest amounts of precipitation recorded in the months of January (109.0 mm) and June (130.4 mm) 2021, the year characterized by the highest amount of precipitation, with a total of 737.2 mm compared to the multiannual average of 436 mm.

During the three years under study, August was dry, with very low amounts - between 2.2 mm in 2020 and 6.8 mm in 2021, compared to the multiannual average of 31.6 mm.

The year with the biggest precipitation quantity among those studied was 2021, while the lowest amount of precipitation was recorded in 2020 – 333.1 mm.

Although the grapevine has a great capacity to adapt to moisture excess and deficit, these alternate periods of water deficit and excess lead to disruption of the plant's physiological processes, affecting vegetative growth and implicitly the production.

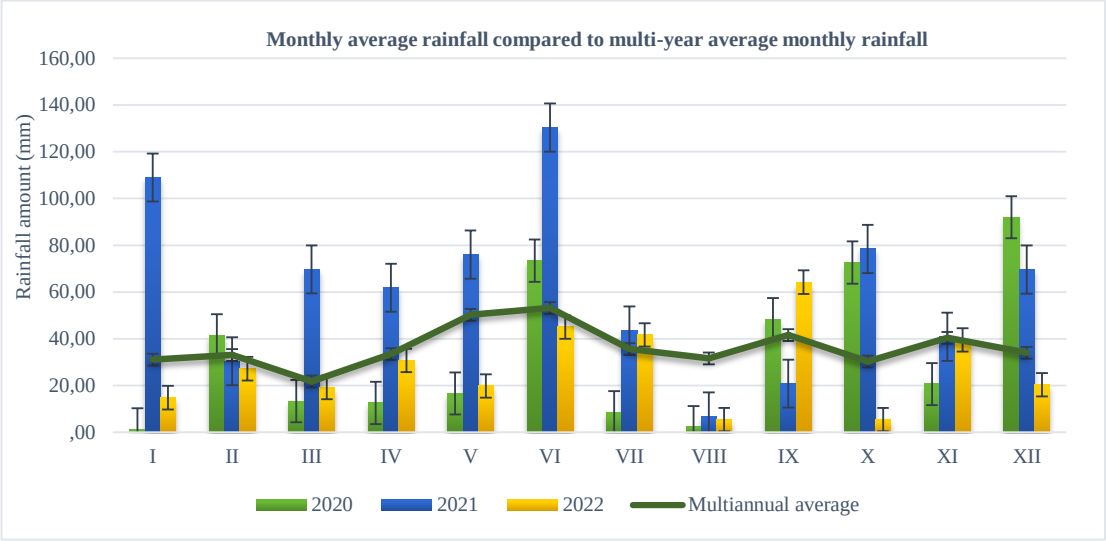


Figure 2. Monthly precipitation compared to multiannual average monthly precipitation (2000-2019) under the conditions of the Murfatlar wine-growing center // Precipitațiile lunare comparativ cu precipitațiile medii lunare multianuale (2000-2019) în condițiile centrului viticol Murfatlar

Grapevine phenology is strongly influenced by temperature. This relationship is so strong that vine phenology can be accurately predicted based on temperature-only dependent models (12). In recent years, observations from different wine regions of the world have shown changes in terms of the development of the grapevine and the ripening time of grapes, a phenomenon also observed in our country and, implicitly, in the Murfatlar vineyard.

Vegetation phenophases were complexly conditioned by the level and cumulative action of climatic factors. All these climate changes influenced the onset and the development of the phenophases of the vine vegetation with influences on the quality of the harvest. The vegetation onset for both varieties (budburst) was reached starting with the second decade of April, with the exception of 2020, for the Columna variety, when budburst occurred on 13. IV. Compared to the reference interval, the start of vegetation for the Columna variety was achieved on average 4 days earlier (on 18. IV compared to 22. IV), flowering and veraison were achieved one day earlier, and full maturity 5 days later. The harvest was carried out on average after 24. IX. For the Mamaia variety the start of vegetation was achieved 3 days later (on average on 23. IV versus 20. IV). Grapes veraison onset was 3 days earlier (on 11 August against 14 August) and full maturity of the grapes was reached during September 2 days earlier than the average period for 2000-2019 (on 15. IX compared to 17. IX). The grapes of the Mamaia variety were harvested 3 days earlier (on 17. IX compared to 20. IX) (Table 1).

Table 1. Vegetative phenophases for the varieties Mamaia and Columna between the years 2020 and 2023 // Fenofazele vegetative pentru soiurile Mamaia și Columna între anii 2020 și 2023

Variety	Year	Budding	Flowering	Veraison	Full Maturity	Harvesting date
Columna	**2020**	13 IV	29 V	10 VIII	16 IX	21 IX
	2021	20 IV	04 VI	20 VIII	27 IX	27 IX
	2022	24 IV	05 VI	09 VIII	19 IX	26 IX
Average 2020-2022		**13 IV -24 IV 18 IV**	**29 V-05 VI 01 VI**	**9 VIII -20 VIII 14 VIII**	**16 IX-27 IX 21 IX**	**21 IX-27 IX 24 IX**
Average 2000-2019		**11 IV-04 V 22 IV**	**21 V-15 VI 02 VI**	**04 VIII-26 VIII 15 VIII**	**04 IX-28 IX 16 IX**	**15 IX- 30 IX 23 IX**
Mamaia	**2020**	21 IV	04 VI	12 VIII	14 IX	14 IX
	2021	26 IV	03 VI	10 VIII	17 IX	20 IX
	2022	26 IV	03 VI	12 VIII	16 IX	21 IX
Average 2020-2022		**21 IV-26 IV 23 IV**	**03 VI- 04 VI 03 VI**	**10 VIII-12 VIII 11 VIII**	**14 IX-17 IX 15 IX**	**14 IX-21 IX 17 IX**
Average 2000-2019		**12 IV-29 IV 20 IV**	**24 V-10 VI 02 VI**	**29 VII-30 VIII 14 VIII**	**10 IX-25 IX 17 IX**	**15 IX-25 IX 20 IX**

High temperatures favor malic acid degradation, directly correlated with must acidity, and accelerate the accumulation of sugars by stimulating photosynthetic activity. Production quality in terms of sugar content and total must acidity (table 2) shows different accumulations depending on the potential of the variety and on the climatic conditions specific for each year.

In 2020, the Mamaia variety recorded a higher sugar content, slightly higher than the reference (described by ampelography), while the Columna variety accumulated a sugar content slightly below its potential (196 g/L). In 2021, both varieties were below the optimal sugar accumulation level, with values slightly below their potential. For the 2022 harvest, both varieties show superior values from this point of view, which can be considered the most favorable year among those studied, a fact that was reflected in the favorable climatic conditions of this wine year. From the point of view of total acidity, in the case of the three studied years, the two varieties presented values specific to the variety.

Table 2. Quality of grapes at the time of harvest for the varieties Mamaia and Columna between the years 2020 and 2022 // Calitatea strugurilor la momentul recoltării pentru soiurile Mamaia și Columna între anii 2020 și 2022

Variety	Year	Sugars (g/L)	Total Acidity (g/L H_2SO_4)	pH
Columna	2020	196.0±2.0 (b)	6.4±0.4 (a)	3.36±0.2 (a)
	2021	193.9±1.4 (a)	6.3±0.3 (a)	3.33±0.1 (a)

	2022	208.7±1.7 (a)	5.4±0.2 (b)	3.52±0.1 (a)
Mamaia	2020	206.6±2.1 (b)	4.9±0.2 (b)	3.78±0.1 (a)
	2021	197.0±1.6 (c)	5.3±0.2 (a)	3.49±0.2 (a)
	2022	210.8±2.5 (a)	5.1±0.2 (ab)	3.46±0.2 (a)

Average values, ± standard deviation (n=3), the letters represent the significance of the p<0.05 difference between variants. The difference between any two values followed by at least one common letter is insignificant

Wine quality was closely related to the quality of the must, as can be seen in table 3, the results obtained did not present significant differences from a statistical point of view. As for alcohol concentration, the highest values were recorded in 2022, the year in which the best harvests were obtained, both quantitatively and qualitatively. The total acidity has values typical of the two varieties, with lower values in 2022, due to the increased accumulation of sugars, inversely correlated with the metabolism of organic acids. In the case of the three years studied, higher values of volatile acidity are observed for the Mamaia variety, the wine of this variety being susceptible to the early development of this defect, a fact that involves concerns and the subject of future research within SCDVV Murfatlar.

Table 3. Wine quality for the Mamaia and Columna varieties between 2020 and 2022 // Calitatea vinului pentru soiurile Mamaia și Columna între anii 2020 și 2022

Variety	Year	Alcoholic content (%vol)	Total Acidity (g/L)	Volatile Acidity (g/L)	Nonreducing Extract (g/L)	Reducing sugars (g/L)	Density (g/cm^3)
Columna	2020	11.48±0.3 (a)	6.35±0.2 (a)	0.24±0.1 (a)	21.4±1.2 (a)	2.6±0.8 (a)	0.99198±0.0003 (a)
	2021	11.35±0.6 (a)	6.18±0.1 (a)	0.22±0.15 (a)	20.8±1.0 (a)	1.3±0.2 (b)	0.99046±0.0002 (b)
	2022	12.17±0.8 (a)	5.94±0.3 (a)	0.28±0.08 (a)	21.6±1.8 (a)	2.8±0.6 (a)	0.98854±0.0001 (c)
Mamaia	2020	12.03±0.4 (a)	5.65±0.2 (a)	0.56±0.04 (a)	24.1±1.5 (a)	4.0±0.5 (a)	0.99372±0.0002 (a)
	2021	11.52±0.3 (a)	5.83±0.1 (a)	0.54±0.1 (a)	25.0±1.2 (a)	2.6±0.4 (c)	0.99295±0.0001(b)
	2022	12.32±0.2 (a)	5.48±0.3 (a)	0.46±0.07 (a)	23.8±1.6 (a)	3.2±0.6 (ab)	0.99213±0.0002 (c)

Average values, ± standard deviation (n=3), the letters represent the significance of the p<0.05 difference between variants. The difference between any two values followed by at least one common letter is insignificant

The data concerning the sensory evaluation of the wines were processed in the form of sensory profiles, presented graphically in Figure 3. Although the analyzed wines present similar aromatic vectors, differences can be observed due to environmental factors specific to each year that influenced the complexity of the aromas. The aromatic characteristics, in the case of both varieties, were best highlighted in the conditions of the year 2022. In case of Columna variety, the predominant olfactory character is floral, of citrus and exotic fruits, and in case of Mamaia variety, floral and fruity.

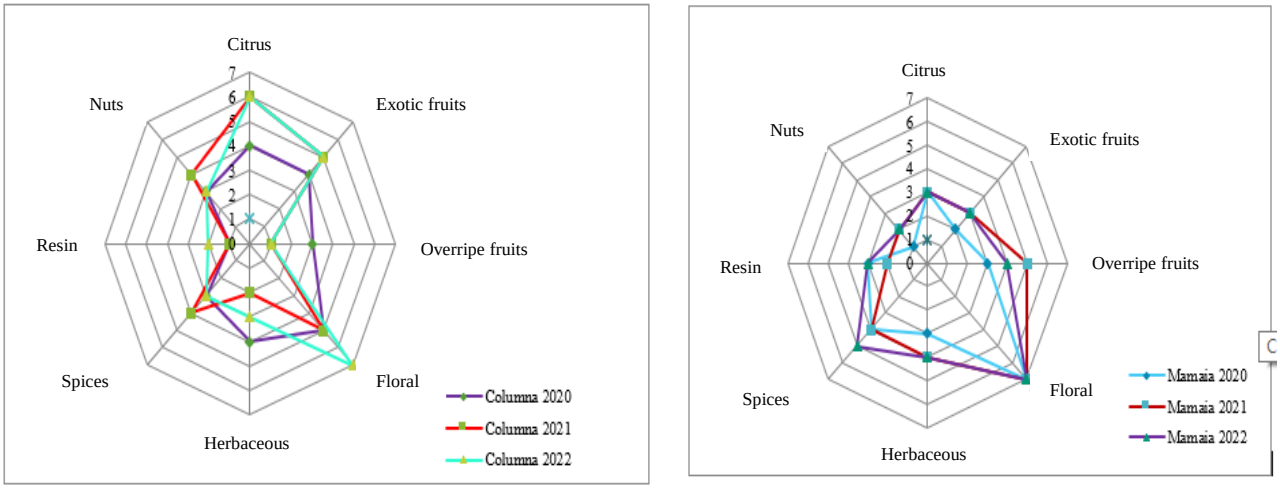


Figure 3. Sensory profile of the wines obtained fromColumna and Mamaia varieties // Profilul senzorial al vinurilor din soiurile Columna și Mamaia

Grape production was significantly influenced by weather conditions, as can be seen from Fig. 4, in 2020 recording the lowest productions for both varieties. The highest productions were achieved in 2022, the difference being 87.1% for the Columna variety and 49.2% for the Mamaia variety compared to 2020.

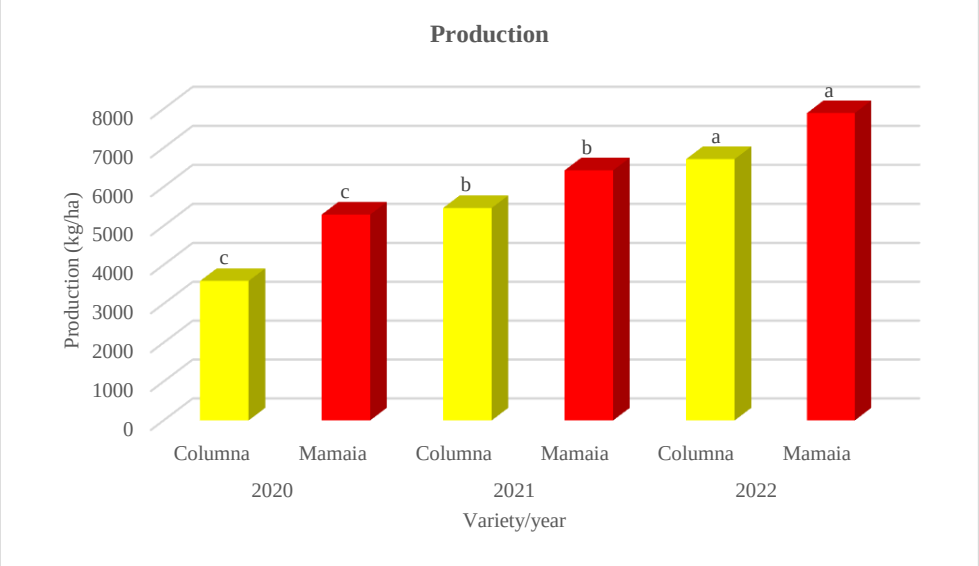


Figure 4. The production obtained for Columna and Mamaia varieties between 2020 and 2022 in the Murfatlar viticultural center // Producția obținută pentru soiurile Columna și Mamaia între anii 2020 și 2022 in centrul viticol Murfatlar

CONCLUSIONS

The increasing trend of air temperature values observed in recent years, especially in the months of February and March, by approximately +2°C, determines the early initiation of budding and the exposure of the buds to bad weather. The increase in temperature values observed during the ripening period, in the months of July and August, of 3-3.5°C determined changes in the metabolism and the processes of growth and development of the vine, influencing the quality and quantity of the obtained wine.

The water regime characterized by the alternation of dry periods with periods of excess rainfall also influenced the production of grapes, causing disruption of the plant's physiological processes and affecting vegetative growth.

The year 2022 met the most favorable conditions for plant development, which can be seen in terms of production, with a difference of 87.1% for the Columna variety and 49.2% for the Mamaia variety compared to 2020, in terms of quality of the must and wine, in 2022 both varieties accumulating over 200 g/L of sugars in the must, but also of the sensory profile.

Due to climate change, grapevine is increasingly exposed to conditions less favorable to its optimal development, a context that will bring new challenges to the European wine sector. Although this plant has physiological strategies for survival, such as the deep root system or the stomatal control of evapotranspiration, viticulture is strongly dependent on the climate. In order to effectively face these challenges, it is necessary to design innovative strategies, adapt viticultural technological practices, but also minimize the contribution that the wine industry itself has to this phenomenon.

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GOLIA - GRAPEVINE VARIETY WITH INCREASED FROST RESISTANCE

GOLIA - SOI DE VIŢĂ-DE-VIE CU REZISTENŢĂ SPORITĂ LA GER

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Rezumat. *Obţinerea unor noi soiuri valoroase de viţă-de-vie, cu producţie şi calitate superioară, care să posede însuşiri de rezistenţă genetică sporită la boli şi ger este rezultatul lucrărilor de cercetare din domeniul ameliorării. În acest context, la Staţiunea de Cercetare - Dezvoltare pentru Viticultură şi Vinificaţie Iaşi, în urma lucrărilor de hibridare sexuată între soiurile Sauvignon × Şarba, a fost omologat în anul 1999 soiul de viţă-de-vie Golia pentru vinuri albe de calitate. Soiul Golia a fost studiat în perioada 2015 - 2022, comparativ cu Fetească albă, unul dintre soiurile consacrate din sortimentul podgoriei Iaşi. Rezultatele obţinute privind vulnerabilitatea soiurilor la acţiunea temperaturilor scăzute, apreciată prin procentul de ochi viabili, atestă faptul că soiul Golia prezintă o rezistenţă genetică sporită la ger. De asemenea, faţă de datele înscrise la omologare, s-a remarcat un potenţial de producţie apropiat de normalul biologic al soiului şi o tendinţă de creştere a acumulărilor de zaharuri din must, acestea fiind condiţionate de schimbările climatice din ultimii ani. Astfel, în perioada de referinţă, valoarea medie a producţiei a fost de 3,57 kg/butuc la soiul Golia, fiind apropiată de cea a soiului martor Fetească albă (3,88 kg/butuc). La maturitatea deplină strugurii au acumulat în medie 217 g/L zaharuri şi 5,65 g/L aciditate (acid tartric). Vinul obţinut se remarcă prin tăria alcoolică (13,10 % vol.) superioară martorului Fetească albă (11,57 % vol.), extractul sec nereducător (19,0 g/L) şi conţinutul ridicat de compuşi fenolici (0,47 g/L).*

Cuvinte cheie: *plasticitate ecologică, rezistența la ger, soiuri noi, struguri pentru vin, Vitis vinifera* L.

Abstract. *Obtaining new valuable grapevine varieties, with superior grape yield and quality, possessing an increased genetic resistance to diseases and frost, is the result of breeding research programs. In this context, at the Research - Development Station for Viticulture and Oenology Iaşi, as a result of the sexual hybridization between the Sauvignon × Şarba varieties, was homologated in 1999 the new variety Golia, intended for quality white wines. The Golia variety was studied in the period 2015 - 2022, compared to Fetească albă, one of the most renowned varieties of the Iaşi vineyard assortment. The results obtained regarding the vulnerability of the varieties to low temperatures, assessed by the percentage of viable buds, attest that Golia variety has an increased genetic resistance to frost. Also, compared to the homologation data, was noted a production potential close to the normal biological yield of the variety and a tendency to increase the accumulation of sugars in the must, under the influence of the climate changes in recent years. Thus, in the study period, the average yield was 3.57 kg/stock for the Golia variety, very close to the control variety Fetească albă (3.88 kg/stock). At full maturity, grapes accumulated an average sugar amount of 217 g/L and a total acidity of 5.65 g/L (as tartaric acid). The obtained wine stands out for its alcoholic strength (13.10% vol.), superior to the Fetească albă variety (11.57 % vol.), a higher non-reducing dry extract (19.0 g/L) and polyphenolic content (0.47 g/L).*

Keywords: *ecological plasticity, frost resistance, new varieties, wine grapes, Vitis vinifera L.*

INTRODUCTION

The main risk factors for the grapevine are represented by the winter frost, assessed by the critical value of the absolute minimum temperature, the drought, due to small amounts of precipitation, combined with absolute maximum temperatures higher than 30°C, as well as other climatic factors of an accidental nature (rain in the winter and hail during the vegetation period). The vineyards from the N-E of the country, located towards the northern limit of the grapevine growing, are more and more affected by the climatic changes that have occurred in the last decades. The change in the environmental conditions causes changes in plant metabolism, in the unfolding of growth and development processes, with positive or negative influences on the quality and vitality of plants (Martin T., 1968).

In order to support the grapevine growers, scientific research in the field of plant breeding has accessed a topic aimed at creating new valuable genotypes, with superior yield and quality, and possessing properties of increased genetic resistance to diseases and frost (Oprea Șt. and Moldovan S.D., 2007; Calistru Gh. *et. al*., 1997). In this direction, at the Research - Development Station for Viticulture and Oenology Iaşi was homologated and patented the grapevine variety for white wines Golia, which stands out for its increased genetic resistance to frost, medium tolerance to drought and gray mold, and good resistance to downy mildew and powdery mildew. The research started in 1979 and was materialized through the homologation in 1999 of the new variety, named Golia, as the result of sexual hybridization between the Sauvignon × Șarba varieties. The genetic material was obtained by grafting on several rootstocks in order to assess the affinity to grafting and production (Calistru Gh. *et al.*, 1999). The variety was patented in 2004, after specific tests carried out by specialists from the State Institute for Testing and Registration of Varieties, who confirmed its biological value, authors: Dănulescu Dumitru, Calistru Gheorghe, Damian Doina and Crăcană Alexandru.

MATERIAL AND METHOD

The Golia variety was studied in the 2015 - 2022 period, compared to Fetească albă variety, one of the renowned varieties in the assortment of the Iasi vineyard. The plantations where the research was carried out are at full production capacity, located on a plot with a slope of 7-8 %, predominantly southern exposition, cambic chernozem soil, with planting distances of 2.2 m between rows and 1.2 m between plants, bilateral cordon system, with a stem height of 0.8 m. The agrotechnical measures, correlated with the level of climatic factors, consisted of dry pruning, spring plowing on the intervals maintained as a "black field", weed control with manual weeding on the row, and mechanical mowing of long-term grass strips, carrying out treatments for diseases and pests, and specific green works (weeding, tying shoots, pruning). The research focused on observations and determinations regarding the vegetation phenophases, frost resistance, behavior to the attack of pathogenic agents, the evaluation of the quantitative and qualitative production, expressed by the average number of grapes per vine, the average weight of a grape, calculated yield, the sugar and total acidity content of grape must and the main characteristics of the obtained wines (OIV, 2012).

RESULTS AND DISCUSSIONS

The overall analysis of the main climatic elements from the study period (2015-2022), compared to the multiannual average, highlighted the increase in the average annual temperature from 9.8 °C (multiannual value), up to 11.1 °C, with a thermal plus of 1.3 °C; the increase of the thermal balances during the vegetation period and the average temperatures in the months of July, August and September; the reduction in the amount of annual rainfall and during the vegetation period, and the increase in the number of days with temperatures > 30 °C, from 17.3 days to 42.4 days (table 1).

Table 1. Synthesis of the main climatic elements of the years 2015 - 2022 compared to multiannual averages // Sinteza principalelor elemente climatice ale anilor 2015 - 2022 comparativ cu mediile multianuale

Climatic elements analyzed	Multiannual average (1981-2010)	Average 2015 - 2022
Global thermal balance (Σt°g)	3168.4	3384.8
Active thermal balance (Σt°a)	3048.9	3274.9
Useful thermal balance (Σt°u)	1386.0	1604.9
Average temperature in July (°C)	21.0	22.6
Average temperature in August (°C)	20.3	22.5
Average temperature in September (°C)	15.6	17.3
Absolute air minimum temperature (°C)	-27.2/ 28.12.1996	- 21.0/ 01.01.2015
Absolute soil surface minimum temperature (°C)	-35.0/ 26.01.2010	-27.4/01.01.2015
Air average annual temperature (°C)	9.8	11.1
Maximum air temperature (°C)	42.3/20.07.2007	37.3/05.08.2017
Annual rainfalls (mm)	579.6	540.4

Rainfalls during the vegetation period (mm)	398.1	323.3
Insolation during the vegetation period (h)	1448.2	1481.6
Average maximum temperatures in August (°C)	26.9	31.4
Average temperature in Ist and IInd decades of June (°C)	19.1	20.5
Number of days with max. temperatures > 30°C	17.3	42.4
Duration of the bioactive period (days)	169.0	171
Real heliothermal index (IHr)	2.0	2.4
Hydrothermal coefficient (HC)	1.3	1.0
The viticultural bioclimatic index (Ibcv)	7.1	9.4
Oenoclimatic suitability index (IAOe)	4106.1	4681.3
Huglin heliothermal index (IH)	2095.2	2269.7
Night cooling index (CI)	11.2	11.9

Regarding the main synthetic bioclimatic indicators, it was noted: the increase of the real heliothermic index (IHr), from 2.0 to 2.4, ensuring a good maturation of the grapes; the decrease of the hydrothermal coefficient (HC), as a result of the registration of smaller amounts of rainfall during the vegetation period; the increase in the values of the viticultural bioclimatic index (from 7.1 to 9.4), which indicates higher heliothermic resources on the background of lower water resources during the growing season; the increase in the values of the oenoclimatic suitability index (IAOe), from 4106.1 to 4681.3, which confirms that the Copou - Iaşi viticultural center has favorable conditions for the growing of white grape varieties and medium favorability for the production of red wines. The increase of the Huglin heliothermic index (IH) from 2095.2 to 2269.7 classify the vineyard area, for the reference period, in the "warm temperate" climate category.

The main ampelographic characters

At bud burst the rosette is fuzzy, yellowish-green. The top of the shoot and the first leaves are covered with long, dense, light-green hairs. Young leaves are hairy, light-green. The adult leaf is small (12-14 cm long), orbicular, lobed (3 to 5 lobes), with a light-green limb, hairy along the nervures on the lower side. The lateral sinuses are opened in the U-shape and the petiolar sinus is V-shaped, more or less open. The leaf teeth are small and with convex edges. Insertion of the first inflorescence is at nodes 4-5, being distributed 1-2 per shoot. The flower is hermaphrodite, functionally normal. The grapes are compact, cylindro-conical, multiaxial and branched, small in size (114 g), having a length of 9-14 cm. The berries are small, uniform, round in shape, with a green-yellow colored skin with a persistent pistillar point, elastic and non-adherent to the pulp. The content of the pulp is white-greenish, juicy, sweet-sour, with a specific aroma (figure 1).



Figure 1. Golia variety // Soiul Golia

Agrobiological features

In the conditions of the Iași vineyard, the Golia variety unfold the phenological spectrum in a period of 175 to 180 days of active vegetation, 2-3 days later than the control variety Fetească albă, respectively from the second half of April, when bud burst starts and until the end of October, with the yellowing and falling of the leaves. The flowering takes place in the first decade of June, grape veraison starts in the middle of August and the grape maturity is reach in the IV-V epochs together with the other varieties in the assortment (tables 2 and 3).

Table 2. The phenology of the Golia variety in the Copou-Iaşi viticultural center (2015 - 2022) // Fenologia soiului Golia în centrul viticol Copou-Iaşi (2015 - 2022)

No.	Phenophase	Year								Limits
		2015	2016	2017	2018	2019	2020	2021	2022	
1.	Bud burst	27 IV	13 IV	16 IV	19 IV	25 IV	15 IV	30 IV	26 IV	13 – 30 IV
2.	Flowering	7 VI	9 VI	6 VI	25 V	11 VI	12 VI	22 VI	08 VI	25 V – 22 VI
3.	Grape veraison	12 VIII	14 VIII	16 VIII	29 VII	07 VIII	16 VIII	20 VIII	10 VIII	29 VII – 20 VIII
4.	Grape maturity	16 IX	12 IX	13 IX	17 IX	30 IX	24 IX	07 X	22 IX	12 IX -07 X

Table 3. The phenology of the Fetească albă variety in the Copou-Iaşi viticultural center (2015 - 2022) // Fenologia soiului Fetească albă în centrul viticol Copou-Iaşi (2015 - 2022)

No.	Phenophase	Year								Limits
		2015	2016	2017	2018	2019	2020	2021	2022	
1.	Bud burst	21 IV	10 IV	12 IV	14 IV	23 IV	13 IV	29 IV	21 IV	10 – 29 IV
2.	Flowering	5 VI	2 VI	2 VI	21 V	7 VI	6 VI	18 VI	4 VI	21 V – 18 VI
3.	Grape veraison	4 VIII	5 VIII	31 VII	21 VII	02 VIII	6 VIII	5 VIII	2 VIII	21 VII – 6 VIII
4.	Grape maturity	14 IX	18 IX	12 IX	25 IX	13 IX	24 IX	27 IX	21 IX	12 IX -27 X

Behavior to climatic stress factors - frost resistance

Specialized literature shows that the frost resistance limit of grapevine is between -15 °C ... -18 °C for buds of table grape varieties, -18 °C ... -21 °C for wine varieties, the annual canes are affected at temperatures of -20 °C ... -21 °C and the perennial wood at -22 °C ... -24 °C (Martin *et al.*, 1972; Oşlobeanu *et al*., 1991).

In the period 1998-2022, temperatures below the freezing limit of the grapevine were recorded in the years: 2001, 2003, 2006, 2010, 2012, 2014, 2015, 2017 and 2018. Analysis of the absolute minimum temperatures in the winter months showed that the lowest value was recorded on January 26th, 2010, respectively -27.0 °C in the air and -35.0 ^{o}C at the surface of the soil. Also, the average of the lowest absolute minimum temperatures in the air was -18.2 ^{o}C and on the soil surface -23.6 ^{o}C (table 4). These values confirmed that the Copou-Iaşi viticultural center is located in the semi-protected grapevine growing area.

Table 4. Absolute minimum temperatures recorded in the Copou-Iasi viticultural center (1998 -2022) // Temperaturile minime absolute înregistrate în centrul viticol Copou Iaşi (1998 -2022)

Year	in the air			at soil surface		
	month	day	t °C	month	day	t °C
1998	XII	03	-19.0	XII	24	-24,0
1999	XII	24	-13.0	II	20	-19,3
2000	I	25	-15.9	I	26	-22,2
2001	**XII**	**18**	**-20.4**	**XII**	**18**	**-24,5**
2002	XII	26	-19.8	XII	26	-21,0
2003	**I**	**13**	**-21.6**	**I**	**13**	**-30,6**
2004	I	31	-17.0	I	31	-19,0
2005	II	08	-19.4	II	06	-27,6
2006	**I**	**23**	**-25.1**	**I**	**25**	**-29,0**
2007	II	24	-19.6	II	24	-25,0
2008	I	05	-19.5	I	05	-24,2
2009	XII	19	-17.0	XII	21	-29,0
2010	**I**	**26**	**-27.0**	**I**	**26**	**-35,0**
2011	I	05	-14.8	I	25	-20,5
2012	**II**	**12**	**-26.7**	**II**	**08**	**-33,0**
2013	I	09	-14.3	I	29	-20,5

2014	**I**	**31**	**-20.6**	**I**	**31**	**-22,5**
2015	**I**	**01**	**-21.0**	**I**	**01**	**-27,4**
2016	I	04	-17.5	I	03	-18,2
2017	**I**	**20**	**-18.7**	**II**	**11**	**-27,1**
2018	**I**	**24**	**-19.7**	**I**	**24**	**-25,5**
2019	I	8	-12.0	I	8	-22,5
2020	I	7	-8.4	I	9	-7,0
2021	I	19	-16.3	I	19	-22,1
2022	I	25	-11.6	I	21	-13,8
		average	**-18.2**		**average**	**-23.6**

In order to highlight the levels at which temperature drops are possible and to appreciate the degree to which they act destructively on the various grapevine parts, the lowest absolute temperature was analyzed, establishing the number of days with negative temperatures lower or equal to -15 ^{o}C, -20 ^{o}C and -25 ^{o}C, considered as climatic accidents for grapevine, the frequency of the years in which these low temperatures occur, as well as their periodicity. Thus, in the air, the *temperatures considered dangerous for the grapevine* (≤ -15 ^{o}C), at which the fruit buds are affected, were recorded in the analyzed period in 60% of the years in January, in 32% of the years in February and only 16% of the years in December.

Very dangerous temperatures (≤ -20 ^{o}C), which affect buds and annual wood, were recorded in 20% of the years in January, in 4.0% of the years in February and 4.0% of the years in December. The periodicity of temperatures ≤ -20 ^{o}C was 5 years in January, 25 years in February and 25 years in December (table 5).

Table 5. Frequency of negative temperatures recorded in the Copou Iasi viticultural center (1998 – 2022) // Frecvența temperaturilor negative înregistrate în centrul viticol Copou Iași (1998 – 2022)

Analyzed elements	Negative temperature groups					
	≤ - 15°C		≤ - 20°C		≤ - 25°C	
	air	soil	air	soil	air	soil
January						
Number of days	28	75	9	25	3	13
Number of years	15	17	5	16	2	5
Frequency of years (%)	60,0	68,0	20,0	64,0	8,0	20,0
Periodicity of the years	1,7	1,5	5,0	1,6	12,5	5,0
February						
Number of days	19	34	6	19	1	14
Number of years	8	14	1	8	1	6
Frequency of years (%)	32,0	56,0	4,0	32,0	4,0	24,0
Periodicity of the years	3,1	1,8	25,0	3,1	25,0	4,2
December						
Number of days	12	46	1	15	-	2
Number of years	4	10	1	6	-	2
Frequency of years (%)	16,0	40,0	4,0	24,0	-	8,0
Periodicity of the years	6,3	2,5	25,0	4,2	-	12,5

Temperatures considered extremely dangerous for the grapevine (≤ -25 ^{o}C), which affect all parts of the plant, were recorded with a frequency of 8% of the years in January and 4% of the years in February. In December, during the analyzed period, no temperatures ≤ - 25 ^{o}C were recorded. The frequency of years with temperatures ≤ -25 °C was 12.5 years in January and 25.0 years in February.
At the soil surface, a much higher frequency of very low temperatures was found compared to those in the air. These temperatures led to significant losses of main buds, damage to the annual and multiannual wood and, implicitly, to the obtaining of grape yields far below the average of thermally normal years. In conclusion, from the analysis of the lowest temperatures recorded in the winter months, it was found that, in all years, the lowest temperatures considered as climatic accident were in January.

The results obtained regarding the vulnerability of the studied varieties to the action of low temperatures, assessed by the percentage of viable buds, attest that Golia variety has an increased

genetic resistance to frost, a particularly important aspect for the vineyards of the Moldavian area and for the entire country (table 6).

Table 6. Genetic resistance characteristics // Însuşirile de rezistenţă genetică

Analyzed elements	Golia	Fetească albă
Frost resistance assessed in % viable buds		
✓ average 1985 – 1997	75	68
✓ average 1998 – 2022	83	46
✓ average in years with minimum temperatures > -20°C	53	18
✓ average in the years with min. temperatures between -15°C and -20°C	78	21
Resistance to cryptogamic diseases noted in the OIV scale		
✓ downy mildew	7-8	7-8
✓ powdery mildew	9	9
✓ gray mold	5-7	6-7

In the conditions of an average number of anti-cryptogamic treatments, Golia variety showed a resistance specific to *V. vinifera* L. varieties, respectively, good to downy mildew and powdery mildew, and medium to gray mold.

Regarding the elements that define the fertility of the studied varieties, it was found that, compared to the control variety, the percentage of fertile shoots was higher for Golia variety, 56% during the homologation period and 73% in the 2015-2022 interval (table 7).

In the conditions of an approximately equal bud load attributed by pruning, the average number of inflorescences varied depending on the hereditary specifics of the varieties. The values of the absolute fertility coefficient were supraunitary, the relative coefficient falling between 0.67 (Fetească albă) and 1.08 (Golia).

Table 7. Evolution of the agrobiological and productivity characteristics of the Golia variety compared to the control // Evoluţia însuşirilor agrobiologice şi productive ale soiului Golia comparativ cu martorul

No.	Parameters	Golia		Fetească albă (control)	
		Average 1992-1995 (at homologation)	Average 2015 - 2022	Average 1992- 1995	Average 2015 - 2022
1.	Fertile shoots (%)	56	73	48	71
2.	Absolute fertility coefficient (c.f.a.)	1.36	1.44	1.32	1.31
3.	Relative fertility coefficient (c.f.r.)	0.77	1.08	0.67	0.94
4.	Absolute productivity index (i.p.a.)	162	120	149	123
5.	Relative productivity index (i.p.r.)	95	106	78	90
6.	Yield (kg/vine)	3.51	3,37	2,79	3,88
7.	Average weight of a grape (g)	117	86	113	104
8.	Weight of 100 berries (g)	117	116	167	158
9.	Sugars (g/L)	187	217	183	193
10.	Total acidity (g/L as tartaric acid)	6.36	5.65	4,66	5.06
11.	Alcoholic potential (% vol.)	11.0	12.58	10,8	11.35

The Golia variety showed high values of the absolute and relative productivity indices, close to the control variety. Comparing the results regarding the fruiting capacity, it can be concluded that Golia variety reached its biological potential and presents a good adaptability to the conditions of the studied ecosystem.

The analysis of the technological characteristics of grape production completed the elements of knowledge for the Golia variety. The number of grapes/stock and the yield obtained (kg/stock), confirmed the data on the fertility of Golia variety, compared to the control. In comparison with the initial approval data (at homologation), in the reference period 2015 - 2022, there were changes in the quantitative level of grape yield obtained, as a result of the climate changes recorded in recent years. Thus, the average value of the grape production was 3.57 kg/stock for Golia variety, very close to that

of the control variety Fetească albă (3.88 kg/stock).

The quality of the harvest, assessed by the average weight of a grape, the weight of 100 berries, the sugar content and the total acidity of the must, reflects both the specific genetic character of the variety and the influence of climatic factors on these elements. Golia variety is characterized by a higher sugar accumulation potential than the control variety (average values of 217 g/L for the Golia variety, respectively 193 g/L for the Fetească albă), under conditions of an average acidity of 5.65 g/L as tartaric acid.

On the background of global warming, the wines obtained from the Golia variety showed in recent years a higher alcohol concentration, varying between 12.90 and 13.40 % vol., with a balanced total acidity (5.55 - 6.47 g/L acid tartric), completed by a low pH, which confers to the wine a pleasant sensation of freshness (table 8).

Table 8. The main compositional characteristics of wines //
Principalele caracteristici de compoziție ale vinurilor

No.	Analyzed parameters	Golia	Fetească albă (control)
1.	Alcoholic concentration (% vol.)	13.10	11.57
2.	Total acidity (g tartaric acid /L)	5.93	5.42
3.	Volatile acidity (g acetic acid /L)	0.44	0.37
4.	Free SO_2 (mg/L)	28	28
5.	Total SO_2 (mg/L)	87	88
6.	Reducing sugars (g/L)	2.13	1.21
7.	pH	3.31	3.43
8.	Total dry extract (g/L)	21.13	16.63
9.	Non-reducing extract (g/L)	19.00	15.43
10.	Density (g/cm^3)	0.9912	0.9910
11.	Total polyphenols (g EAG/L)	0.47	0.44
12.	Polyphenol index (OD 280 nm)	8.74	7.93
13.	Color intensity (Ic)	0.109	0.10
14.	Color tint (T)	4.95	3.47
15.	d 420%	78.54	71.75
16.	d 520 %	15.92	20.25
17.	d 620 %	5.55	8.00

Sensory, the wines of Golia variety were appreciated as being of high refinement and elegance, standing out for their yellow-green color of medium intensity, bright limpidity, low content of reducing sugars (dry wines), balanced concentrations of phenolic compounds and a special extractive richness and texture. From the olfactory point of view, the wine of the Golia variety is characterized by its fine aromas of green fruit and fresh green pepper, with citrus notes, reminding of those of the genitors (Sauvignon blanc × Şarba). All this are completed by a superior full body and a high persistence of taste, which makes the wine of the Golia variety appreciated as a complex, balanced, ample and generous.

In the Copou-Iaşi viticultural center, the wine of the Fetească albă variety showed lower alcohol concentrations compared to the wine of the Golia variety, rarely exceeding 12.00 % vol., due to a lower biological capacity to accumulate sugar in grapes. The total acidity was balanced (5.17 - 5.85 g/L as tartaric acid), even slightly lower in years with long and warm autumns, completed by a higher pH (3.4-3.5). Compared to the wine of the Golia variety, the wine of Fetească albă presented a lower non-reducing extract, on the background of a lower content of phenolic compounds, which is sensorially translates into a lighter-body perception, lower extractiveness and taste persistence. On the other hand, the yellow-green color, with bright golden hues, the finesse and delicacy of the floral aromas (grapevine flowers, linden), as well as the textural harmony, represent the basic characteristics of the wines obtained from the grapes of the Fetească albă variety.

CONCLUSIONS

The results regarding the vulnerability to low temperatures, assessed by the percentage of viable buds, attest the fact that Golia variety possess an increased genetic resistance to frost, a particularly important aspect for the vineyards located in the areas where this phenomenon occurs with a high frequency.

In comparison with the data obtained at the homologation, during the research period (2015 – 2022), changes were found in the quantitative level of grape production, as a result of the climate changes recorded in recent years.

The quality of the harvest, assessed by the average weight of a grape, the weight of 100 berries, the sugar content and the total acidity of the must, reflects both the specific genetic character of the variety and the influence of the climatic factors. Golia variety was characterized by a higher sugar accumulation potential than the control variety Fetească albă.

Due to global warming, in recent years, the wines obtained from the Golia variety grapes showed higher alcohol concentrations, varying between 12.90 and 13.40 % vol., with a balanced acidity (5.55 - 6.47 g/L tartaric acid), which confers to the wine a pleasant sensation of freshness. Also, the wines of the Golia variety were appreciated for their yellow-green color, bright limpidity, low content of reducing sugars (dry wines) and balanced concentrations of phenolic compounds.

The variety is included in the assortment of vineyards from the region of Moldavian Hills (Dealurile Moldovei), for obtaining quality white wines, being also recommended for plantation in other viticultural areas with climatic conditions similar to those of the Iasi vineyard (Odobeşti, Blaj).

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CONTRIBUTIONS TO THE IMPROVEMENT OF APRICOT AND PEACH ASSORTMENTS AT RSFG CONSTANȚA

CONTRIBUȚII LA ÎMBUNĂTĂȚIREA SORTIMENTULUI DE CAIS ȘI PIERSIC LA SCDP CONSTANȚA

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Abstract

The improvement of apricot and peach the assortments, with new cultivars which are more productive and resistant to frost and specific diseases, with fruit of good quality is one the basic objective of the breeding programs of the two species. The purpose of this paper is to present the apricot cultivar - De Valu and the peach cultivar - Florica, that were obtained by the team of researchers from RSFG Constanța, patented after 2020. The mentioned cultivars have constant yields and valuable fruits, so it's deserved to be extended in Romanian orchards.

Key words: breeding, cultivars, yields, fresh consumption

Rezumat

Îmbunătățirea continuă a sortimentelor de cais și de piersic cu soiuri noi, care să asigure un consum de fructe o perioadă cât mai mare de timp, productive, rezistente la ger și la bolile specifice, constituie obiective de bază în programele de ameliorare a celor două specii, deosebit de apreciate de consumatori. Scopul acestei lucrări este de a prezenta soiul de cais- De Valu și soiul de piersic- Florica, soiuri ce au fost obținute de către colectivul de cercetători de la SCDP Constanța, brevetate după anul 2020 și care datorită producțiilor constante și a calității fructelor merită să fie extinse în livezile din România.

Cuvinte cheie: ameliorare, soiuri, producții de fructe, consum proaspăt

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is a species highly appreciated by consumers, due to its fruits of exceptional quality, very fine and aromatic, which can be consumed both fresh and processed in different forms. According to FAO data, in 2020 the total production of apricots worldwide was 3,719,974 tons, with Asia in first place with a percentage of 65% of the total, followed by Europe with 20%. Africa follows with 12%, North America with 2%, South America with 1% and Australia and New Zealand below 1%, Fig. 1.

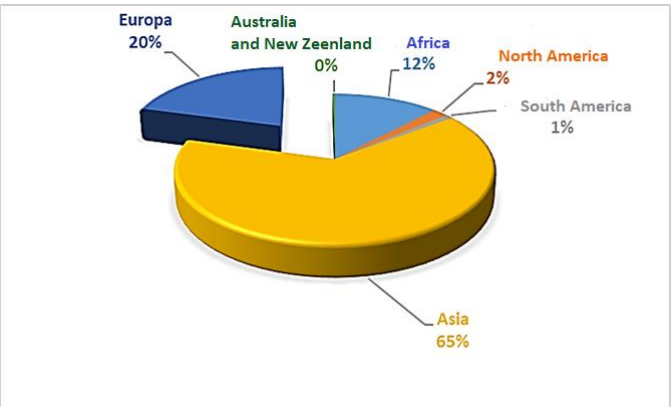


Fig. 1. Apricot yield in 2020 represented by continents // Producția de caise în anul 2020 reprezentată pe continente

In our country, apricots are cultivated in the south-east, south-west and west, where the pedoclimatic conditions are favourable for their growth and fruiting. Since 1977, the apricot breeding program carried out at RSFG Constanta has aimed to broaden the range, to obtain late flowering varieties with qualitative fruits, superior productive characteristics, biological resistance to abiotic and biotic stress factors (Cociu, V., 1991; Cociu, V., p. 1993; Topor, E., 1997, 2006, 2007; Opriţă V. A, 2018, 2020). There were registered 15 varieties of apricot, of which three were patented, de Valu being the last variety of apricot patented in 2020.

Peach (*Prunus persica* (L.) Batsch] is one of the most valuable fruit species, due to the biological characteristics of the trees and the quality of the fruits. Trees bear fruit quickly, and in favorable growing areas, yields are high and constant. The fruits can be eaten fresh or in the form of jam, preserve, compote, juices, etc. Currently, there are a large number of varieties acclimatized or created in our country, which provide fresh fruit from the last decade of June to mid-September. Peach culture is spread over all continents, at the level of the year 2022 large areas holding Asia (72%), Europe (15%), Africa (6%) and South America (4%), figure 2.

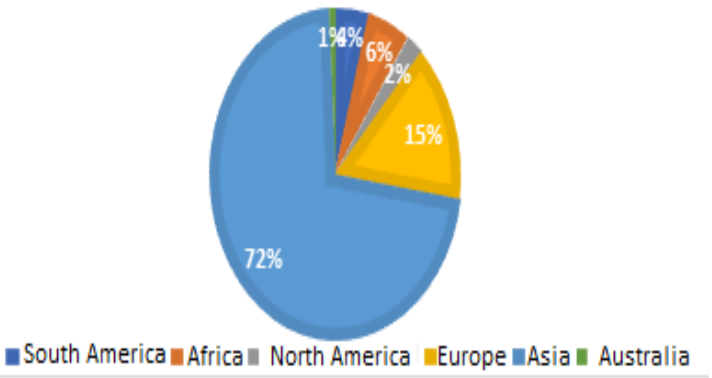


Fig. 2- Peach area by continents, 2020 / Suprafața ocupată cu piersic pe continente, 2020 (sursa FAOSTAT)

FAO data show that the world production of peaches in 2020 was 24,569,744 tons (table 1), with peaches ranking third, after citrus fruits and apples, in the total number of fruits produced worldwide.

Table 1. Production of peaches and nectarines by continents and in the main growing countries (thousands of tonnes) // Producția de piersici și nectarine pe continente și în principalele țări cultivatoare (mii tone)

The continent/ The country	Production years					% of total 2020	
	2016	2017	2018	2019	2020	Worldwide	Continental
Worldwide total	23 263	24 165	24 307	24 841	24 567	100	-
d.c. Europa	3 930	4 254	4 040	4 245	3 656	14,9	-
North America	881	790	728	643	582	2,5	-
Asia	16 196	16 851	17 250	17 717	18 075	73,5	-
South America	1 013	876	960	906	905	3,8	-
Africa	935	1 100	1 037	1 035	1 047	4,5	-
Australia	80	82	82	86	81	0,5	-
USA	855	764	700	617	560	-	96,2
China	13 585	13 988	14 386	14 770	15 016	-	83,0
Italy	1 427	1 250	1 090	1 224	1 015	-	27,8
France	207	221	184	202	178	-	4,9
Spain	1 421	1 799	1 450	1 545	1 306	-	35,8
Greece	528	640	968	926	890	-	24,4

As in other regions of the world, in Romania, peach cultivation coincides as an area with that of the vine. Currently, in our country, there are approx.1700 ha cultivated with peach. At the level of 2020, in Romania the production of peaches and nectarines was 15,900 tons (https://www.fao.org/faostat/en/#data/QCL). Unfortunately, although the population appreciates the native varieties, on the market there is a massive import of peaches from Mediterranean countries.

Between 1977-2022, the peach breeding program at RDRP Constanța aimed at resistance to diseases and pests, frost, drought, trees habits, fruit quality (size, shape, weight, color, pulp texture, sugar content, acidity, aroma), the productivity, the ornamental character of flowers and leaves (Cociu V., 1981; Ionescu Pr., 1991; Dumitru L.M., 2003, 2007, 2010, 2013; Gavăt C., 2021). During this period, 32 varieties of peach, nectarine, pavia were created, both standard, semi-dwarf and dwarf; four varieties of peach were obtained variety patents.

The purpose of this paper is to present the two varieties, apricot (de Valu), respectively peach (Florica), that was patented after 2020 and which due to the constant production and quality of the fruits deserve to be expanded in Romanian orchards.

MATERIAL AND METHOD

RSFG Constanta is located in the south-eastern area of the country. The climate of the area is semi-arid, with hot and very dry summers and temperate winters. The average annual temperature is 10.7 °C and the annual rainfall is approx. 400 mm, with an uneven distribution during the year. The soil is calcareous chernozem, formed on loess, with a clayey texture (Păltineanu et al., 2015).

The germplasm stock of the two species, apricot and peach, consists of a large number of genotypes, which have been used in breeding work since 1977. Directed hybridization was used as a working method, followed by self-pollination of hybrids and rigorous selection in hybrid offspring

regarding resistance to frost, drought, diseases, plant habits, flowering phenology, followed by observations on fruit quality, production, etc. (Cociu V., 1989).

De Valu (synonym Orizont clonă 1) cultivar was obtained at RSFG Constanta and then was tested in competition microculture and enrolled at ISTIS for registration in 2017.

Florica (synonym X sel.V.T) cultivar obtained at RSFG Constanța through individual selection, resulting from self-pollination of the hybrid H 25 11 68; it was studied in 1998 and enrolled at ISTIS for registration in 2019. Peach selection was tested in a competition culture at UASVM Bucharest.

After registration at ISTIS, fruit quality was assessed by fruit size, skin and pulp colour, consistency, succulence, pulp aroma and taste, stone size and easy pulp separation according to UPOV descriptors (https://www.upov.int/test_guidelines/en/list.jsp). Determinations were made regarding fruit content in dry matter (%) and titratable acidity (mg%).

Since 2011, the trees of the patented varieties can be found in the demonstration plots at RSFG Constanța, on rows 9 and 10 the De Valu variety grafted on Constanța 14 and on rows 25 and 26 the Florica variety grafted on Tomis 1.

RESULTS AND DISSCUSIONS

De Valu apricot cultivar

Characteristics of the variety: it is self-fertile, does not require pollinators, and can be planted alone in a plot; it is resistant to the main specific apricot diseases, free from viruses;

The tree is of medium vigour, with an erect posture, it bears fruit predominantly on May bunches. The flower is medium-sized, and white in colour, the stigma is positioned higher in relation to the stamen. Table 2 shows the average yield in the last 5 years (2018-2022). **The fruit** is oblong, slightly elongated, and *large in size* (70 g). The skin is light orange, the covering colour absent or very little on the sunny side. The pulp is orange, very juicy, flavourful, with fine texture and medium firmness. The core is medium-sized, with a sweet kernel, non-stick to the pulp.

Table 2. Average fruit yield in De Valu compared with Goldrich (Control)/ Producția medie de fructe* la soiul De Valu comparativ cu Goldrich (Martor)

Cultivar	Fruit -Average yields									
	2018		2019		2020		2021		2022	
	Kg/tree	t/ha	Kg/tree	t/ha	Kg/tree	t/ha	Kg/tree	t/ha	Kg/tree	t/ha
De Valu	18.0	11.2	21.0	13.1	4.0	2.5	19.0	11.2	17.0	10.6
Goldrich	10.0	6.2	11.0	6.8	3.0	1.8	10.0	6.2	17.0	10.6

*planting density of 625 trees/ha





Fig. 3 și Fig. 4- De Valu

Table 3. Fruit characteristics (multiannual data) // Caracteristici de calitate ai fructelor (date multianuale), Valu lui Traian

Cultivar	Average fruit weight (g)	Soluble solid content (%)	Acidity (mg%)	Fruit destination
De Valu	75 - 90	11.5 – 14.0	0.8 – 1.1	Fresh consumption and proccesing
Goldrich (Mt)	72 - 84	11.5 – 13.6	1.4 – 1.9	Fresh consumption and proccesing

Florica peach cultivar

The characteristics of the cv.: it is self-fertile, does not require pollinators, and is resistant to frost; it is tolerant to the main diseases, requires treatments at the warning; requires fruit thinning and irrigation; the average yields are shown in table 4.

The tree is of medium vigour, with an erect posture, it bears fruit mainly on mixed branches; Medium flowering, abundant every year; The flower is campanulate, on type 5, the shape of the petals is medium oval, and dark pink in colour; The stigma is positioned lower in relation to the stamen.

The fruit is medium-sized, 150-170 g (table 5, fig. 5), round in shape, with the pistil extremity slightly deepened and with the prominence of the middle suture. The background colour of the epidermis is yellow, the covering dark red, and marbled; the thickness is medium and the adhesion to the pulp is weak. The pulp is yellow-orange (fig. 6), with the intensity of anthocyanin pigmentation around the core weak, firm, and very crunchy, with a pleasant taste and intense aroma. The core is non-adherent, and medium in size (7.1 g).





Fig. 5 and Fig. 6-Florica cv.

Fruit ripening period: late, the 3rd decade of August, after the Southland variety, *extends the varietal assembly line with dessert peaches* until the Flacăra cultivar ripens (01.09).

Table 4. Average fruit production* in the Florica cv. compared to the Flacăra cv. (control) /Producţia medie la soiul Florica comparativ cu soiul Flacăra (martor)

Cultivar	Average fruit yield									
	2018		2019		2020		2021		2022	
	Kg/tree	t/ha	Kg/tree	t/ha	Kg/tree	t/ha	Kg/tree	t/ha	Kg/tree	t/ha
Florica	25.5	21.2	26.0	21.6	27.2	22.9	27.7	23.0	28.0	23.3
Flacăra	24.8	20.6	25.4	21.2	26.0	21.7	27.2	22.6	27.7	23.0

*planting density of 833 trees/ha

Table 5. Fruit quality characteristics (multiannual data), Valu lui Traian / Parametrii calitativi ai fructelor (valori multianuale)

Cultivar	Average fruit weight (g)	Dry substance (%)	Acidity (mg%)	Fruit destination
Florica	160-180	14.5	0.65	Fresh consumption and proccesing
Flacăra (Mt)	180-200	14.0	0.76	Fresh consumption and proccesing

CONCLUSIONS

1. The pedoclimatic conditions in southeastern Romania are favourable for the growth and fruition of apricot and peach. It is recommended to observe all the agrotechnical links specific to apricot and peach culture (irrigation, thinning, etc.).

2. **The De Valu cv.** has constant yields, it is tolerant to the main diseases and pests. The late bloom offers it the advantage of passing over the late frosts in spring better than other apricot cv. the fruit aspect is pleasant, it has a specific flavour, and a big fruit. It is recommended both for fresh consumption and canning.

3. **The Florica cv.** *has good resistance to handling, transport and temporary storage*. It can be used for fresh consumption as the fruits are very crunchy and aromatic or in the form of compote and preserve.

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CHANGES IN PHENOTYPES OF *PINOT NOIR* AND *CHARDONNAY* VARIETIES DUE TO CLIMATE CHANGE IN THE MINIȘ VINEYARD

MODIFICĂRI ÎN FENOFAZELE SOIURILOR *PINOT NOIR* ȘI *CHARDONNAY* DATORATE SCHIMBĂRLOR CLIMATICE, ÎN PODGORIA MINIȘ

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Abstract
Pinot noir and Chardonnay varieties are relatively well suited to be cultivated in the area delimited for the Miniș PDO and the influences of the oenoclimate, particularly the Active Heat Balance and the Useful Heat Balance, can be modelled with the help of cultivation technology. There is a close link between the cultivation characteristics of the two varieties, which means that in areas where Pinot Noir is suitable for cultivation, the variety for white wines should be Chardonnay, especially for sparkling base wines. The distribution of sugar accumulation values was almost symmetrical during the period studied, the averages being extremely close, which defines a close correlation between the two varieties.

Keywords: Pinot noir, Chardonnay, sugar accumulation correlation, phenophase, climate change

Rezumat
Soiurile Pinot noir și Chardonnay se pretează relativ bine pentru a fi cultivate în arealul delimitat pentru DOP Miniș, iar influențele directe ale enoclimatului, cu precădere bilanțul termic activ și bilanțul termic util, pot fi modelate cu ajutorul tehnologiei de cultură. Există o legătură strânsă între caracteristicile de cultură ale celor două soiuri, ceea ce presupune că este recomandabil ca în zonele unde se pretează cultivarea Pinot noir, să se asigure varianta de soi pentru vinuri albe cu soiul Chardonnay, mai ales pentru vinul bază spumante. Distribuția valorilor acumulărilor de zaharuri a fost aproape simetrică, în perioada studiată, mediile fiind extrem de apropiate, ceea ce definește o corelație strânsă între cele două soiuri.

Cuvinte cheie: Pinot noir, Chardonnay, corelație, acumularea zahărului, fenofază, schimbări climatice

INTRODUCTION

Grapevine phenology is a topic of great interest in the context of climate change, and this is evident in the multitude of studies, by grapevine variety, by area, at local, national or continental level. It is difficult to establish the pioneer in this direction, because similar research has been carried out in different wine-growing areas of the country and in most, if not all, wine-growing areas in the world. Observations, experiments and trials have been carried out on grapes for wine, table grapes and raisins.
Mountains are considered the best indicators of climate change. At higher altitudes (Dolomite Alps), the effects of global temperature rise are expected to be more pronounced. Phenological advance is more pronounced in grapevine varieties grown at higher altitudes. Phenophase duration and growing season length are more affected in varieties grown at low altitudes [1]. In a study for the period between 1964 and 2015, Chinese researchers demonstrated that the dominant factor leading to changes in the start and end points of plant phenophase was temperature and that the phenological response to

temperature changes is particularly sensitive, further demonstrating that there is a correlation between phenophase and climate change [4].

Early phenological development, shortened phenophase, poor grape berries development, early maturity with lower yield and lower quality are the consequences of the challenges of climate change [10]. The occurrence of extreme climatic phenomena does not bring changes in all phenological phases, with shoot development and flowering recording significantly more extreme coincidence events than other phenophases. The varieties studied in Switzerland, *Chardonnay* and *Pinot noir*, did not differ in their response to extreme temperatures for the area [13]. In southern England, studies on *Chardonnay* show that the date of the last spring frost advances at a higher rate than grapevine budbreak, indicating an overall decrease in bud frost risk in susceptible phenotypes [8].

On the basis of data obtained in Slovakia during studies carried out between 1985 and 2018, the influence of temperatures: mean-maximum, mean and sum of effective temperatures on the occurrence of phenophases was evaluated. The results of the observations showed an earlier flowering by five to seven days, an earlier start of flowering by 7 to 10 days, an earlier berry softening by 18 days, and harvest dates were advanced on average by 8 to 10 days [2]. The greatest influence was exerted by the average amplitude of air temperatures 10 days before the start of the bleeding phase of the grapevine [9].

Studies comparing phenologically two varieties from a specific area (Douro, Portugal) have also been the subject of predictions for the future and the testing of several phenological models for budbreak, flowering and dormancy [5]. Although phenology is not the only characteristic to consider for grapevine adaptation to climate change, it plays a major role in the current distribution of the variety-vineyard binomial [6].

The results showed that in the conditions of the Giurgiu area, southern Romania, the varieties analysed entered vegetation faster and had a slightly higher percentage of fertile shoots than the average recorded in other growing areas. Fertility coefficients and productivity indices were also high in the studied area. [14] For the bud-break and flowering phenophases, the grapevine varieties analysed in the PDO Ștefănești area showed a similar behaviour with the variation of the identified climatic parameters: average air temperature being the main factor, followed by minimum temperature. [15]

In the area delimited by PDO Odobești, the phenological spectrum of the grapevine, its fertility and productivity, the quantity and quality of grape production were studied and determined for five years, and the data obtained were compared with the *Sauvignon blanc* variety, which is widely cultivated in that area [3]. Murfatlar PDO shows that, in addition to oenoclimatic factors, also the growing system influences the duration of phenological stages [12]. In the area of the PDO Tarnave, the warming of the climate has led to an earlier flowering, with an average of 8 days, correlated with an earlier ripening, with an average of 9-11 days. As regards the start date of the flowering and ripening phenotypes, no clear differences were observed [11].

Knowledge of the HI heliothermal index (Huglin index) was absolutely necessary in the context of climate change and the assessment of the suitability of establishing new plantations with certain wine grape varieties and the favourability of areas with designations of origin [7]. So was the Active and Useful Heat Balance, in addition to a number of other weather and soil parameters [16]. PDO - Protected Designation of Origin belongs to the categories of Geographical Indications in Romania and represents a relatively small area unit compared to the Protected Geographical Indication, which may cover one or more counties [17]. The BBCH scale has been used to define the phenotypes of grapevines [18].

MATERIAL AND METHOD

The experiment was based on observations carried out for three consecutive years, 2020, 2021 and 2022, in *Pinot noir* and *Chardonnay* plantations, in the Miniș PDO, on vine phenophases that are

sensitive to environmental factors and their fluctuations: shoot growth, first leaves, flowering, the veraison period and ripening.

The observations were made directly in the field, in identical time intervals, in the locality of Minis in Arad County, in plantations founded in the autumn of 2008, through the reconversion of vineyards with European funds, at 2.2 m between rows and 1 m between grapevines per row, at a density of 4545 grapevines per hectare.

The plantations have been authorized by the National Office of Vineyards and Wine Products [16], as being able to produce grapes for the production of Miniș Protected Designation of Origin (PDO Miniș) wines.

Data from the weather station in the perimeter of the central unit of the Research and Development Station for Viticulture and Winemaking Minis, Ghioroc, Fn, Arad County, were used for correlation with climatic data. The heliothermal index, Huglin for the studied area was: PDO Miniș, HI=2202 [7].

Phenological observations were made at four time intervals in May: shoot growth and leaflet emergence (BBCH10, BBCH13, BBCH15, BBCH19 and BBCH60); two intervals in June for monitoring flowering (BBCH64 and BBCH69); one interval in August for the veraison phenophase (BBCH85), three intervals in August (BBCH85, BBCH86 and BBCH87) and one in September (BBCH89) for monitoring ripening progress (Table 1).

Table 1. The main phenophases of *Pinot noir* and *Chardonnay*, in PDO Miniș (2020, 2021 and 2022) / Fenofazele principale ale viței-de-vie observate la soiurile *Pinot noir* și *Chardonnay*, în DOP Miniș (2020, 2021 și 2022)

	BBCH10 lăstar (cm)						BBCH 13 lăstar (cm)						BBCH13 frunze degajate pe lăstar (nr)					
Data	4-9 mai						10-12 mai						10-12 mai					
Anul	2020		2021		2022		2020		2021		2022		2020		2021		2022	
Pinot noir	3	4	2	3	5	10	8	10	6	8	10	15	2	3	1	2	3	4
Chardonnay	5	7	4	5	7	15	9	12	8	10	15	20	3	4	2	3	4	5
	BBCH15 lăstar (cm)						BBCH15 frunze degajate pe lăstar (nr)						BBCH19 lăstar (cm)					
Data	17-19 mai						17-19 mai						24-25 mai					
Anul	2020		2021		2022		2020		2021		2022		2020		2021		2022	
Pinot noir	20	23	24	25	20	35	4	5	2	3	5	8	50	52	48	50	45	65
Chardonnay	20	30	28	30	20	40	5	7	3	5	7	9	55	58	58	60	45	70
	BBCH19 frunze degajate pe lăstar (nr)						BBCH60 Înflorit (%)						BBCH 64 lăstar (cm)					
Data	24-25 mai						24-25 mai						2-7 iunie					
Anul	2020		2021		2022		2020		2021		2022		2020		2021		2022	
Pinot noir	5	6	4	5	8	11	0	3	0	0	0	5	50	60	60	70	65	70
Chardonnay	6	8	4	5	8	11	0	0	0	0	0	1	60	70	70	90	70	80
	BBCH64 Înflorit (%)						BBCH69 Înflorit (%)						BBCH85 Ciorchini în pârgă (%)					
Data	2-7 iunie						9-14 iunie						9-aug.					
Anul	2020		2021		2022		2020		2021		2022		2020		2021		2022	
Pinot noir	10	12	12	15	10	15	70	80	80	90	90	100	60	70	80	90	75	80
Chardonnay	50	60	60	65	70	75	80	90	100	100	100	100	80	90	100	100	80	100
	BBCH86 Zaharuri (g/ L)						BBCH87 Zaharuri (g/ L)						BBCH89 Zaharuri la recoltare (g/ L)					
Data	23-24 august						30-aug.						7-13 septembrie					
Anul	2020		2021		2022		2020		2021		2022		2020		2021		2022	
Pinot noir	125	128	139	140	160	165	181	182	179	180	204	206	220	222	218	220	239	240
Chardonnay	130	132	159	160	195	200	190	195	189	190	230	235	235	238	239	240	240	241

RESULTS AND DISCUSSIONS

The sum of active temperatures in the months of observations (May, June, July, August and September) showed an increasing trend during the summer months and a sharp decrease in September, especially in 2021 and 2022 (Figure 1).

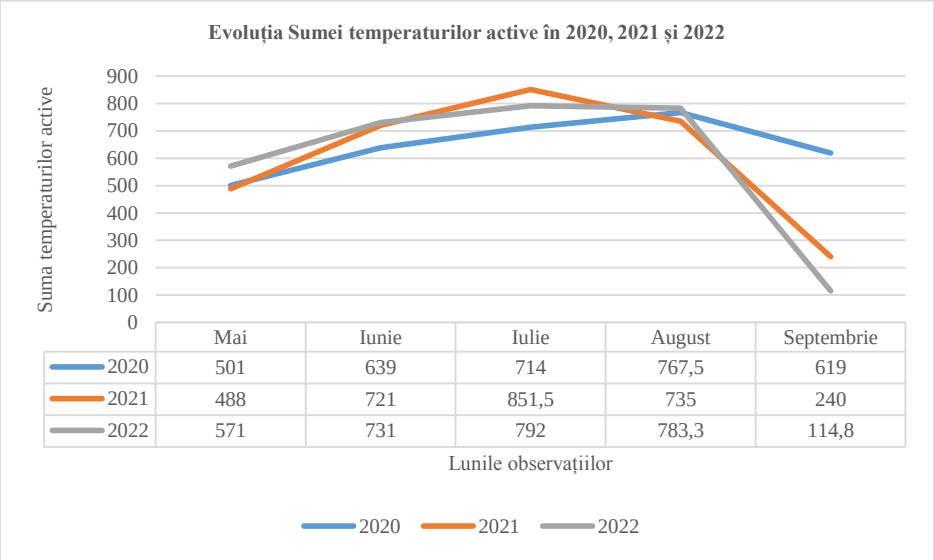


Fig 1. The evolution of the sum of active temperatures for grapevine, in PDO Miniș (2020, 2021 și 2022) / Evoluția Sumei temperatuilor active pentru vița-de-vie, în DOP Miniș (2020, 2021 și 2022)

However, it is considered normal when considered in the context of the entire growing season of the vine in the Minis area. The trend in sugar accumulation from the time the grapes enter the veraison until harvest maturity, in accordance with the production requirements and correlated with the provisions of the specification for the Miniș PDO, is parabolic, with a slight inclination to the right, analysed in relation to the variable Sum of active temperatures in May, June, July, August and September (Figure 2).

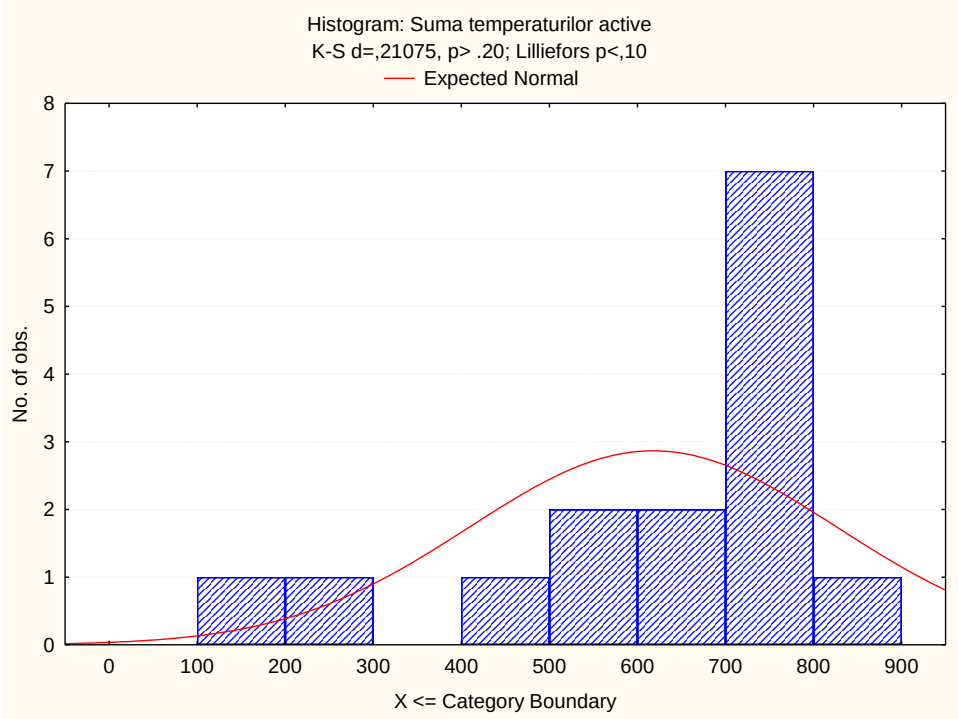


Fig. 2. The unimodal curve of the sum of active temperatures for grapevine, in PDO Miniș / Curba unimodală a sumei temperatuilor active pentru vița-de-vie, în DOP Miniș (2020, 2021 și 2022)

During the study period, there is a more abrupt drop in active temperatures in September, with the sum of useful temperatures evolving almost identically in 2021 and 2022. In July, useful temperatures peak more sharply in 2021 than in 2020 and 2022. Throughout the period with useful vineyard temperatures, 2021 was steeper than adjacent years (Table 2).

Tabelul nr. 2. The sum of useful temperatures in PDO Miniș (2020, 2021 și 2022) / Suma temperaturilor utile în DOP Miniș (2020, 2021 și 2022)

Luna/ Anul	2020	2021	2022
Mai	191,0	178,0	261,0
Iunie	339,0	421,0	431,7
Iulie	404,0	541,5	482,0
August	457,5	425,0	473,3
Septembrie	112,0	242,0	93,0

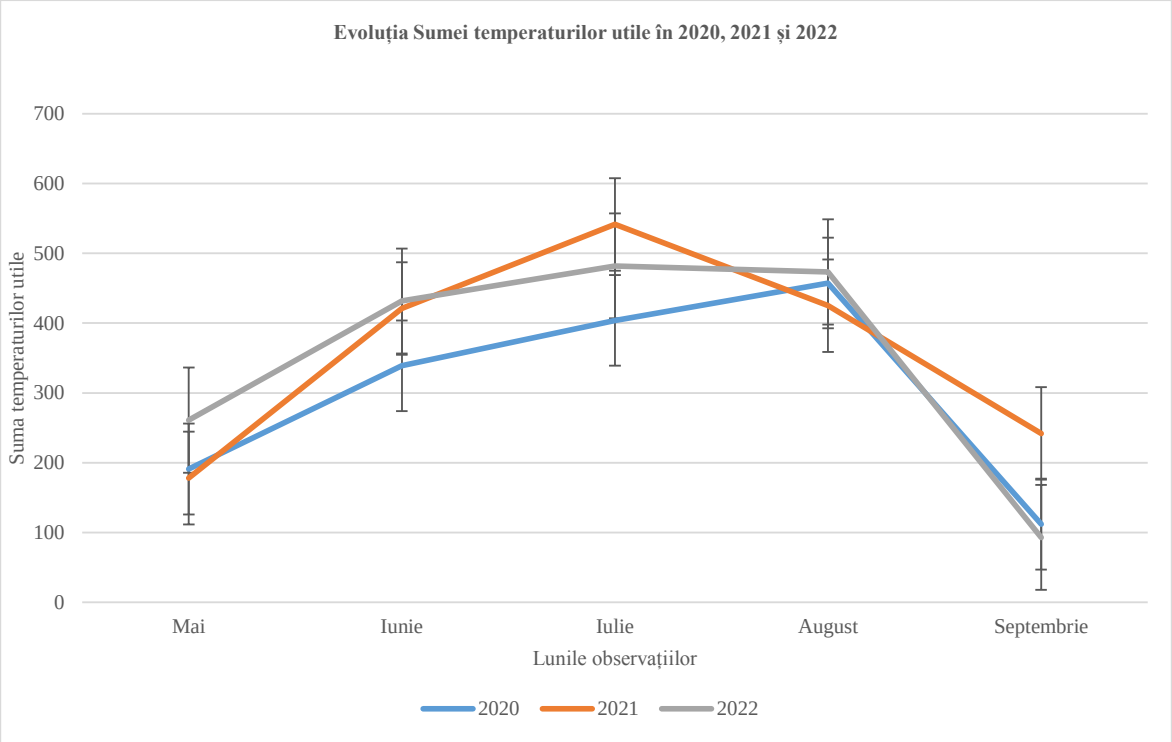


Fig. 3. The evolution of the sum of useful temperatures for grapevine, in PDO Miniș (2020, 2021 și 2022)/ Evoluția sumei temperatuilor utile pentru vița-de-vie, în DOP Miniș (2020, 2021 și 2022)

Shoot growth dynamics in the first phenophase of active vegetation are clearly in favour of *Chardonnay* in each of the three years of observations (Figures 4, 5 and 6), although by the time the first leaflets are released (BBCH11), when the shoots are about 10-15 cm, both *Pinot noir* and *Chardonnay* behave almost identically, which means that the swelling of the buds, the bud break and the pink bud were on the same coordinates, confirming the literature, which locates in the cooler areas the areas suitable for growing these varieties in tandem, i.e. where *Chardonnay* is found, *Pinot noir* is also the best expressed.

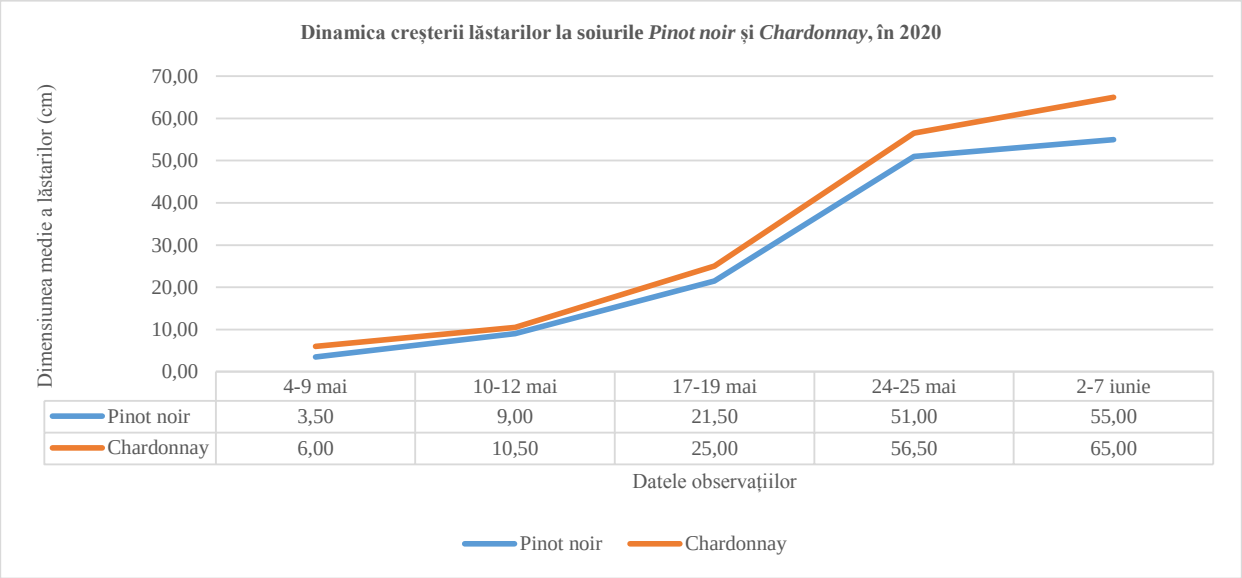


Fig. 4. Dynamics of shoot growth of *Pinot noir* and *Chardonnay* cultivars, in PDO Miniș, in 2020 / Dinamica creșterii lăstarilor la soiurile *Pinot noir* și *Chardonnay*, în DOP Miniș, în 2020

An increase in shoot length was associated with a greater number of leaves on the shoots and higher amounts of organic substances resulting from photosynthetic processes, which led to the accumulation of sugars in the grapes. Distancing the graph for *Chardonnay* towards the end of May 2021 shows a reaction directly proportional to the useful temperature peak in July 2021, to which the variety responds much more sensitively than *Pinot noir*. Even though throughout the vegetative phenophase the *Chardonnay* variety grew only 2 cm more than *Pinot noir*, from the second half of May 2021 onwards, there were significant differences between the shoots of the two varieties, up to 10 cm in the case of *Chardonnay* (Figure 5).

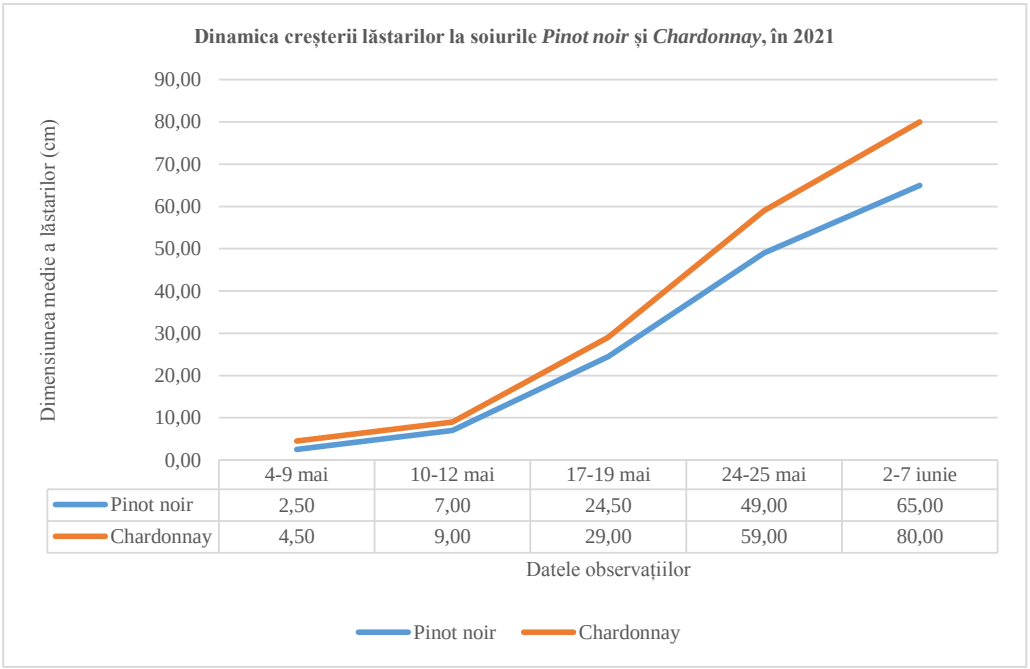


Fig. 5. Dynamics of shoot growth of *Pinot noir* and *Chardonnay* cultivars, in PDO Miniș, in 2021 / Dinamica creșterii lăstarilor la soiurile *Pinot noir* și *Chardonnay*, în DOP Miniș, în 2021

The 2022 plots show an almost perfect tandem between shoot growth dynamics in both varieties, correlated with cumulative effective temperatures (Figure 6).

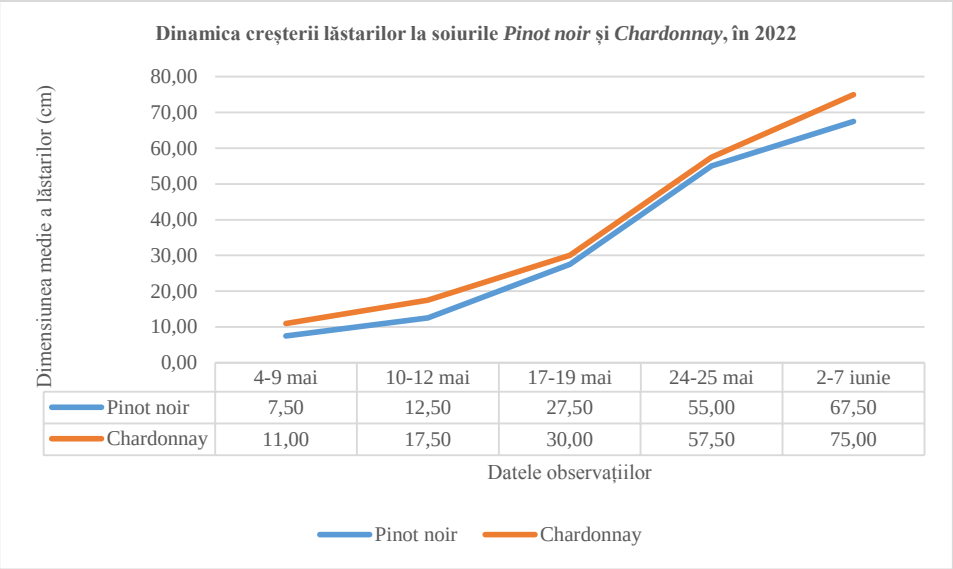


Fig. 6. Dynamics of shoot growth of *Pinot noir* and *Chardonnay* cultivars, in PDO Miniș, in 2022 / Dinamica creșterii lăstarilor la soiurile *Pinot noir* și *Chardonnay*, în DOP Miniș, în 2022

The year 2020 is atypical compared to 2021 and 2022 in terms of vegetative growth dynamics for each variety (Figures 7 and 8), with the accumulation of useful temperatures leading to a sharp slowdown in Pinot noir. If the percentage of bunches entering the veraison had been significantly higher than for Chardonnay, we could have attributed this stagnation to the physiological processes being directed towards grape ripening and seed maturation.

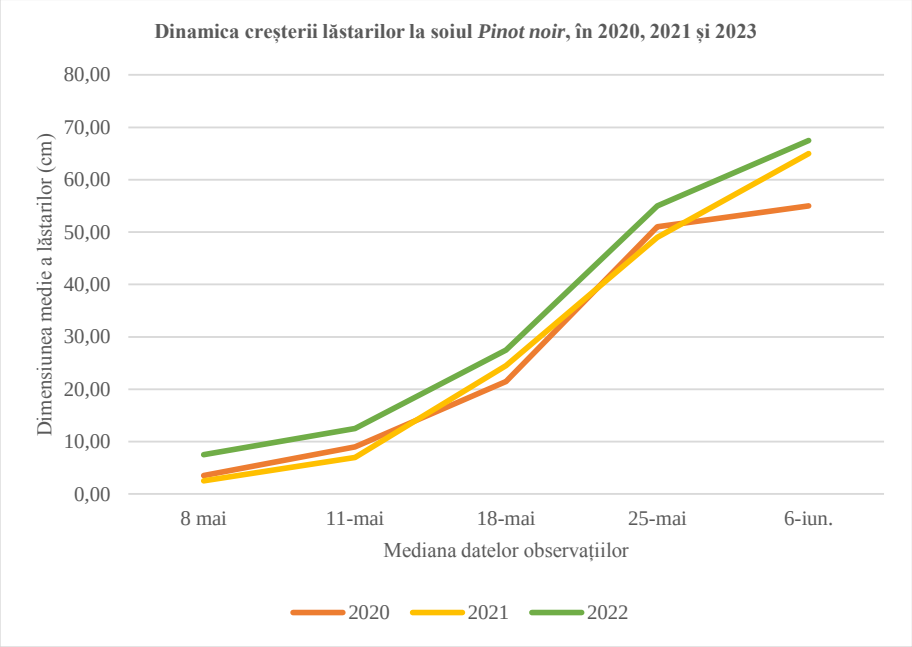


Fig. 7. Dynamics of shoot growth of *Pinot noir* cultivar, in PDO Miniș, in 2020, 2021 and 2022 / Dinamica creșterii lăstarilor la soiul *Pinot noir*, în DOP Miniș, în 2020, 2021 și 2022

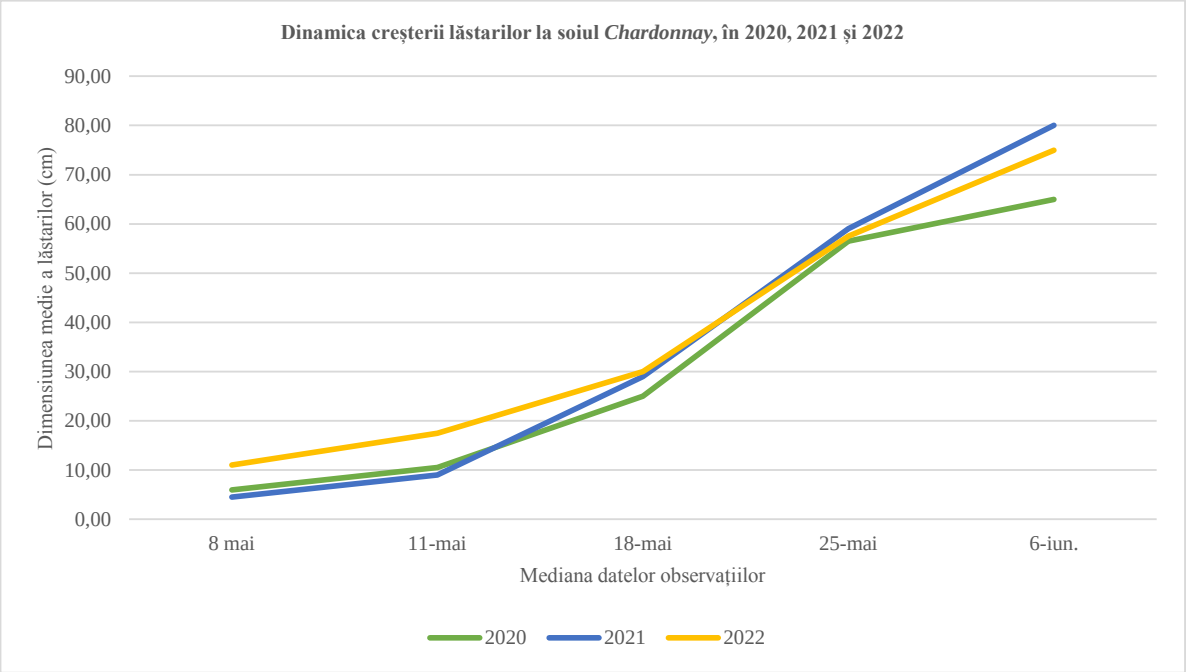


Fig. 8. Dynamics of shoot growth of *Chardonnay* cultivar, in PDO Miniș, in 2020, 2021 and 2022/ / Dinamica creșterii lăstarilor la soiul *Chardonnay*, în DOP Miniș, în 2020, 2021 și 2022

The *Chardonnay* variety responds much more quickly to the consequences of the useful temperature peak in July 2021, with 100% of the clusters having entered the veraison, compared to *Pinot noir* with only 80%. In years with a normal evolution of useful temperatures during the veraison period, the *Chardonnay* variety is constant in triggering the phenomena preceding grape ripening, whereas *Pinot noir* also reacts to other environmental factors, either biotic or anthropic (Figure 9).

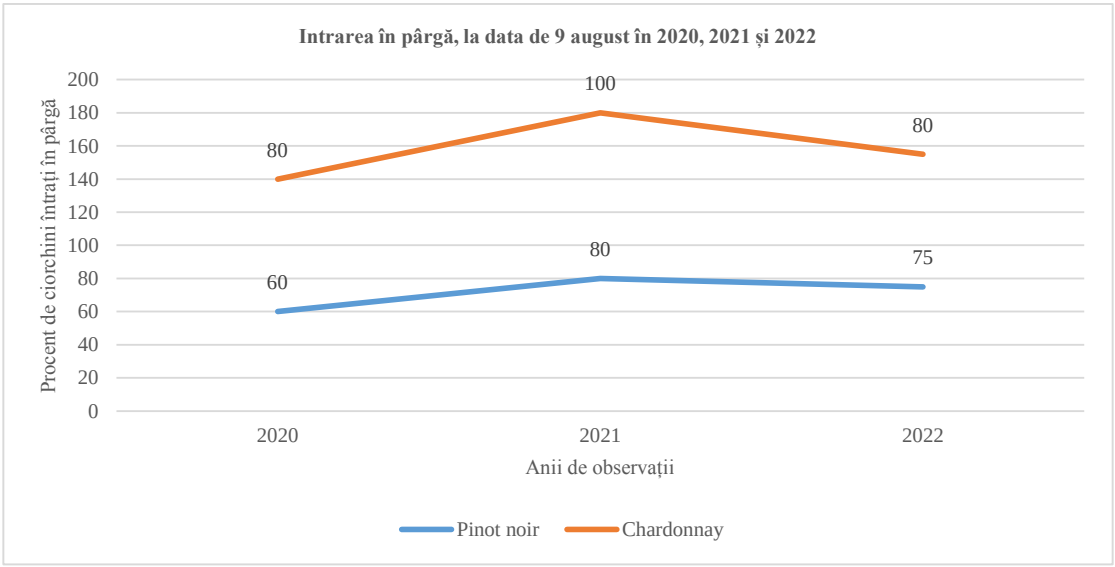


Figura nr. 9. The ripening percentage of the clusters in Pinot Noir and Chardonnay cultivars/ Procentul ciorchinilor intrați în pârgă la soiurile *Pinot noir* și *Chardonnay*, în DOP Miniș, în 2020, 2021 și 2022

Ripening was determined at three different intervals in August and September, but the same in each of the study years. The result, illustrated in Figure 10, reveals an almost parallel trend between the two varieties, as they react effectively identically to environmental factors.

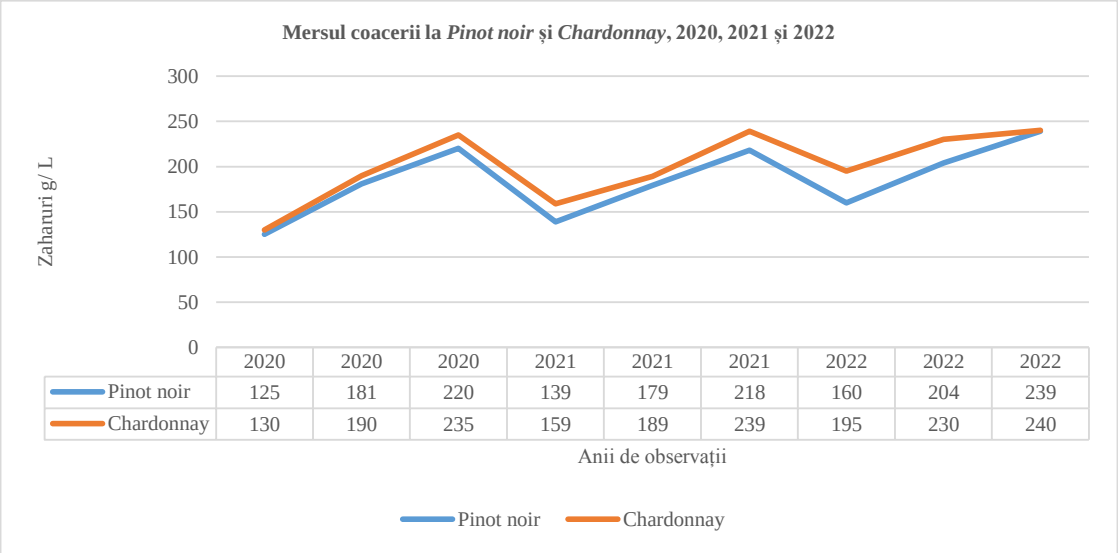


Fig. 10. The ripening progress – sugar accumulation (g/ L) in *Pinot Noir* and *Chardonnay* varieties/
Mersul coacerii – acumularea de zaharuri (g/ L), la soiurile *Pinot noir* și *Chardonnay*, în DOP Miniș, în 2020, 2021 și 2022

Increasing the number of replicates/observations for each variety would have led to a decrease in the variance affecting the mean of each result. The small number of observations, over only three consecutive years, implies the disadvantage that they are subject to errors related to the number of grapevines on which observations were made, the homogeneity of the plots in which the grapevines were studied and the like, including data sensitive to genetic variation (Figure 11).

F-Test Two-Sample for Variances

	Chardonnay	*Pinot noir*
Mean	209,4	195,8
Variance	1121,4	1199,457143
Observations	15	15
df	14	14
F	0,934922941	
P(F<=f) one-tail	0,450795179	
F Critical one-tail	0,402620943	

Fig. 11 Variance analysis of *Pinot noir* and *Chardonnay* cultivar./
Analiza varianței la soiurile *Pinot noir* și *Chardonnay*, în DOP Miniș, în 2020, 2021 și 2022

The distribution of sugar accumulation values is almost symmetrical for the two varieties over the period studied, with the averages being extremely close. There is a strong correlation between the two varieties, with F = 0.935, very close to 1, with a positive trend (Figure 12).

With a confidence level of 50-60%, the experiment would require a much larger number of observations for greater accuracy of the results. The averages of sugar accumulation are very close: 195.8 g/L for *Pinot noir* and 209.4 g/L for *Chardonnay*. Given that 1% alcohol by volume is theoretically obtained by fermenting 17 g of sugars in the presence of yeasts of the genus *Sacharromyces*, *Pinot noir* grapes could produce a wine with 11.58% vol. alc. and *Chardonnay* grapes a wine with 12.31% vol. alc. From both varieties, the possible wines are in line with the specifications for the Miniș PDO, which stipulate a minimum alcoholic strength of 11.5% vol. alc. [17].

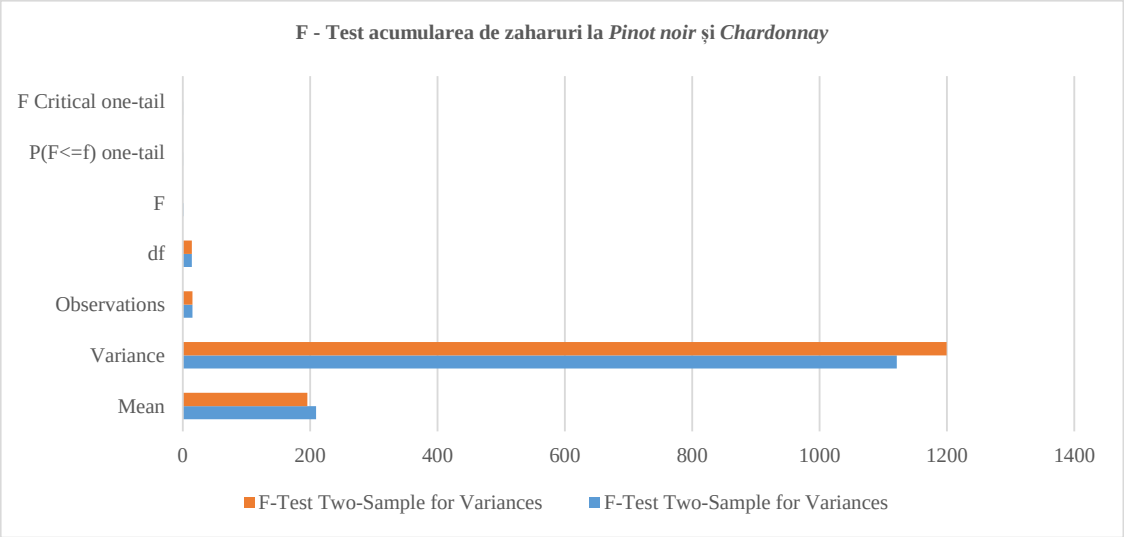


Fig. 12. The F test for sugar accumulation of *Pinnot noir* and *Chardonnay* grapevine /
Testul F, pentru acumularea de zaharuri la soiurile *Pinot noir* și *Chardonnay*, în DOP Miniș, în 2020, 2021 și 2022

The mean standard error shows the value of the discrepancy between the measurements, at the same date, of sugar accumulation in the grapes of the two varieties. Being so far from zero (8.94 for *Pinot noir* and 8.64 for *Chardonnay*), it shows that the discrepancy arises due to the small number of samplings. Kurtosis defines a slight tail to the left (-0.518) in *Pinot noir*, with no outliers to the right (0.353) in *Chardonnay*. This means that we have a univariate normal distribution. Skewness-test shows skewness to the left (negative): (-0.518) for *Pinot noir* and more pronounced (-0.978) for *Chardonnay*. The tabular T-test shows lower values for *Chardonnay* than for *Pinot noir* for the same confidence interval of 95-98% (Figure 13).

Pinot noir		*Chardonnay*	
Mean	195,8	Mean	209,4
Standard Error	8,942248572	Standard Error	8,646386528
Median	204	Median	222
Mode	220	Mode	190
Standard Deviation	34,6331798	Standard Deviation	33,48731103
Sample Variance	1199,457143	Sample Variance	1121,4
Kurtosis	-0,51866804	Kurtosis	0,353075986
Skewness	-0,517971018	Skewness	-0,977616955
Range	112	Range	109
Minimum	128	Minimum	132
Maximum	240	Maximum	241
Sum	2937	Sum	3141
Count	15	Count	15
Largest(1)	240	Largest(1)	241
Smallest(1)	128	Smallest(1)	132
Confidence Level(95,0%)	19,1792157	Confidence Level(95,0%)	18,54465472

Fig. 13. Descriptive statistics of sugars accumulation of Pinot Noir and Chardonnay varieties /
Statistică descriptivă pentru acumularea de zaharuri la soiurile *Pinot noir* și *Chardonnay*

At a critical F of 4.196, with df 15, in the F-table we have the alpha value in the range 2.64 - 2.80. At P-value = 0.283, the null hypothesis is rejected, so there is a link between the variables defining the environmental factors and the results of the observations (Figure 14).

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Pinot noir	15	2937	195,8	1199,457143
Chardonnay	15	3141	209,4	1121,4

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1387,2	1	1387,2	1,195420411	0,28355651	4,195971819
Within Groups	32492	28	1160,428571			
Total	33879,2	29				

Fig.14. One-way ANOVA test for sugar accumulation of Pinot noir and Chardonnay /
Testul ANOVA, cu un singur factor, pentru acumularea de zaharuri la soiurile *Pinot noir* și *Chardonnay*

The recommendation would be to continue the observations, even with extension on varieties created within the research unit, as was done in 2019-2022, in other wine-growing areas in Romania [17].

CONCLUSIONS

Pinot noir and *Chardonnay* varieties are relatively well suited to be cultivated in the area delimited for the Miniș PDO, the former being the most cultivated grape wine variety in this area, according to ONVPV statistics [18].

The direct influences of the enoclimate, especially the active thermal balance and the useful thermal balance, especially in the phenophases of intense shoot growth, entry into veraison and ripening of the grapes, can be shaped by the use of cultivation technology, by green harvesting, shading with white lime film, partial defoliation.

There is a close link between the growing characteristics of the two grape wine varieties, which means that it is advisable in areas where *Pinot noir* is suitable for growing, to provide the variety for white wines with *Chardonnay*, especially for the base wine for sparkling wines.

Further research is needed, in conjunction with environmental factors and especially the response to extreme weather events: late frosts, early frosts, hail, sunburn. More observations will make the results more accurate.

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RESEARCH REGARDING THE GROWTH, DEVELOPMENT AND FRUITING PROCESSES OF SOME ROOTSTOCK- SCION COMBINATIONS IN A HIGH DENSITY APPLE ORCHARD, IN NORTHERN TRANSYLVANIA

CERCETĂRI PRIVIND PROCESELE DE CREŞTERE, DEZVOLTARE ŞI FRUCTIFICARE A UNOR COMBINAŢII SOI-PORTALTOI LA SPECIA MĂR, ÎNTR-O PLANTAŢIE DE MARE DENSITATE, ÎN NORDUL TRANSILVANIEI

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Abstract
Researches have been effectuated in a high density apple orchard (1666 trees/ha) located in Northern Transylvania at FRDS Bistrita, in a highland area called "Bistrita hills" established in the year 2001 with apple varieties resistant to scab (Venturia inaequalis), Florina and Generos, grafted on rootstock M26 conduced as slender spindle training system. The registering and analysing of data focused on two main time periods: early development stage of young orchard in the first 7 years, during 2001-2008 and the full maturity stage during 2019-2021. In the 20 years lifespan of the orchard at both cultivars there have been registered a positive fructification tendency starting with the first years, thus at Florina was 3.56-56.85 to/ha registered and at Generos 5.79-51.07 to/ha. Growth characteristics evaluated by the trunk cross sectional area indicator showed increased values at Florina/ M26 combination at the full development of trees in 2021 (143.36 cm^2) when compared with Generos/M26 variant (126.25 cm^2). Productivity index showed higher values in early stage during 2003-2008 at Florina (0.20-0.93) and at Generos (0.38-0.73) while at maturity during 2019-2021 at Florina ranging 0.15-0.26 and at Generos 0.14-0.26, growth and production beeing more equilibrated. Growth and yield capacity of trees was influenced in the period 2001-2021 beside the fruit growing technology also by the evolution of climatic factors (drought, hail falling, negative temperatures during blossom).

Keywords: high density orchard, rootstock, variety, yield, trunk cross sectional area

Rezumat
Cercetările au fost efectuate într-o plantaţie de măr de mare densitate (1666 pomi/ha) localizată în Nordul Transilvaniei la SCDP Bistrita, într-o zonă colinară denumită "Dealurile Bistriţei", înfiinţată în anul 2001 cu soiuri de măr rezistente la rapăn (Venturia inaequalis), Florina şi Generos, altoite pe portaltoiul M26 având sistem de conducere a pomilor fus subţire. Înregistrarea, analiza datelor s-a focusat asupra a două importante perioade de timp: perioada de tinereţe a plantaţiei în primii 7 ani (2001-2008) şi perioada de maturitate deplină (2019-2021). În cei 20 de ani de viaţă a plantaţiei, la ambele soiuri s-a înregistrat o tendinţă pozitivă de fructificare încă din primii ani, astfel la soiul Florina s-a înregistrat o producţie de 3.56-56.85 to/ha, iar la soiul Generos valorile au arătat 5.79-51.07 to/ha. Caracterisiticile de creştere evaluate prin suprafaţa secţiunii trunchiului au arătat valori mai ridicate la Florina/M26 la dezvoltarea maximă a pomilor (143,36 cm^2), comparativ cu soiul Generos/M26 (126,25 cm^2). Indexul productivităţii a arătat valori mai ridicate în perioada 2003-2008 (Florina 0,20-0,93, Generos 0,38-0,73), iar la maturitate între 2019-2021 la Florina s-au înregistrat valori de 0,15-0,26, respectiv la Generos între 0,14-0,26. Creşterea şi fructificarea pomilor a fost influenţată în perioada 2001-2021 pe lângă tehnologia de cultură şi de evoluţia factorilor climatici (secetă, grindină, temperaturi negative în timpul înfloritului).

Cuvinte cheie: plantatie de mare densitate, portaltoi, soi, producţie, secţiunea transversală a trunchiului

INTRODUCTION

The apple (*Malus domestica*, *Borkh*) takes the second-place in terms of importance in national fruit production after the plum species, and recent data from the last statistical year 2020 showed a

slight decrease of the apple areas and yields (52.3 thousand ha) compared to the values recorded in 2015 (55.8 thousand ha). This phenomenon can be due both to the extensive process of reconversion of old fruit plantations and to the clearing of aging orchards, respectively the preparation of land for new orchards (INS, 2021). The total production of apples registered by the INS shows a significant increase from 476.6 thousand tons (2015) to 551.4 thousand tons (2020), this positive trend contributes to an increase in the production of fruit on the consumer's table and can be a cause of the phenomenon of intensification and the increase in the productivity and density of fruit plantations. The reconversion process of the non-performing plantations started in 2015 and continues today, producers are increasingly opting for high-density plantations and production systems. Many fruit plantations were established at the beginning of the 1950`s with densities of 150-250 trees/ha, with a globular canopy and a tall trunk (Cepoiu, 1989, 2005), later after the 1970`s, an intensification phenomenon began and the growth planting densities using less vigorous rootstocks with planting densities of 1,250 trees/ha (Ghena, 2003, Meland, 1996, Stefan, 1993). Due to the lack of high-performance irrigation systems, of water resources in the area or other causes, in the vast majority of current fruit plantations, low-vigor M9 or M26 type rootstocks were not chosen, which require or impose an irrigation system, so the MM106 rootstock still predominates, or other rootstocks from abroad with great vigor that resist even in more difficult eco-pedological conditions in more clayed soils, drier areas with lower amounts of precipitation (Isac, 1998). After the revolution (1989), at the national level in the early 2000`s, more advanced culture systems (thin spindle) began to be known for the apple species, with rootstocks that increased less vigor, higher productivity (M26, M9, M27), but many of these they were still in the testing phase (Gonda, 2003). In Europe, in the large apple-producing countries such as Germany, France, Spain, there are very modern technologies with high-density culture systems, with irrigation and/or fertigation systems having an assortment of varieties and rootstocks resistant to eco-pedological conditions, reaching productions of 40-50 tons/ha. At the beginning of the 2000`s, the main interest was the use of varieties with genetical resistance, especially in apple scab (*Venturia inaequalis*) from abroad, such as `Florina', `Prima', `Priam', `Priscilla', `Wanda', and Romanian cultivar `Generos` to reduce production costs with phytosanitary treatments (Branişte, 1999) and to have apples as healthy as possible with a low content of pesticides. The establishment of these fruit plantations means substantial financial efforts, but these costs are amortized over time if satisfactory production levels are reached. Among the fruit tree rootstocks with vegetative propagation in Romania, the MM106, M9, M26 rootstocks are authorized for propagation, and among them, at the level of the 2000`s, the most optimal candidate for orchards with medium-high density with medium-sub-medium vigor that can withstand more difficult conditions of drought without the application of irrigation systems was the rootstock M26, selected and approved by the East Malling research station in England. The promotion in culture of varieties with genetic resistance to diseases with its many valences constituted a major interest in the fruit-growing of Bistrita-Năsăud county, since the beginning of the 2000`s, thus efforts were undertaken to capitalize on the genetic progress of international and national improvement alike, materializing in a demonstration plot with a high-density planting system in different technological variants. The most valuable characteristic of varieties from a horticultural point of view is productivity and quality, properties that come from the genetics of the species that can be influenced by eco-climatic, biological and technological factors. Productivity is influenced by some genetic peculiarities such as: the vigor of the trees, the differentiation fruit buds, the branching capacity, the behaviour during the fertilization process, the precocity of fruiting, the resistance to stress factors. The aim of this multi-year study (2001-2021) was to investigate the growth, development and fruiting processes of some apple scab-resistant varieties (Florina and Generos) grafted on M26 rootstock in the eco-

pedological conditions of Northern Transylvania, in the fruit-growing region of Bistriţa. The aim of this research was to know the productivity of the two relevant apple varieties from the national orchard (Florina and Generos respectively) using the M26 rootstock of medium vigor to explore a very common soil both in Transylvania and in other areas of Romania.

MATERIAL AND METODS

The research was carried out in a high-density apple orchard (1,666 trees/ha) located in the Northern Transylvania at FRDS Bistriţa, in a hilly area called "Bistriţa Hills", established in 2001 with apple cultivars resistant to apple scab (*Venturia inaequalis*), Florina and Generos, grafted on the M26 rootstock with a thin spindle tree management system. The terrain of the research station is located at altitudes between 330-410 m and the studied plot is at an altitude of 345 m. The orchard under study is located on a hill with a 10% slope, with South East exposure. The soil type is typical brown `argilloiluvial`-the term used in the old pedological systematization system at the establishment of the research plot and novadays called eutricambosoil, weak-medium pseudoglayed, loamy, on clays and gravel, medium to low permeability. The experiment was bifactorial, with completely randomized blocks, with three replicates. The biometric, phytometric and production data were analyzed with the statistical program XLSTAT, version 2022. The recording, analysis of the data focused on two important time periods: the youth period of the plantation in the first 7 years (2001-2008) and the period of full maturity (2019-2021).The following phytometric descriptors were analyzed: trunk cross sectional area -TCSA (cm^2), average shoot growth (cm), number of fruits per tree, average fruit weight, fruit yield (kg/tree), fruit total production (to/ha), productivity index (kg/cm^2). The trunk circumference was measured 20 cm above the grafting point, and the phytometric growth descriptors were recorded in synthetic tables, the number, the fruit weight was measured in the orchard and in the laboratory immediately after harvesting the fruits that were ripe for consumption from those 3 replicates.

RESULTS AND DISCUSSIONS

The analysis from national statistical data (INS, 2021) regarding the areas of agricultural land planted and cultivated with apple fruit trees shows a decreasing trend in the last 5 years (Fig.1).

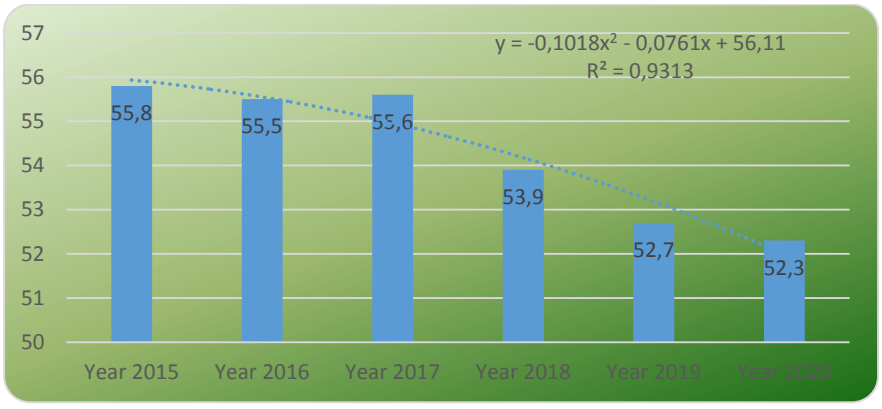


Figure 1 The cultivated area in Romania with apple orchards (thousand ha) // Suprafaţa cultivată în România cu specia măr (mii ha)

The total production of fruits in Romania has increased in the last five years (Fig.2), obtaining relevant fruit productions at national level.



Figure 2. The total of apple fruit production obtained in Romania (thousands of tons) // Producţia totală de mere în România (mii de tone)

The trunk cross-section area (TCSA) achieved the greatest increase in Florina cultivar grafted on M26 rootstock in the first 4 years of growth and development (43.18 cm^2) and in the period of maximum maturity (in the 17-18 years of the trees' life) there was a growth of only 18.4 cm^2 (Table 1). In case of Generos cultivar, the increase of growth was slower in the first five years of the tree`s life (34.32 cm^2) and in the last years, during the period of maximum maturity, a value of 18.98 cm^2 was recorded, slightly higher than the Florina variety. In the last years of research, a slower growth of the trunk was observed in both varieties, but the growth was continuous in the last years as well (Fig. 3).

Table 1. Trunk growth characteristics of Florina and Generos varieties grafted on M26 rootstock/Dinamica creşterii trunchiului la soiurile Florina şi Generos, altoite pe portaltoiul M26

Trunk cross- sectional area (cm^2)	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Diference TCSA (2008-2003)	Year 2019	Year 2020	Year 2021	Diference TCSA (2021-2019)
Florina/M26/1,666 trees/ha	10.5	14.6	30.49	36.14	46.37	53.68	**43.18**	124.96	131.32	143.36	**18.40**
Generos/M26/1,666 trees/ha	7.6	9.5	23.01	29.14	36.26	41.92	**34.32**	107.27	131.32	126.25	**18.98**

The values for the Florina/M26 cultivar (Table 1) from the initial data of 10.5 cm^2 of TCSA, increased to the level of 143.36 cm^2. The data presented in figure 3 showed an increase from 7.6 cm^2 to the level of 126.25 cm^2 for Generos cultivar.

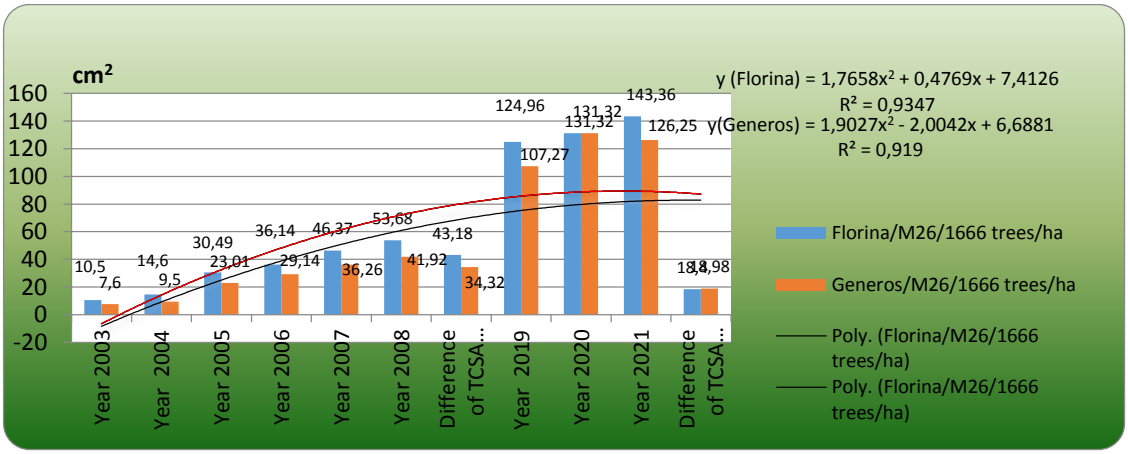


Figure 3. The dynamics of trunk cross sectional area growth -TCSA (cm^2) in Florina and Generos varieties // Dinamica creşterii suprafeţei secţiunii trunchiului la soiurile Florina şi Generos

In the first 5 years, the trees developed harmoniously, the crown of the trees was formed according to the standard methodology of forming the shape of a thin spindle crown (Fig. 4).



Figure 4. View from the high density orchard (1,666 trees/ha) in the first years of development // Livadă de mare densitate (1.666 pomi/ha), în primii ani după plantare

The average growth of the shoots in the first 5 years of the tree`s life (2003-2008) was moderate at Florina cultivar with average values of 35.00 cm and 36.73 cm for Generos cultivar (Table 2).

Tabel 2. The shoot growth characteristics of the Florina and Generos cultivars grafted on the M26 rootstock/Dinamica creşterii lăstarilor la soiurile Florina şi Generos, altoite pe portaltoiul M26

Shoot growth (cm)	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Average (2008-2003)	Year 2019	Year 2020	Year 2021	Average (2019-2021)
Florina/M26/1,666 trees/ha	31.3	32.1	46.7	40.3	30.6	29	**35.00**	68.34	43.18	46.51	**52.68**
Generos/M26/1,666 trees/ha	30	34.3	50.6	37.9	35.3	32.3	**36.73**	63.05	44.25	58.88	**55.39**

After 17-18 years after folowing the technological orchard management in the experimental field, the recorded values showed a more pronounced an average growth of 52.68 cm for the Florina cultivar and 55.39 cm for the Generos cultivar. By advancing in age towards the maturity of the trees, the tendency of the growth of the shoots can be observed (Fig. 5).

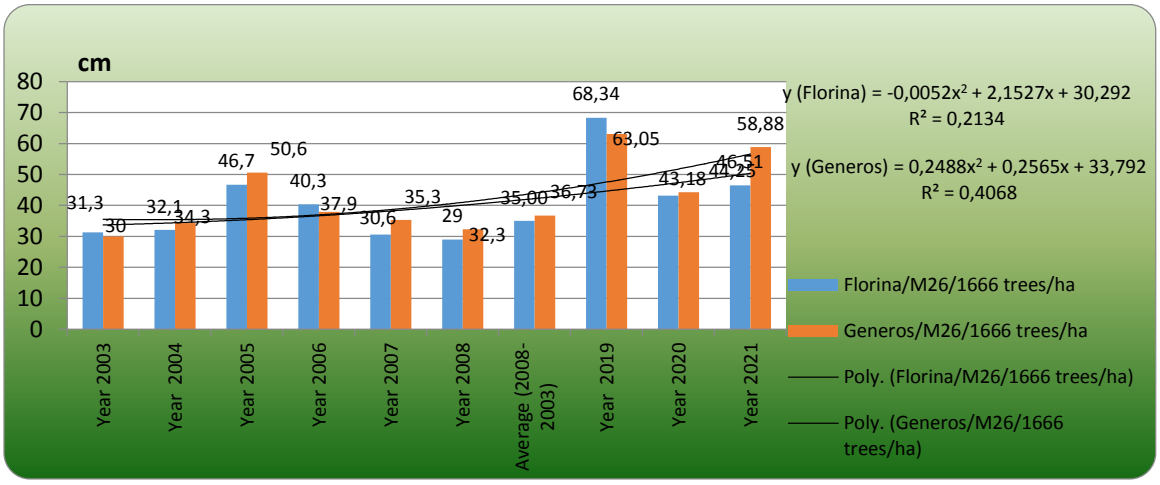


Figure 5. The tendency of the average shoots growth in Florina and Generos varieties // Dinamica creşterii lăstarilor la soiurile Florina şi Generos

The number of fruits per tree in the first years had values between 129 fruits/tree for Florina and 128 fruits/tree for the Generos cultivar (Table 3).

Table 3. The number of fruits per tree (piece) in the Florina and Generos varieties grafted on the M26 rootstock // Numărul de fructe/pom la soiurile Florina şi Generos altoite pe portaltoiul M26

No fruits/tree (pieces)	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Average (2008-2003)	Year 2019	Year 2020	Year 2021	Average (2019-2021)
Florina/M26/1,666 trees/ha	29	125	138	170	147	166	**129.17**	118	202	203	**174.33**
Generos/M26/1,666 trees/ha	45	87	147	134	136	219	**128.00**	82	159	154	**131.67**

At the end of the experiment, the trees developed more intensively, the generative fruiting processes intensified and the number of fruits per tree increased, reaching an average number of 174 fruits for the Florina variety and 131 fruits for the Generos cultivar (Fig. 6).

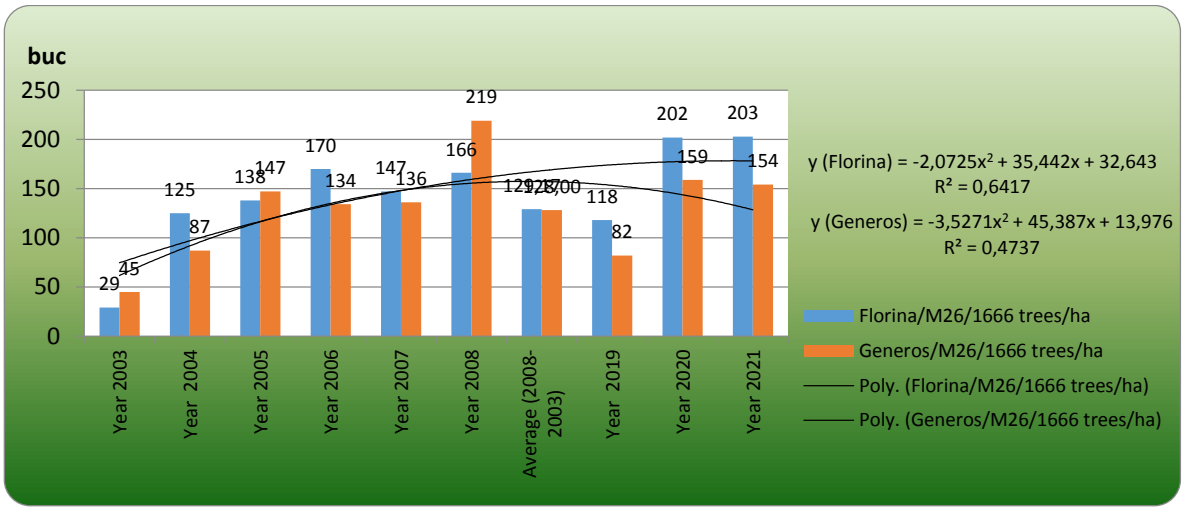


Figure 6. The number of fruits (piece/tree) in the Florina and Generos cultivars // Numărul de fructe (buc./pom) la soiurile Florina şi Generos

The average fruit weight was 131.33 g for the Florina cultivar in the first 5 years of life and 119.67 g for Generos, in the last years of full maturity when the trees reached the technological maturity of production, fruit weights of 168.92 g were achieved for Florina and 190.85 g at Generos (Table 4).

Table 4. Fruit weight per tree for Florina and Generos varieties grafted on M26 rootstock // Greutatea fructelor/pom la soiurile Florina şi Generos altoite pe portaltoiul M26

Average weight of fruits (g)	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Average (2008-2003)	Year 2019	Year 2020	Year 2021	Average (2019-2021)
Florina/M26/1,666 trees/ha	72	108	125	170	147	166	**131.33**	167	170	170	**168.92**
Generos/M26/1,666 trees/ha	65	77	87	134	136	219	**119.67**	190	191	192	**190.85**

The evolution of the average fruit weight in the experimental variants from the beginning to the maximum technological maturity is presented in figure 7.

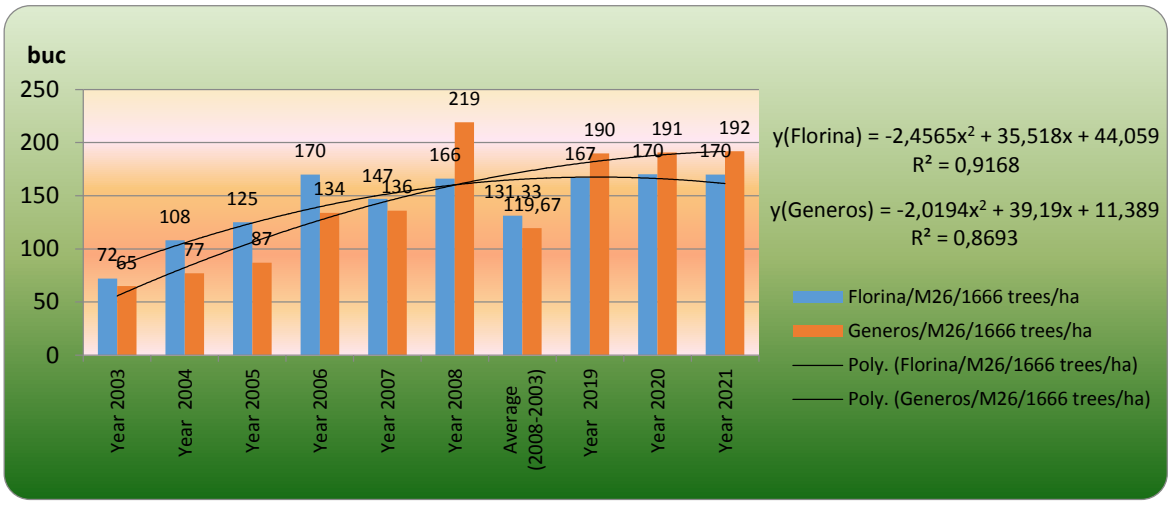


Figure 7. Fruit weight of the Florina and Generos cultivars in 2003-2021 // Greutatea fructelor la soiurile Florina şi Generos în perioada 2003-2021

The average fruit production per tree was 17.4 kg/tree at Florina and 15.32 kg/tree at Generos and in the years 2019-2021 the values of 29.51 kg/tree at Florina and 25.18 kg/tree at the Generos variety were recorded (Table 5).

Table 5. Fruit production kg/tree for Florina and Generos varieties grafted on M26 rootstock // Producţia de fructe, kg/pom, la soiurile Florina şi Generos altoite pe portaltoiul M26

Fruit yield (kg/tree)	Year 2003	Year 2004	Year 2005	Year 2006	Year 2007	Year 2008	Average (2008-2003)	Year 2019	Year 2020	Year 2021	Average (2019-2021)
Florina/M26/1,666 trees/ha	2.14	13.6	17.25	23.46	22.19	24.19	**17.14**	19.59	34.48	34.46	**29.51**
Generos/M26/1,666 trees/ha	2.93	6.7	12.87	19.16	19.58	30.66	**15.32**	15.65	30.28	29.6	**25.18**

The trend of the evolution of fruit production kg/tree is presented in figure 8. We observe a significant increase in fruit production with the advancing age of the trees towards maximum technological maturity.

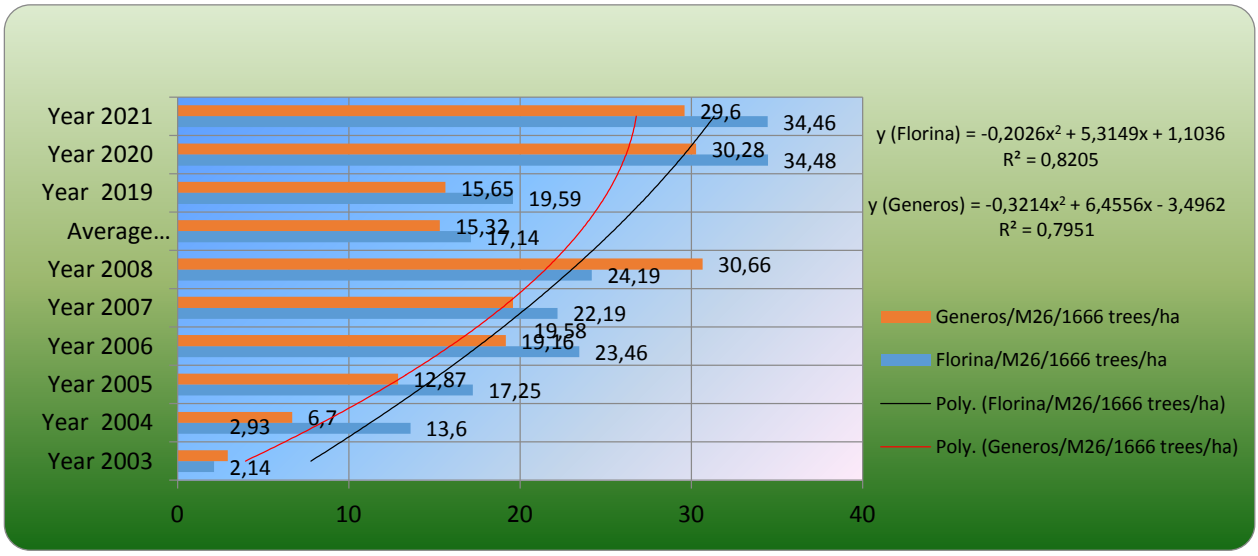


Figure 8. Fruit production trend kg/tree in Florina and Generos varieties // Dinamica creşterii producţiei, kg/pom, la soiurile Florina şi Generos

Fruit production per hectare was 26.62 to/ha in the first 5 years of tree development in Florina and 27.23 to in the Generos cultivar and at the maximum maturity of development it was 49.10 to/ha in the Florina variety and 42.37 to/ha in the cultivar Generos (Table 6).

Table 6. Fruit production to/ha in Florina and Generos varieties grafted on M26 rootstock // Producţia de fructe, t/ha, la soiurile Florina şi Generos altoite pe portaltoiul M26

Fruit Production (to/ha)	Year 2003	Year 2004	Year 2005	Anul 2006	Year 2007	Year 2008	Average (2008-2003)	Year 2019	Year 2020	Year 2021	Average (2019-2021
Florina/M26/1,666 trees/ha	3.56	29.7	19.5	29.7	36.96	40.3	**26.62**	32.67	57.79	56.85	**49.10**
Generos/M26/1,666 trees/ha	5.79	13.5	32.2	28.2	32.62	51.07	**27.23**	26.97	50.88	49.25	**42.37**

We observe a significant increase in fruit production in the last 17-18 years (Fig. 9) since the beginning of the experiment, the trend of continuous growth is described with a 2nd degree equation with a coefficient of determination of 0.75-0.8.

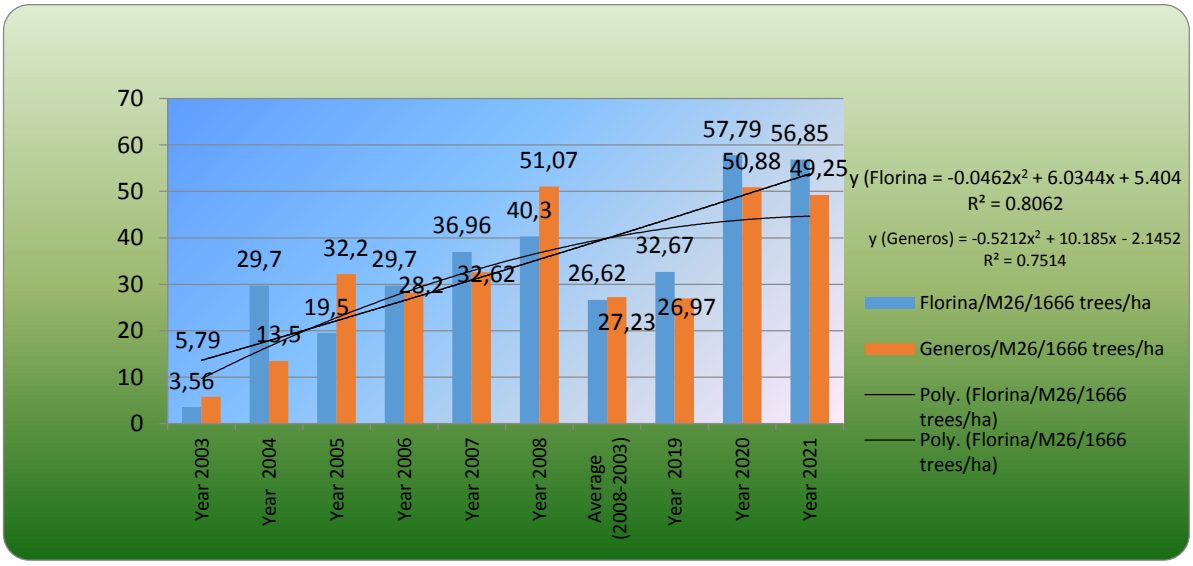


Figure 9. The evolution trend of fruit production to/ha in Florina and Generos varieties // Evoluția producției de fructe, t/ha, la soiurile Florina și Generos

The productivity index (Fig. 10) recorded higher values in the first 5 years of growth and fruiting, namely 0.55 for the Florina variety and 0.60 for the Generos variety, and in recent years the productivity index capped at lower values of 0.21 for the variety Florin respectively 0.20 for the Generos variety, following the "plateau" phase of the fruit plantation.

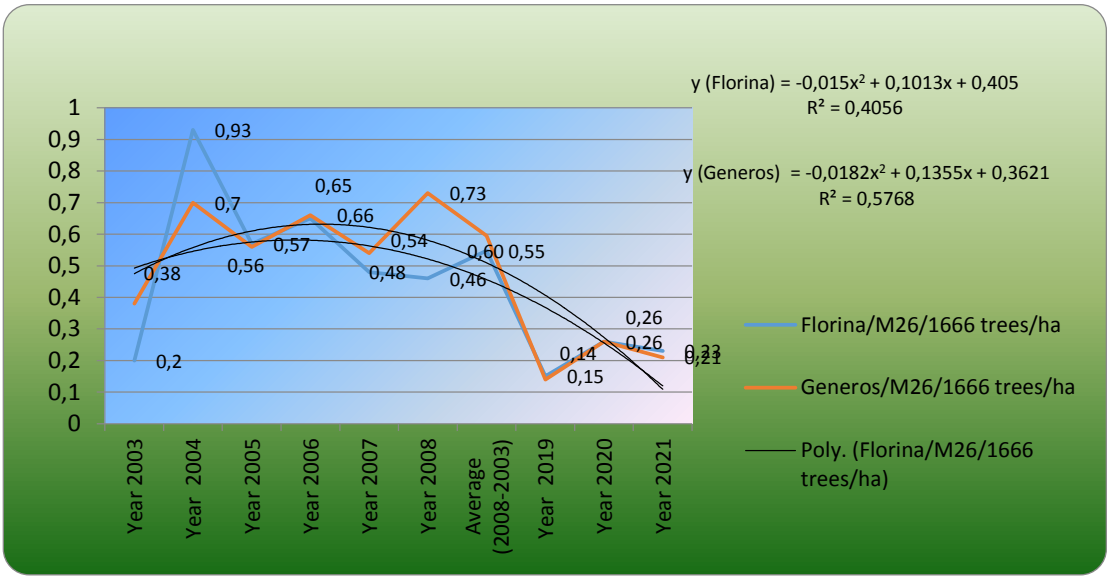


Figure 10. The evolution of the productivity index (kg/cm2) in the Florina and Generos varieties during the experimental period // Evoluția indexului de productivitate (kg/cm^2) la soiurile Florina și Generos în perioada de studiu

CONCLUSIONS

The reported yield per hectare in the ecopedological conditions from FRDS Bistrita at the maximum maturity of the experimental plantation (17-18 years after planting) was satisfactory, reaching a level of 42-49 to/ha for the Florina and Generos varieties grafted on the M26 rootstock. The

fruit production obtained was slightly higher in the Florina / M26 cultivar compared to the Generos / M26 cultivar, but the values were close under the conditions of applying a conventional chemical maintenance technology but with a reduced phytosanitary treatment scheme. The M26 rootstock adapted well to the local eco-pedological conditions, making good use of the typical brown argiloiluvial soil (eutricambosoil) resources, very widespread in Transylvania. Varieties resistant to apple scab (*Venturia inaqualis*) contributed to obtain quality fruits with the possibility of applying technological links in organic fruit growing.

Micu I. (1995) researched the adaptation and fruiting of some apple varieties with genetic resistance to diseases from the Transylvanian plateau and found that the Florina cultivar produced well in the V-XII year after planting and the production obtained was 24.6 tons/ha. In the pedoclimatic conditions of Timişoara, Iordănescu Olimpia (2005) observed the fact that in the eighth year from planting the Florina and Generos varieties grafted on the M26 rootstock in a thin spindle tree management system at a reduced density of 1250 trees/ha can be obtained 14.79 kg/tree for the Florina variety and 12.63 kg/tree for the Generos variety. Similar to the same varieties, Branişte (2005) observed that satisfactory yields can be obtained even in years V-IX from planting of 19.5 kg/tree for Florina and 15.4 kg/tree for Generos cultivars. Our results confirm those reported by the authors of the mentioned studies, at the same biological age, in the young phase of the trees (the first 5-6 years of production) and at maximum maturity, an increase in the level of production by approximately 40% is noted in both varieties.

ACKNOWLEDGEMENTS

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GENETIC RESOURCES USED FOR FRUIT TREES, BERRIES AND STRAWBERRY ROMANIAN BREEDING PROGRAMS

RESURSE GENETICE FOLOSITE ÎN PROGRAMUL ROMÂNESC DE AMELIORARE LA POMI, ARBUȘTI FRUCTIFERI ȘI CĂPȘUN

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Abstract

Fruit genetic resources are an important treasure of traits valuable for breeding and for long-term sustainable fruit production. The research undertaken on ex situ collections for most important fruit species has as results the new registered varieties and launched into fruit production. The pomological characterization (used descriptors, data management, pests and diseases evaluation) allowed accumulating of new knowledge mainly for apple, pear, plum, sweet cherry, strawberry, blueberry and other berries used as genitors in breeding programs, in the last 20 years, at Research Institute for Fruit Growing Pitești, Romania. Growing interest in fruit genetic resources is related to modern breeding for long-term sustainable.

Key words: bank gene, fruit trees, berries, strawberry.

Rezumat

Resursele genetice pomicole reprezintă un tezaur valoros pentru ameliorare și pentru producția durabilă de fructe pe termen lung. Cercetările întreprinse asupra colecțiilor ex situ existente la cele mai importante specii pomicole s-au concretizat în noile soiuri înregistrate și lansate în producție. Caracterizarea pomologică (descriptori folosiți, managementul datelor, evaluarea comportării la principalele boli și dăunători) a permis acumularea de noi cunoștințe, în principal, pentru genotipurile de măr, păr, prun, cireș, căpșun, afin și alți arbuști fructiferi, utilizate ca genitori în programele de ameliorare derulate, în ultimii 20 de ani, la Institutul de Cercetare-Dezvoltare pentru Pomicultură Pitești, România. Interesul tot mai mare pentru resursele genetice pomicole este legat de ameliorarea modernă pentru durabilitate pe termen lung.

Cuvinte cheie: colecții, pomi, arbuști fructiferi, căpșun.

INTRODUCTION

National, regional and local genebank, botanical gardens, arboreta, private companies and NGOs host fruit genetic resources collections (Bramel and Volk, 2019; Flachowsky and Höfer, 2010; Gassmann at al., 2017). A few of the still existing collections were established already in the 1920s, including the UK National Fruit Collection in Brogdale, currently maintained by the University of Reading, and the collection of the N.I. Vavilov Research Institute of Plant Industry in Russia (Loskutov, 1999; Kellerhals et al., 2021). In Germany, the collection of Julius Kűhn - Institut in Dresden-Pillnitz was established around 1930, and the Balsgard collection in Sweden in the late 1940s (Bramel and Volk, 2019). The collection at the Chinese Academy of Agricultural Sciences was established in the 1950s, and, in France, the INRAE collection in 1946 (Roux-Cuvelier et al., 2021).

In Romania, fruit genetic resources are collected and conserved as *ex situ* collections, organized according to biological requirements of species, in different research units, continuation of activity started in 1957, in the same period with organized the Research Institute of Horti-Viticulture. In 1959, the first official catalog of fruit genetic resources with 1,536 genotypes was published (Braniște and Cociu, 2006). This important treasure was the result of the work combined with passion of some

Romanian personalities in horticulture, such as prof. Constantinescu, prof. Ștefănescu, Ph. eng. Botez, Ph. Doc. Cociu, who collected and introduced into the collections everything that was most valuable, at that time, from Romania and abroad.

The pomological collection is a real laboratory, which offers the initial material used in cross combinations, but, in the same time, the possibility for establishing the genetic value of the parental forms, by carrying out hybridological analyzes of segregation of various characters and characteristics from parents (Cociu et al., 1989; Butac M., 2002).

For accessions held by the parties of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) in the Multilateral System, germplasm transfer is performed with a Standard Material Transfer Agreement (SMTA). For germplasm held by non-ITPGRFA parties or not held in the Multilateral System, the exchange could be under the conditions of the Nagoya Protocol or in other specific bilateral agreements (Bramel and Volk, 2019).

The European Cooperative Programme for Plant Genetic Resources (ECPGR) is a collaborative program among most European countries aimed at ensuring the long-term conservation and facilitating the increased utilization of plant genetic resources. The EURISCO catalog provides online passport information on accessions conserved in European plant genetic resources collections. Different working groups on *Malus/Pyrus, Prunus, Vitis* or berries, inside regular meetings, try to improve the management collections. ECPGR decided to establish a European conservation system, focused on existing ex situ collections to create a European Genebank Integrated System (AEGIS) aimed at conserving the genetically unique accessions for Europe and making them available for breeding and research (Kellerhals, 2009).

Presently, the commercial fruit growing is the main factor generating genetic erosion, by restricting of cultivated species and varieties. In addition, the modification of biotic and abiotic factors led to changes in the biological cycle of diseases and pests and, thus, increased the vulnerability of commercial genotypes, and, their defense mechanism became limited and easily defeated. For this reason, the care for fruit genetic resources is manifested by completing, concentrating and restoring the germplasm fund, but also the "intelligent" use of genetic resources, as the main source for food security. Access to a germplasm fund is very important when carrying out breeding programs, the information provided (origin, heritability, pedoclimatic adaptation, phenotypic data, resistances / susceptibilities to biotic and abiotic factors) being essential for the breeder in order to choose the genitors (Militaru et al., 2018).

Collecting and preserving of fruit genetic resources was carried out at Research Institute for Fruit Growing Pitesti, Romania since 1970 with the following objectives: maintaining the heritage of current genetic resources in fruit growing; permanent completion of the germplasm fund with old, traditional and new autochthonous forms, but also with foreign varieties; germplasm extension for some fruit species and berries; inventory, monitoring and evaluation of fruit genetic resources to identify new potential genitor useful for breeding programs.

MATERIALS AND METHODS

Dynamic and sustainable conservation of autochthonous genetic resources involves specific activities, starting with identification and collection of new genotypes, their vegetative propagation (usually, by grafting or cutting), followed by conservation in pomological collections organized *ex situ*, in parallel with evaluation and phenotypic characterization, according to international recognized descriptors (figure 1).

The total fruit genetic resources (fruit species, berries and strawberries) preserved at Research Institute for Fruit Growing (RIFG) Pitești, Romania counts about 2,000 accessions, of which 1,639 of

fruit trees, 111 strawberry, 109 currant, 69 blueberry and 95 of other berries (table 1). All accessions are maintained as trees or plants in the field collection.

Woody plant species are grafted on high vigor rootstocks and planted at 4 m between rows and 2-3 m between trees. For berries, the plants are placed at 3 m distance between rows and 50 cm between plants per row. The experimental device is made to illustrate the historical evolution: wild or semi-cultivated species, autochthonous varieties, introduced varieties. Inside each group, the genotypes are planted in order of harvest maturity.

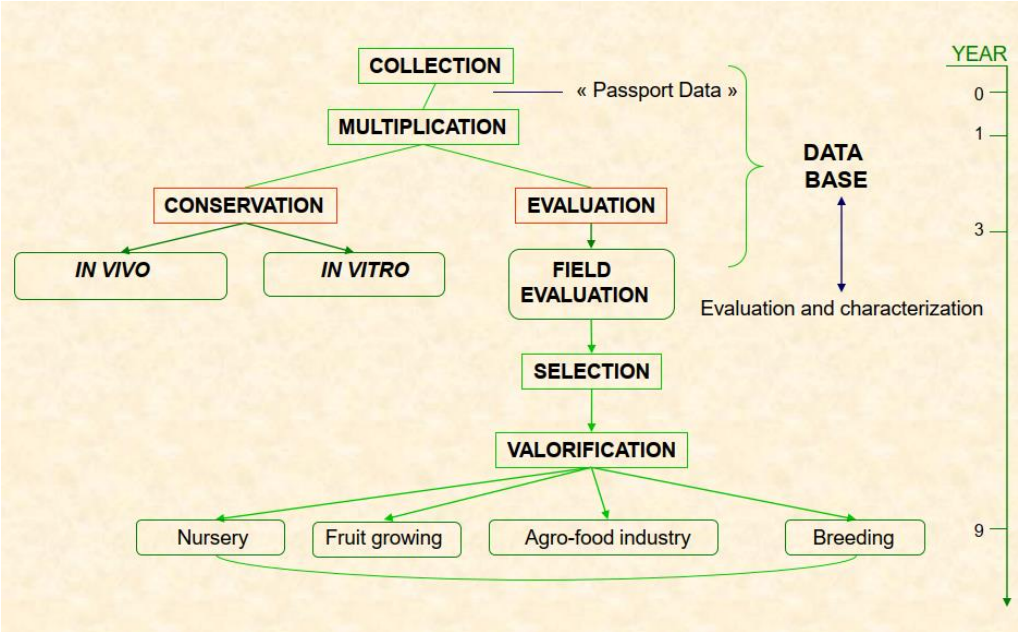


Figure 1. Work methodology // Metodologie de lucru

The periodic reorganization of collections is an absolutely necessary work, which is done at different time periods, depending on biological requests of fruit species. Thus, it is best to refresh the biological material preserved in strawberry, at a maximum interval of 4-5 years, then for some small fruit species (raspberry, currant, gooseberry, and blackberry) at 8-10 years and after a longer period (20-25 years) for other genera and species such as pome, stone and nuts.

Table 1. Fruit genetic resources preserved at RIFG Pitești, Romania // Resurse genetice conservate la ICDP Pitești, România

Species	No. of preserved genotypes	Genetic drift varieties (Source: ISTIS, 2022)
Apple	602	18 (Ardelean, Aromat de vară, Delicios de Voinești, Fălticeni, Florina, Frumos de Voinești, Golden Delicious, Goldspur, Idared, Jonagold, Jonathan, Kaltherer Böhmer, Mutsu, Pionier, Romus 3, Starkrimson, Voinea, Wagener premiat)
Pear	208	18 (Aniversare, Argessis, Aromată de Bistrița, Carpica, Conference, Contesa de Paris, Curé, Favorita lui Clapp, Haydeea, Olivier de Serres, Napoca, Republica, Timpurii de Dâmbovița, Untoasă Bosc, Untoasă de Geoagiu, Untoasă Hardy, Williams, Williams roșu)
Quince	13	0
Plum	440	16 (Alina, Anna Späth, Carpatin, Centenar, d'Agen, Gras ameliorat, Gras românesc, Ialomița, Pescăruș, Piteștean, Renclod Althan, Rivers timpuriu, Stanley, Tuleu gras, Vâlcean, Vinete de Italia)
Sweet cherry	162	9 (Boambe de Cotnari, Germersdorf, Hedelfinger, Izverna, Jubileu, Ponoare, Silva, Stella, Van)
Sour cherry	144	12 (Bucovina, Crișana 2, Ilva, Engleze timpurii, Mocănești 16, Pitic, Schattenmorelle, Timpuriu de Cluj, Timpuriu de Osoi, Timpurii de Pitești, Țarina, Vrâncean)
Walnut	48	5 (Geoagiu 65, Germisara, Jupânești, Novaci, Sibișel 44)
Hazelnut	22	2 (Tonda delle Langhe, Uriașe de Halle)
Strawberry	111	5 (Benton, Gorella, Premial, Red Gauntlet, Senga Sengana)

Currant	109	10 (Mărgăritar, Abanos, Perla neagră, Record,Tinker, Tsema, Houghton Castle, Jonkheer van Tets, Red Lake, Roşu timpuriu)
Blueberry	69	4 (Blueray, Coville, Pemberton, Weymouth)
Gooseberry	14	0
Raspberry	22	5 (Cayuga, Citria, Malling Exploit, Ruvi, The Latham)
Blackberry	21	2 (Darrow, Thornfree)
Seabuckthorn	5	1 (Şerpeni)
Honeysuckle	10	0
Chokeberry	2	1 (Nero)
Elderberry	3	2 (Ina, Nora)
Cornel	12	0
Rosehip	6	1 (Can)
Total	**2,023**	**111**

In order to harmonize the data, the germplasm evaluation and characterization was done according to some descriptors, recognized at international level. For each genotype, the fruit use, the plant use, status of sample and virus disease status are noted. In addition, a set of specific descriptors are used for each species, especial the fruit characteristics (size, shape, color) and susceptibility to some specific diseases and pests with major economic impact (table 2).

Table 2. Descriptors used for germplasm characterization and evaluation // Descriptori folosiți pentru caracterizarea și evaluarea germoplasmei

Apple	season of flowering, colour of flower button, harvest maturity, fruit size, fruit shape, over colour, ground colour, susceptibility to *Venturia inaequalis*
Pear	season of flowering, colour of flower button, harvest maturity, fruit size, fruit shape, over colour, ground colour, resistance to *Erwinia amylowora, r*esistance to *Psylla* sp.
Plum	season of flowering, harvest maturity, fruit size, fruit shape, over colour, adhesion of the kernel to the pulp, susceptibility to *Monilia laxa, Monilia fructigena,* PPV
Sweet and sour cherry	harvest maturity, over colour, juice colour, fruit cracking, susceptibility to *Monilia laxa, Monilia fructigena, Blumeriella jaapii*
Walnut	growth habit, tree vigour, dichogamy, season of flowering, tipul înfloritului, harvest maturity, nut size, nut shape, shell colour, shell thickness, ease of removal of kernel halves, kernel flavour, susceptibility to *Xanthomonas juglandis, Gnomonia juglandis, Cydia* sp.
Hazelnut	growth habit, tree vigour, flowering precocity, shape of nut apex, size of nut basal scar in relation to nut size, double (twin) kernels, first male bloom date, first female bloom date, dichogamy, nut maturity date, nut falling, susceptibility to *Xanthomonas corylina, Phyllactinia corylea, Balaninus nucum*
Strawberry	growth habit, vigour of the plant, fruit size, fruit shape primary fruits, external fruit colour, flesh colour, fruit firmness, susceptibility to *Botrytis cinerea, Phytophtora fragariae*
Currant	plant vigor, plant growth habit, number of basal shoots, number of flowers, fruit size, fruit shape, beginning of flowering, beginning of fruit ripening
Blueberry	growth habit, size of corolla tube, fruit size, intensity of bloom, skin color, sweetness, acidity, time of beginning of vegetative growth, time of beginning of flowering, time of beginning of fruit ripening
Gooseberry	plant vigor, plant density, number of basel shoots, shoot princkes, fruit size, fruit shape, fruit color, time of bud burst, time of beginning of flowering, time of fruit ripening
Raspberry	plant habit, for varieties which fruit on previous season's cane in summer: length and color of dormant cane, fruiting lateral: attitude and length, spines presence, spines density, fruit shape in lateral view, fruit size of single drupe, fruit color, glossiness, firmness, adherence to plug, main bearing type, length of fruiting period on previous year's cane
Blackberry	growth habit, number of new canes, length of dormant cane, spines, number of spines, leaf type, color of petal, length of fruiting lateral, fruit size, ratio length/width, fruit color, time of beginning of flowering, time of fruit ripening

RESULTS AND DISCUSSION

Apple collection has 1.2 ha and it was organized in the spring of 2010, by transferring, for safety reasons, of preserved biological material from Research Station for Fruit Growing Voinești, Dâmbovița. It includes a number of 602 genotypes grafted on the MM106 rootstock, with 2 trees/genotype planted at 4 m between rows and 2.5 m between trees per row. From 602 genotypes, 7 are *Malus* species, 82 local varieties and 513 foreign varieties. The characterization and evaluation of genotypes is based on the ECPGR (European Cooperative Program for Plant Genetic Resources) descriptors, updated and completed by Marc Lateur et al., in 2022.

In the last 20 years, the main donors used in apple cross combinations have been:

- for fruit quality and attractiveness: 'Gala' clones ('Mitchgla', 'Venus Fengal', 'Decarli Fendeca'), 'Golden Orange', 'Red Jonaprince', 'Falstaff', 'Auriu de Bistrița';
- for late ripening and good storage: 'Goldrush', 'Enterprise', 'Generos';
- sources for scab resistance: 'Crimson Crisp', 'Goldrush', 'Enterprise', 'Topaz', 'Red Topaz', 'Rubinola', 'Luna', 'Galiwa';
- for powdery mildew resistance: 'Ariwa';
- for earliness: 'Dalinbel', 'Romus 3'.

Pear collection, established in 2 years (2009 and 2010), includes 208 accessions, of which 13 species, 68 autochthones varieties and 127 foreign varieties. Each accession is represented by 2 trees grafted on *Pyrus communis* rootstocks, planted at 3.5 m between rows and 2 m between trees per row, with linear distribution. Characterization and evaluation of conserved genotypes is based on ECPGR descriptors (Lateur et al., 2022).

As a result of data collected out of evaluation of tree's and fruit' characteristics, genotypes common used in breeding activity, in the last 18 years, were:

- 'Monica' for nice appearance;
- 'Angelys', 'Williams', 'Max Red Barlett', 'Abate Fetel', 'Packham's Triumph', 'Rocha' for fruit quality and appearance;
- 'Euras', 'Orizont', 'Isadora' for good storage capacity and resistance to fire blight (*Erwinia amylowora)*;
- 'Tse Li', 'Isadora' for tolerance to *Psylla* sp.;
- 'Packham's Triumph', 'Xenia', for high productivity;
- 'Paradise', for red skin;
- 'Cu miezul rosu' (local variety), for red flesh.

Plum collection, reorganized in 2018 and 2019, has 440 genotypes, with 2 trees of each genotype, grafted on the 'Myrobolan' rootstock, planted at a distance of 4 x 3 m. As structure, the collection has 5 species of *Prunus*, 170 autochthonous varieties and 265 foreign varieties.

The characterization and evaluation of genotypes is based on the ECPGR (European Cooperative Program for Plant Genetic Resources) descriptors.

In the last years, the main genitors used in plum cross combinations have been:

- for fruit quality and attractiveness: 'Romanța', Toptaste', 'Andreea', 'Boranka', 'Timocanka', 'Milenium';
- for productivity: 'Stanley', 'Čačanska lepotiča', 'Romanța', 'Jojo', 'Haganta';
- for self-fertility: 'Stanley', 'Čačanska lepotiča', 'Romanța', 'Jojo';
- for Plum pox virus resistance/tolerance: 'Jojo', 'Haganta', 'Hanita', 'Andreea';
- for earliness: 'Tita', 'Piteștean', 'Carpatin', 'Topfirst'.

Sweet cherry collection was planted in spring 2013, having 162 accessions (one species, 35 autochthonous varieties and 126 foreign varieties). Each accession is represented by two trees grafted on 'Mahaleb' rootstock, planted at 5 x 4 m.

In the last years, for different objectives we used in cross combinations the following genitors:

- for fruit quality: 'Van', Techlovan', 'Kordia', 'Regina', 'Rubin', 'Tentant', 'Vanda';
- for productivity: 'Van', 'Skeena';
- for self-fertility: 'Stella', 'Skeena', 'New Star', 'Lapins';
- for fruit cracking resistance: 'Kordia', 'Regina', 'Lapins', 'Adriana';
- for earliness: 'Bigarreau Burlat', 'Merchant', 'Bellise', 'Spectral';
- for lateness: 'Kordia', 'Regina', 'Skeena', 'Areko', 'Sylvia'.

Sour cherry collection, planted on 2010, has 0.8 ha and contained 144 accessions (one species, 50 autochthonous varieties and 93 foreign varieties), with three trees per genotype, grafted on 'Mahaleb' rootstock and planted at 4 x 3 m.

In the sour cherry germplasm fund there are very valuable genotypes, some of them being used in controlled hybridizations, such as:

- for fruit quality and red colour: 'Rival', Sumadinka', 'Erdy Nogy', 'Stelar', 'Schatenmorelle', 'Vaskova', 'Vatinska';
- for productivity: 'Erdy Nogy', 'Sumadinka';
- for self-fertility: 'Nana', 'Schattenmorelle';
- for earliness: Țarina', 'Timpurii de Pitești', 'Mari timpurii', 'Dropia'

Strawberry and berries collection. According to the breeding objectives, in the last 15 years, the main donors used in berry cross combinations have been:

- strawberry: 'Mira' and 'Benton' for disease resistance, 'Mira' and 'Garda' for fruit quality, 'Albion' and 'San Andreas' for day neutral cultivars;
- blueberry: 'Simultan' and 'Bluecrop' for adaptability to local conditions of soil and climate, 'Simultan' and 'Delicia' for fruit quality, 'Duke' and 'Berkeley' for wide ripening season;
- currant: 'Abanos' for productivity and processing, 'Poli 51' for fruit quality and chemical content;
- raspberry: 'Opal' for frost resistance, 'Triple Crown' and 'Chester' for fruit quality;
- blackberry: 'Darrow' for frost resistance, 'Polka' and 'Benefis' for fruit quality and 'Opal' for wide ripening season (Table 3).

Table 3. Objectives and genitors for berry breeding programs //
Obiective și genitori pentru programele de ameliorarea ale arbuștilor fructiferi

Strawberry	Genitor	Blueberry	Genitor	Currant	Genitor	Raspberry	Genitor
Disease resistance (anthracnose, red stele)	Benton Idea Mira	**Adaptability to local conditions of soil and climate**	Simultan Azur Augusta Safir Bluecrop Blueray	**Productivity**	Tsema Kantata 50 Abanos Geo Deea	**Frost resistance**	Opal Star Gustar Ruvi Citria
Fruit quality (size, shape, firmness, colour)	Queen Elisa Mira Clery Garda Tecla Asia	**Fruit quality (size, appearance)**	Bluecrop Spartan Simultan Delicia Augusta	**Fruit quality and chemical content**	Tsema Kantata 50 Poli 51 Deea Perla Neagră	**Fruit quality (size, color, firmness)**	Latham Polka Polana Benefis
Early season Late season Day neutral	Premial Alba Clery Argentera Mira Albion San Andreas	**Wide ripening season**	Simultan Duke Bluecrop Blueray Berkeley	**Processing**	Perla Neagră Poli 51 Abanos	**Wide ripening season**	Heritage Citria Opal Ruvi

Walnut collection, planted on April 2019, with planting distance 8 x 6 m, grafted trees on *Juglans regia*, has 48 genotypes, of which 33 are autochthonous.

Hazelnut collection, organized on 2020 and completed in 2021, has 22 genotypes planted at 4 m between rows and 3 m between plants.

CONCLUSIONS

The fruit genetic resources collected at RIFG Pitesti contributes to the sustainable preservation of biodiversity of different fruit growing species and to ensure the availability of this biological material for distinct user purposes (breeding, research, agro-food industry).

Traditional fruit breeding and evaluation of fruit genetic resources was based on phenotypic characterization. For future, the request is to combine both, phenotyping and genotyping characterization, as common practice. However, field evaluation regarding pests and diseases resistance or susceptibility is important for breeding and for replanting traditional varieties. The information on

the fruit quality of genetic resources are useful for breeding or specific processing and consumption purposes.

The selection from wild flora still offers the possibility of identification and utilization of valuable genotypes adapted to different soil conditions and climate changes.

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CARBOHYDRATE RESERVES IN SOME GRAPEVINE CULTIVARS FROM SCDVV BLAJ

REZERVA DE CARBOHIDRAȚI A UNOR SOIURI DE VIȚĂ-DE-VIE CULTIVATE LA SCDVV BLAJ

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Abstract
Carbohydrate reserves are crucial for the growth cycle of grapevine because they provide the carbon source that supports the start of vegetation. There is scientific evidence that shows that the carbohydrate amount from the reserves stored over winter influences the new root and bud development, the shoot growth and the development of the inflorescences in the following season. The present study assesses the overwintering carbohydrate reserve status of several grapevine varieties cultivated at SCDVV Blaj. The studied cultivars were: Italian Riesling, Fetească regală, Fetească neagră, Fetească albă, Neuburger, Traminer roz and Sauvignon blanc, all located at Crăciunelu de jos grapevine plantation. The soluble sugars content, the starch content and the amount of total carbohydrates were determined using the classic anthrone reagent method. All of the samples were assessed as possessing a sufficient carbohydrate reserve for the first phenophases of this year's (2023) vegetation period, most of them being classified as very well-matured, having a total carbohydrate content higher than 16 g % DW.

Keywords: grapevine, soluble sugars, starch, total carbohydrates, reserve

Rezumat
Rezerva de carbohidrați îndeplinește un rol crucial în ciclul de creștere a viței-de-vie fiind principala sursă de carbon necesară pornirii în vegetație. Mai multe rapoarte științifice evidențiază faptul că această rezervă de carbohidrați înmagazinată susține creșterea noilor rădăcini, dezmuguritul, creșterea lăstarilor și dezvoltarea inflorescențelor în sezonul următor. Prezentul studiu evaluează starea rezervei de carbohidrați din perioada de repaus vegetativ a mai multor soiuri de viță-de-vie cultivate la SCDVV Blaj. Soiurile studiate au fost: Riesling italian, Fetească regală, Fetească neagră, Fetească albă, Neuburger, Traminer roz și Sauvignon blanc, toate situate în plantația viticolă Crăciunelu de jos. Conținutul de zaharuri solubile, conținutul de amidon și cantitatea de carbohidrați totali au fost determinate prin metoda reactivului anthronă. Toate probele au fost evaluate ca având o rezervă suficientă de carbohidrați pentru primele fenofaze de vegetație ale acesui an (2023), cele mai multe dintre ele fiind clasificate ca foarte bine maturate, având un conținut total de carbohidrați mai mare de 16 g% DW.

Cuvinte cheie: viță-de-vie, zaharuri, amidon, carbohidrați, rezervă

INTRODUCTION:

Vineyards from Transylvania's viticultural zone often face a lot of challenges related to climatic conditions. Cold winters with low temperatures are quite common, and this can have a negative impact on the grapevine's health, destroying the buds and the vascular tissues, in extreme cases, leading to the complete loss of the grapevines. Another challenge of this area is the late spring frosts which can destroy the young shoots leading to the loss of the grape harvest (Țâra *et al.*, 1992; Răcoare *et al.*, 2022). The cold resistance of the winter buds is conditioned by the maturation degree of the canes, which is determined by the total content of carbohydrates (Noronha *et al*., 2018; Călugar *et al*., 2019; Popova, 2021). Also, in grapevines, the carbohydrate reserves act as a buffering mechanism that allows

the functioning of cellular processes, growth and stimulation of defense mechanisms when metabolic demands exceed energy intake. They help perennial plants to resist biotic and abiotic stress factors including the attack of diseases and pests (Noronha *et al.,* 2018). Therefore, through an adequate management, the stress of the grapevine can be minimized by ensuring the optimal accumulation and storage of carbohydrates in buds and woody tissues (Țâra *et al*., 1999; Iliescu *et al*., 2012).

Carbohydrates are produced by leaves and stored as starch and soluble sugars (mostly glucose, fructose and sucrose) in the perennial organs (Bennet *et al*., 2005; Pellegrino *et al*., 2014; Călugăr *et al*., 2022). Starch is stored as granules within the xylem and phloem ray cell. Areas of high starch concentrations can be seen after staining with iodine at the whole-tissue level (Călugăr *et al.,* 2022). In the wood, starch concentrations are higher than those of sugars during the growing season, but some of the starch is converted to sugars prior to the dormancy period in order to help the grapevine protection from cold damage during winter (Noronha *et al*., 2018; Călugăr *et al*., 2019; Popova, 2021). At all phases of development, starch is the primary storage carbohydrate in the roots. Fine root development that occurs in early spring is most likely provided by carbohydrates stored in the permanent roots (Zapata *et al*., 2004; Pellegrino *et al.,* 2014;). In order to assist the early shoot growth, localized carbohydrate reserves and possibly sugars diffused from the bleeding sap are utilized. As the phloem function of the canes and older wood restarts in the weeks following bud break, reserves from more distant sections of the vine are progressively used (Zapata *et al*., 2004; Pellegrino *et al.,* 2014;). The transport of reserve carbohydrates to the shoot reaches a maximum amount at the stage of 8-10 leaves and then it declines as the canopy photosynthesis becomes sufficient (to meet the carbohydrate demand) (Bennett, 2002). After that, the newly assimilated carbon starts to be exported back to the perennial organs of the grapevine (a week or two before flowering). The reserve replenishment may occur at the same time as the fruit ripening if yields are adequate or if the photosynthesis rates are high enough to produce an excess of carbohydrates for preservation. However, if the productions are to high or if circumstances like water stress or high temperatures decrease photosynthesis, the reserve replenishment may be delayed until after harvest. Along with heavy crop loads, renewed mobilization of carbohydrates may occur in the earlier part of the ripening phenophase to help berry ripening (Zapata *et al.,* 2004; Pellegrino *et al.,* 2014;).

The dynamics of the carbohydrate reserve in the perennial organs of grapevines are mainly determined by the growth and maintenance requirements at particular developmental stages. This balance between carbon supply and demand is influenced by manageable factors such as yield, canopy size, and water supply, and by limiting climatic factors such as temperature or seasonal duration that affect photosynthesis and thus the net capacity of carbon assimilation (Holzapfel *et al.,* 2010).

Shoots maturation is a physiological process of reserve substances accumulation, which is highlighted in autumn by the change of the bark color and marks the transition from the active period of vegetation to the dormancy period (Alexandrescu *et al.,* 1994). The maturation process of the shoots starts in mid-August, with the slowing down of growth, from the base of the shoots and gradually advances towards their top. Along the length of the shoot, there is a maturation lag in the sense that the internodes at the base accumulate a greater amount of carbohydrates than those oriented toward the tip of the shoot. The maturation process ends when the leaves fall, therefore at the end of the vegetation period, the tops of the shoots remain unmatured, often being affected by the early frosts in autumn (Țârdea and Dejeu, 1995). Canes maturation shows inconstancy and variations from one year to another and from one cultivar to another, due to environmental factors and polygenic heredity (Oprea and Moldovan, 2007).

Overall, the assessment of the wood maturation degree of grapevines, at the end of the vegetation period is an important indicator that shows if the morphological, physiological anatomical and structural processes proceeded normally. The degree of cane maturation influences the

differentiation of fruit buds, the rooting of cuttings and the formation of callus when grafting the grapevine (Iliescu *et al*., 2010).

This study aims the dosage of carbohydrates (the most reliable laboratory indicator for assessing the degree of wood maturation) from several grapevine cultivars in order to anticipate the course of this year's vegetation period at SCDVV Blaj.

MATERIALS AND METHODS

The study was carried out during dormancy in the winter of 2022-2023. The following grapevine (wine) cultivars: Fetească albă, Fetească regală, Fetească neagră, Italian Riesling, Neuburger, Traminer and Sauvignon blanc, located in the Experimental Base - Crăciunelu de Jos of the Blaj Viticulture and Vinification Research Station (Table 1) were investigated. The determined parameters included canes water content and diameter, grape yield in the fall of 2022, and the carbohydrate content of canes (sugars, starch and total carbohydrate content).

Table 1. Data regarding the identification and characterization of the samples // Date de identificare și caracterizare a probelor

Crt no.	Sample Code	Cultivar	Experimental plot	Establishment year	Training sistem	Soil type	Soil management	Planting distance [m]	GPS coordinates
1	**FA_R**	Fetească albă	Crăciunel reconversion	2007	Demi-high Guyot system with periodic replacement arms	Coluvisol with lotony sand texture	Grass strips	2.0 x 1.2	46.178252 23.818766
2	**FR_R**	Fetească regală	Crăciunel reconversion	2012		Coluvisol with lotony sand texture		2.0 x 1.2	46.180496 23.511440
3	**FN_PM**	Fetească neagră	Crăciunel, mother plantation	2010		Alluvial soil with a sandy texture		2.0 x 1	46.174396 23.850859
4	**RI_PM**	Riesling italian	Crăciunel, mother plantation	2010		Alluvial soil with a sandy texture		2.0 x 1	46.173977 23.849361
5	**RI_R**	Riesling italian	Crăciunel reconversion	2012		Coluvisol with lotony sand texture		2.0 x 1.2	46.180745 23.856663
6	**NB_R**	Neuburger	Crăciunel reconversion	2012		Coluvisol with lotony sand texture		2.0 x 1.2	46.181368 23.857553
7	**TR_PM**	Traminer roz	Crăciunel, mother plantation	2007		Alluvial soil with a sandy texture		2. 0 x 1	46.173307 23.848492
8	**SG_PM**	Sauvignon blanc	Crăciunel, mother plantation	2014		Alluvial soil with a sandy texture		2.0 x 1	46.173307 23.848492

The water content of canes was determined using the oven drying method at a temperature of 105 ˚C until constant mass. The diameter of the canes was measured with the caliper - 24 measurements were made for each sample. The yield per vine were calculated according to the productions per hectare (reported in accounting) for the year 2022, taking into account the density of vines for each cultivar.

For the determination of the carbohydrate content, samples of annual canes (5) of each cultivar, were collected (in three repetitions) on the 19th of January 2023. The canes were then cut into pieces of approximately 20 cm and dried in an oven (ESAC sterilizer, ELECTRONIC APRIL SRL, Cluj-Napoca, Romania) at 55˚C, for approximately 72 hours (until constant mass). Then the samples were ground, sieved (sizes of max 0.7 mm), packed and labeled accordingly, and stored at room temperature until analysis. The maturation degree of the samples was appreciated by the determination of the carbohydrate content using the colorimetric method adapted from DuBois M., *et al*., (1956). The method principle consists in the extraction of sugars with ethyl alcohol and the extraction of starch with

perchloric acid, under determined conditions and the measurement of the color intensity after treating the extracts with the anthrone reagent. The measurement of the color intensity was done spectrophotometrically using a UV/VIS Spectrophotometer (ScanDrop2, Analytik Jena, Konrad, Germany) at 620nm wavelength. The total carbohydrate (TCH) content of the samples sums up the sugar and starch content, expressed as glucose (in grams) and expressed as a percentage of dry weight (d.w).

The results obtained were evaluated as follows:

- TCH < 12 g% d.w = very poorly matured
- TCH between 12 - 14 g% d.w = poorly matured
- TCH between 14 - 16 g% d.w = matured
- TCH between 16 - 18 g% d.w = well matured
- TCH > 18 g% d.w = very matured

Samples were analyzed in triplicate. The obtained data were statistically analyzed with the program Minitab 17 using the One-Way ANOVA method as well as the PCA method. Tukey test with a 95% confidence interval was used for the statistical interpretation of the results. PEARSON correlation was also used to check correlations between variables.

RESULTS AND DISCUSSION

The analyzed samples recorded a normal level of humidity for the period in which the samples were collected, oscillating around 40%. The lowest moisture level 37.7% was observed in the sample of Italian Riesling from the "mother plantation" and the highest moisture level 43.73% was observed in Traminer. From a statistical point of view, the water content of the canes shows significant differences (Table 2). The canes diameter ranged between 6.82 mm in the sample of Italian Riesling from the "mother plantation" and 8.21 mm in the Traminer sample, the differences between cultivars were not statistically significant (Table 2). The grapes yield, for the studied cultivars, in 2022, ranged between 2.3 and 3.9 kg/vine (Table 2). The Neuburger cultivar recorded the highest yield (3.9 kg/vine), exceeding the normal levels. For the other studied cultivars, the productions obtained in 2022 were within the normal limits.

Table 2. Results regarding canes water content and diameter and grape yield obtained in 2022 // Rezultate privind umiditatea si diametrul coardelor și a producției obținute în anul 2022

Crt.no.	Sample code	Humidity [%]	Diameter [mm]	Yield [kg/vine]
1	FA_R	41.42±0.11^{bc}	7.55±0.34^{a}	2.3
2	FR_R	39.72±0.11^{d}	7.97±0.62^{a}	3.1
3	FN_PM	41.97±0.39^{b}	6.88±0.54^{a}	2.4
4	RI_PM	37.7±0.28^{e}	6.82±0.37^{a}	2.9
5	RI_R	41.20±0.42^{bc}	6.88±0.41^{a}	3.1
6	NB_R	40.50±0.14^{cd}	7.45±0.55^{a}	3.9
7	TR_PM	43.73±0.11^{a}	8.21±0.30^{a}	2.3
8	SG_PM	41.35±0.14^{bc}	7.30±0.06^{a}	2.9

*results are presented as means ± standard deviation
**different letters represent statistically significant differences (p<0.05%)

The total carbohydrate content varied between 12.63 g% d.w in the Neuburger cultivar and 17.94 g% d.w in the Fetească albă cultivar (Table 3). From a statistical point of view, the cultivars Fetească albă, Sauvignon and Neuburger show significant differences in terms of total carbohydrate content, and the cultivars Fetească regală, Fetească neagră, Italian Riesling and Traminer do not show significant differences, having a similar content of total carbohydrates.

Table 3. Results regarding the carbohydrate content // Rezultate privind conținutul de carbohidrați

Crt.no.	Sample code	Sugar content [g% d.w]	Starch content [g% d.w]	Total carbohydrate content [g% d.w]	Interpretation
1	FA_R	10.97±0.76^{a}	6.97±0.35^{bc}	17.94±0.41^{a}	well matured canes
2	FR_R	8.19±0.98^{b}	8.48±0.25^{a}	16.67±01.21^{ab}	well matured canes
3	FN_PM	9.53±0.78^{ab}	7.34±0.11^{b}	16.87±0.68^{ab}	well matured canes
4	RI_PM	10.20±0.97^{ab}	6.31±0.24^{c}	16.51±0.74^{ab}	well matured canes
5	RI_R	10.89±0.18^{a}	5.24±0.12^{d}	16.13±0.30^{ab}	well matured canes
6	NB_R	9.24±0.95^{ab}	3.39±0.27^{e}	12.63±0.68^{c}	poorly matured canes
7	TR_PM	8.79±1.19^{ab}	6.88±0.37^{bc}	15.67±0.82^{ab}	matured canes
8	SG_PM	8.96±0.71^{ab}	6.31±0.43^{c}	15.27±1.13^{b}	matured canes

*results are presented as means ± standard deviation
**different letters represent statistically significant differences ($p<0.05\%$)

The location of the cultivars within the Crăciunelu de Jos plantation does not influence the total carbohydrate content, in the sense that there were no statistically significant differences between the samples collected from the "mother plantation" and/or "reconversion" (Table 3).

Regarding the sugar content, significantly higher values were observed in the samples of Feteasca albă and Italian Riesling from the "reconversion" experimental plot, significantly lower values being recorded by the Feteasca regală cultivar. The Italian Riesling samples from the "mother plantation", Neuburger, Fetească neagră, Traminer and Sauvignon recorded a statistically similar sugar content ranging between 8.79 and 10.20 g% d.w (Figure 1 and Table 3).

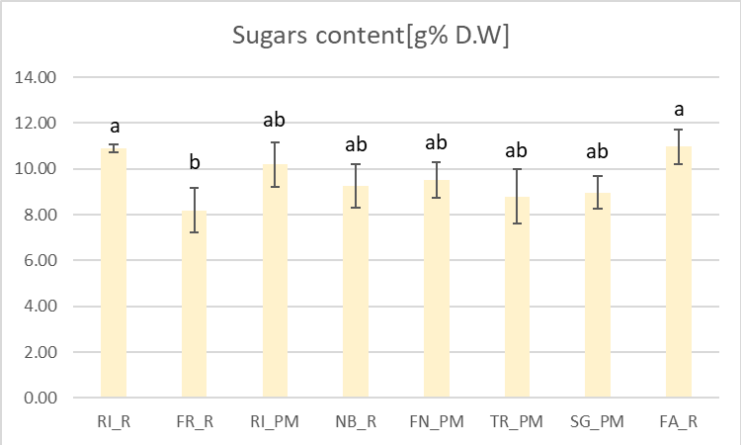


Figure 1. Graphical representation of the sugar content // Reprezentare grafică a conținutului de zaharuri

The starch content ranged between 3.39 and 8.48 g% d.w, lower values than those recorded for the sugar content (Figure 2 and Table 3). The differences are significant between most of the analyzed samples, the highest starch content being recorded by the Fetească regală cultivar, followed by Fetească neagră, Fetească albă and Traminer. A lower starch content was observed in the Sauvignon and Italian Riesling cultivars, the lowest value being recorded in the Neuburger cultivar.

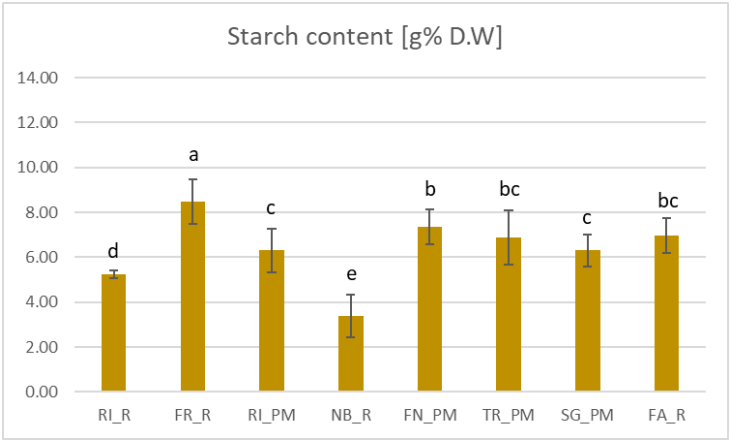


Figure 2. Graphical representation of the starch content // Reprezentare grafică a conținutului de amidon

The correlation between the total carbohydrate content and the yield (per vine) obtained in the year 2022 demonstrated a very strong negative relationship (r=-0.713). In this sense, it can be observed that the Neuburger cultivar, which had the highest yield per vine among the analyzed samples (Table 2), recorded the lowest content of total carbohydrates (Table 3). In the same way, the cultivar Fetească albă, which had the lowest yields, recorded the highest content of total carbohydrates. A weak negative correlation was observed between sugar content and yield, and a strong correlation was observed between starch content and yield.

The PCA analysis of the samples shows that they are differentiated into separate groupings by the 2 studied variables (sugars and starch content). Thus, the Italian Riesling varieties (both the one from the "reconversion" and the one from the "mother plantation") and Fetească albă stand out as having a high sugar content, at the opposite pole are the Sauvignon, Traminer and Fetească regală varieties. With respect to the starch content, the Fetească regală and Fetească neagră cultivars are noted as having a high starch content and the Neuburger variety as having a low starch content (Figure 3).

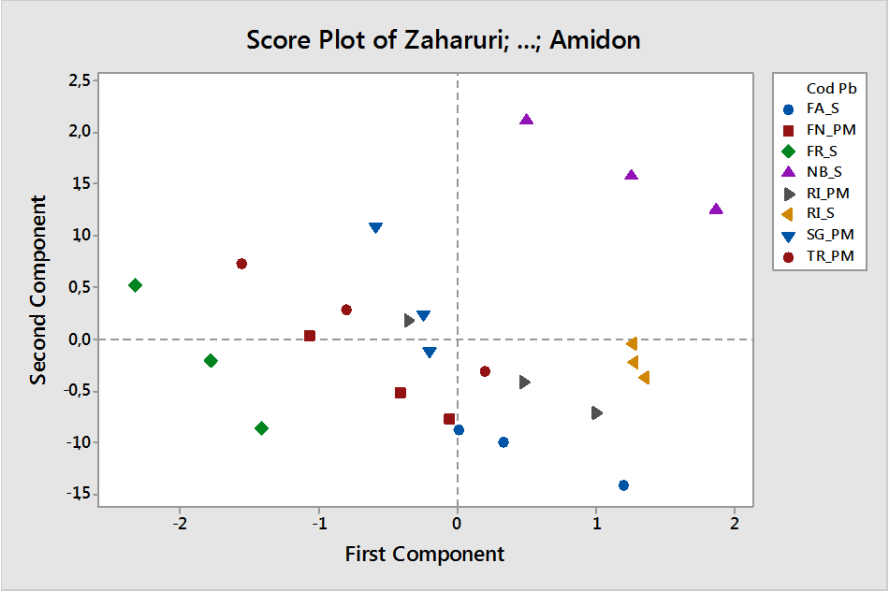


Figure 3. Score plot chart of the PCA analysis regarding the sugar and starch content // Graficul de tip score plot al analizei PCA a conținutului de zahăr și amidon

The results of the present study are consistent with the existing data in the specialized literature. The shoot or cane is considered mature if at the end of the vegetation period, the starch content is greater than 10 g% d.w., or the total carbohydrates are greater than 12 g% d.w. (IRIMIA, 2012). Holzapfel *et al*., (2010) consider that the shoot is fully matured (reaches a maximum degree of lignification), if at leaf fall it has a total carbohydrate content of 19 g% dry matter. Iliescu *et al.*, (2011) report, for the years 2008 and 2009, in the Fetească regală, Sauvignon, Italian Riesling and Traminer cultivars a total carbohydrate content between 13.47 and 17.60 g% d.w as well as slightly higher values for the year 2010 (14.10 and 17.80 g% d.w), the Traminer cultivar recording the highest values in each of the 3 years. Comșa *et al*., (2013) report a carbohydrate content ranging between 18 and 20 g% d.w for Fetească regală, Italian Riesling and Sauvignon cultivars. Cimpoi *et al*., (2020) also report a total carbohydrate content of 15.64 g% (summing the content of soluble sugars 11.38 g %, and the content of starch 4.26 g%), followed by the "Aromat de Iași" cultivar with a total carbohydrates content of 14.97 g%, of which 9.56 g% representing the content of soluble sugars and 5.41 g% the content of starch. Călugăr *et al*., (2022) report for the dormancy period of 2019-2020, a total carbohydrate content between 12 and 18 g% d.w. and a starch content between 3 and 5 g% d.w.

CONCLUSIONS

1. At harvest time, the canes had a water content of approximately 40% and a diameter of approximately 7 mm. None of the samples reached the maximum lignification threshold, but most of the samples exceeded the threshold of 14 g% d.w. total carbohydrates, which means that they are mature enough to expect a normal vegetation period for 2023. Most samples recorded a total carbohydrate content between 16 and 18 g% d.w. being appreciated as well matured.
2. The Fetească albă cultivar was appreciated as well matured, registering a total carbohydrate content of 17.94 g% d.w., and the least matured was the Neuburger cultivar 12.63 g% d.w, being appreciated as poorly matured.
3. The Fetească albă cultivar recorded the highest sugar content of canes (10.97 g% d.w.) while the Fetească regală cultivar recorded the lowest sugar content (8.19 g% d.w.).
4. The starch content recorded lower values than those recorded for the sugar content, ranging between 3.39 g% d.w. in the Neuburger cultivar and 8.48 g% d.w. for the Fetească regală cultivar.
5. The samples from the Fetească cultivar (albă, regală and neagră) and Italian Riesling (from the "reconversion" and the "mother plantation") recorded a total carbohydrate content between 16 and 18 g% d.w. being thus categorized as well matured.
6. A very strong negative correlation was revealed between production and total carbohydrate content, while cultivar location within the plantation had no influence on the carbohydrate content.
7. The results obtained are consistent with the specialized literature, Iliescu *et al.*, (2011), Comșa *et al*., (2013), Cimpoi *et al*., (2020) and Călugăr *et al.*, (2022) reporting similar results.

Based on the results of this study, in the 2023 season, at SCDVV Blaj in the Crăciunelu de Jos vineyard, can be expected a normal vegetation period which can provide a good harvest yield, with the Neuburger cultivar requiring special attention.

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THE STUDY OF THE BICAN ROZ 6 MF CLONE IN THE CLIMATE CONDITIONS OF THE MURFATLAR VINEYARD

STUDIUL CLONEI BICAN ROZ 6 MF ÎN CONDIȚIILE CLIMATICE ALE PODGORIEI MURFATLAR

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Abstract
At Murfatlar Research Station, in the period 2018 – 2022, a study was carried out on 5 clonal elites of the 'Bican roz' variety, selected from existing trunks in the ampelographic collection of the research station. In all 5 years of studies and observations, the clonal elite 80/10/6 was superior to the other elites, this being approved under the name Bican roz 6 Mf in 2022, following testing at ISTIS (State Institute for Testing and Registration Varieties). During the research period, average annual temperatures were 2-3°C higher in comparison with the multiannual average, while precipitation values were situated below the multiannual average (406 mm, average during the study period, compared to 522.6 mm, the multiannual average). For all 5 clonal elites, the agrobiological properties, as well as the technological and agroeconomic characteristics, were compared to those of the original variety. Bican roz 6 Mf is a table grape clone with high growth vigor and 79% fertility. It is a productive clone, with an average grape weight of 714 g, compared to 403 g, the average grape weight for the original variety and with yields of 10.76 kg/trunk, respectively 24 t/ha and commercial production of 75 – 80%.

Key words: table grapes, clonal elites, grapevine, productivity, quality.

Rezumat
La SCDVV Murfatlar, în perioada 2018 – 2022, a fost realizat un studiu pe 5 elite clonale ale soiului 'Bican roz', selectate din butuci existenti în colecția ampelografică a stațiunii. În toți cei 5 ani de studii și observații, elita clonală 80/10/6 a fost superioară celorlalte elite, aceasta fiind omologată sub denumirea de Bican roz 6 Mf în anul 2022, în urma testării la ISTIS (Institutul de Stat pentru Testarea si Inregistrarea Soiurilor). În perioada de cercetare au fost înregistrate temperaturi medii anuale cu 2 – 3°C mai ridicate comparativ cu media multianuală și precipitații sub valoarea medie multianuală (406 mm, media în perioada studiului comparativ cu 522,6 mm, media multianuală). Pentru toate cele 5 elite clonale au fost urmărite însușirile agrobiologice, precum și caracteristicile tehnologice și agroeconomice, comparativ cu cele ale soiului inițial. Bican roz 6 Mf este o clonă pentru struguri de masă, cu vigoare de creștere mare și fertilitate de 79%. Este o clonă productivă, având greutatea medie a unui strugure de 714 g, comparativ cu 403 g, greutatea medie a strugurelui pentru soiul inițial, și cu producții de 10,76 kg/butuc, respectiv 24 t/ha și producția marfă de 75 – 80%.

Cuvinte cheie: struguri de masă, elite clonale, viță de vie, rezistență, calitate.

INTRODUCTION

Grapevine is a light and heat loving plant [11,15] of great socio-economic importance, that uses the energy resources offered by the environment rationally and efficiently, but at the same time

conditions the dynamics of its growth and development is in agreement on how they comply with its vegetation requirements [6,7,13].

The climate changes that are currently happening condition the creation of plant genotypes that will be able to develop and ensure increased productivity in the new pedoclimatic conditions and at the same time will contribute to mitigating desertification processes [1].

The expansion of the grapevine plantations or the introduction of new varieties in a certain area requires the assessment of the ecological favorability of the area allocated for this purpose [4].

The grape breeding achievements obtained thus far, by clonal selection from populations of older varieties, natural mutants and newly created varieties with superior traits, have ensured increased grape production, of superior and relatively constant quality from one year to another [3].

The ecoclimate of the Murfatlar vineyard is characterized by a relatively short period of vegetation, during which a large amount of global radiation and a large heliothermal reserve accumulate, creating favorable conditions for the growth and development of the grapevine.

In ampelographic collections, in addition to the conservation of genetic resources, the aim is to carry out detailed research in order to describe the existing genotypes and the perspective elites that have appeared within the population, which by their characteristics lend themselves to testing in order to obtain valuable clones, these being tested and approved by ISTIS, in order to multiply and establish plantations.

MATERIAL AND METHOD

The team of researchers from Murfatlar Research Station carried out a study during the period 2018-2022 on 5 clonal elites of the 'Bican roz' cultivar, selected from existing trunks in the ampelographic collection of the research station. In this study, the botanical characters and agrobiological properties were established, and the technological and agroeconomic characterization was also carried out.

The choice of clonal elites was made in two stages:

a) in the first stage, field observations were made regarding the vigor, the phytosanitary condition, some morphological components and the production capacity;
b) in the second stage, at the selected clonal elites, determinations were made regarding the quality and quantity of the grapes, the size of the bunches, the number of berries per bunch, but also the quantity of must obtained.

As a result of the observations made, five elite trunks were marked, from which the top elite will be chosen that corresponds to all the criteria chosen by the breeder: consistent production and quality, as well as good resistance to diseases and pests.

RESULTS AND DISCUSSION

During the study period, research was carried out for the creation of varieties and the identification of clonal elites superior to the original 'Bican roz', a cultivar for table grapes, which, along with good quality and productivity, possesses properties of resistance to biotic and abiotic environmental factors, and which requires a minimum of phytosanitary treatments, helping to reduce the pollution of the viticultural ecosystem as well as the final products.

The climate of the Murfatlar vineyard is continental, with hot summers and moderate winters, creating the most favorable natural conditions for the cultivation of grapevine.

Climatic indices express the interaction of several elements of the climate (temperature, precipitation, relative air humidity, real insolation, etc.) and are used to characterize the viticultural

potential at the macroclimatic level [10].

In table 1, a series of climatic elements are analyzed, as well as a series of climatic indices that are used worldwide, such as: Huglin heliothermic index [9] and cool night index [16].

Table 1. Climatic indicators for the Murfatlar Wine Center // Indicatori climatici ai Centrului Viticol Murfatlar

Analyzed climatic elements	Multiannual average	2018	2019	2020	2021	2022
Global thermal balance, Σt °g, °C	**5203.5**	5,379.6	5534.4	4999.6	4396.8	5053.3
Active thermal balance, Σt °g, °C	**4689.8**	4,815.5	5061.8	4500.5	3693.3	4573.8
Useful thermal balance, Σt °g, °C	**2538.7**	2,427.2	2811.8	2270.5	1850.1	2228.8
Absolute minimum air temperature,°C	-15.6	-12.8	-10.5	-13.9	-13.1	-10.6
Average annual temperature °C	12.3	14.6	14.8	14.9	13.8	13.9
Maximum air temperature, °C	37.8	37.4	39	33.9	39.5	36.0
Σ annual precipitation, mm	559.7	696.2	311.4	267.9	661	383.7
Σ active period precipitation, mm	367.6	364.3	180.4	161.7	339.3	206.7
Σ real active period insolation, (h)	1708.4	1453.9	1329.7	1574.6	1300.6	1401
No. of days with max. temperatures > 30°C	51	78	98	57	44	64
Active period, days	192	202	206	213	213	198
Real heliothermic index (IHr)	4.5	3,4	4.5	5.7	3	4.5
Hydrothermic coefficient (CH)	0.9	0.8	0.4	0.4	0.9	0.6
Bioclimatic index (Ibcv)	12.8	8.7	22.6	20.3	6.4	12.3
Oenoclimatic aptitude index (IAOe)	5492.7	5125.0	5847.5	5457.0	4700.3	5250.7
Huglin heliothermic index (IH)	3130.1	3280.0	4583.7	4584.0	2856.3	3976.9
Cool night index (IF)	12.8	14.1	14.4	14.5	10.4	12.9

Analyzing the data from table 1 we can see an increase in thermal balances: global, active and useful, in comparison to the multiannual average. The sum of real insolation during the growing season (1401 hours), is lower than the multiannual average (1708.4 hours); the sum of the annual precipitation (383.7 mm) is lower compared to the multiannual average (559.7 mm); the sum of the precipitation during the growing season (206.7 mm, is significantly smaller (367.6 mm), whereas the cool night index (12.8) has slightly increased (12.9 in 2022).

Due to climate changes, the number of days with maximum temperatures > 30°C increased (64 days) compared to the multiannual average (51 days) and the Huglin heliothermic index (IH) increased (3976.9) compared to the multiannual average which is 3130, 1.

Grapevine fertility represents the ability of the plant to form fruiting organs every year as the initial basis for the grape harvest and can be appreciated under two aspects: potential and actual fertility. In the climatic conditions of the last year of observations on the elites taken in the study, the fertility values recorded for each elite were close to normal or even higher, compared to the initial variety (Table 2).

Table 2. Observations and determinations on the fertility of clonal elites // Observații și determinări privind fertilitatea elitelor clonale

Variety	Clonal elites	Total Buds/ trunk	Dead buds/ trunk	Total shoots /trunk	Fertile shoots	Sterile shoots	Inflorescence primordia /trunk	Fertility %
'BICAN ROZ'	80/2/7	27	6	21	16	5	18	85
	79/5/3	26	7	19	12	7	18	90
	79/2/5	29	9	21	14	7	17	83
	80/5/2	27	8	19	14	5	17	87
	80/10/6	32	5	27	22	5	25	94
	Average	**28**	**7**	**21**	**16**	**6**	**19**	**88**
'Bican roz' initial variety		25	10	15	8	7	12	53

Pruning the vine is one of the most important works applied during the rest period, by which a large part of the wood grown on the trunk (80–85%) is removed annually [5].

The total amount of annual and multi-annual wood that is removed during the fruiting cut depends on the planned production, the load of fruit on the trunk and the vigor of growth on each elite in the previous year. In this case, the elite that has the higher amounts of annual and multiannual wood in comparison with the initial variety, is the clonal elite 80/10/6 (Table 3).

Table 3. The amount of annual and multiannual wood removed after pruning (average during the study period) // Cantitatea de lemn anual și multianual eliminat la tăiere (media în perioada studiului)

Variety	Clonal elites	Annual wood amount g/trunk	Multiannual wood amount g/trunk	Total amount of wood g/trunk
'BICAN ROZ'	80/2/7	652	453	1105
	79/5/3	650	471	1121
	79/2/5	666	494	1160
	80/5/2	699	436	1135
	80/10/6	799	515	1314
	Average	**693**	**474**	**1167**
'Bican roz' initial variety		548	275	823

During the 5 years of observations, the clonal elite 80/10/6 was remarked, which after being tested by ISTIS was approved as Bican roz 6 Mf in 2022 (Figure 2).



Figure 1. 'Bican roz' initial variety // 'Bican roz' soi inițial



Figure 2. Bican roz 6 Mf // Bican roz 6 Mf

The ampelographic main features of this clone, as compared to the Bican roz initial variety (Figure 1), are:

- The budburst is medium, the rosette is green with rare trichomes. The clonal elite has good fertility (79%) and great vigor. The young shoot is green in colour, with very rare trichomes, the positions is semi-erect and the tendrils are very long. The color of the adaxial side of the young leaf is green with anthocyanin areas, with soft hairs between the main ribs, which are dense on the abaxial side. The shoot has a green and red color of the dorsal part of the internode, the red on the ventral part.
- The adult leaf has a slightly shorther petiole than the median rib, pentagonal limb of medium size, green with red, medium embossed on the superior side, soft dense hairs between main ribs on lower side. The upper lateral sinuses are deep, with five slightly overlapping lobes, the teeth are medium

size, the tooth length/width ratio is medium; rectilinear shape, the petiolar sinus presents semi-open lobes.

- ❖ The flower has fully developed stamens and gynoecium. The bunch has a cylindrical-conical shape, long, yellow color, very large, peduncle long, compactness and ripening are medium.
- ❖ The berry is troncovoid, large, pink, with a fairly easy pedicel detachament. The berry is also characterized by high epidermal thickness, semi-crunchy pulp, moderately firm skin, absent or very weak anthocyanin pigmentation, with a different taste and fully formed seeds.

The development of vegetation phenophases is specific to the ecoclimate of the Murfatlar vineyard. The active vegetation period begins with the budburst and ends in autumn when the leaves fall. Bican roz 6 Mf buds in the second half of April, blooms in early June, starts the verasion in the second half of August and ripening in the second decade of September, having a vegetation period of 199 days, compared to the initial variety that has 206 days (Table 4).

Table 4. Phenological data of Bican roz 6 Mf clone, compared to the initial variety // Date fenologice ale clonei Bican roz 6 Mf, comparativ cu soiul inițial

Variety	Year	Calendaristic dates						No. of days vegetation period
		Budburst	**Flowering**	**Veraison**	**Full maturity**	**Harvest**	**Leaf fall**	**Budburst – Leaf fall**
Bican roz 80/10/6	2018	19.04	05.06	14.08	11.09	17.09	30.10	202
	2019	26.04	04.06	08.08	20.09	24.09	26.10	201
	2020	27.04	12.06	12.08	10.09	14.09	11.11	201
	2021	24.04	08.06	17.08	17.09	23.09	04.11	195
	2022	22.04	06.06	10.08	12.09	18.09	10.11	196
Average Bican roz 6 Mf		**19-17.04**	**04-12.06**	**08-17.08**	**10-20.09**	**14-24.09**	**30-11.11**	**199**
'Bican roz' initial variety		28.04	15.06	20.08	25.09	28.09	12.11	206

In order to appreciate the quality of the production and to recognize table varieties, a series of grape characters specific to each variety are used [8,12].

According to table 5, the values of the absolute and relative fertility coefficients, as well as the values of the absolute and relative productivity indices are higher compared to those of the initial variety. For table grapes, the winter bud is affected by temperatures of -18 ±3 ^{o} C, depending on the biological particularities of the varieties. The clone Bican roz 6 Mf has a weak resistance to frost (-18°C), like the initial variety [2].

Table 5. Agrobiological characterization of the Bican roz 6 Mf clone, compared to the initial variety // Caracterizarea agrobiologică a clonei Bican roz 6 Mf, comparativ cu soiul inițial

Clones	Year	Fertility, fertile shoots %	Absolute fertility coefficient	Relative fertility coefficient	Absolute production index	Relative production index	Growth vigour	Cold resistance, %viable buds
Bican roz 80/10/6	2018	75	1,2	0.8	680	453	big	78
	2019	86	1.4	0.9	1173	838	big	73
	2020	68	1.3	0.9	996	689	big	72
	2021	76	1.0	0.4	794	325	big	70
	2022	90	1.1	0.5	642	295	big	84
Average Bican roz 6 Mf		**79**	**1,2**	**0.7**	**857**	**520**	**big**	**75**
'Bican roz' initial variety		56	1.1	0.6	443	242	big	60

The Bican roz 6 Mf is superior to the initial variety in terms of the quality and quantity of the grapes, in that the start of verasion is faster, the thickness of the epidermal is medium and the grape is large and long, compared to the initial variety, where the epidermal is thin and the grape is medium (Table 6).

Table 6. Technological characterization of the clone Bican roz 6 Mf, compared to the initial variety // Caracterizarea tehnologică a clonei Bican roz 6 Mf, comparativ cu soiul inițial

Clones	Year	Average bunch weight, g	Weight of 100 berries, g	Average berry weight, g	Must sugar content, g/l	Must total acidity g/l H_2SO_4	Grape production kg/trunk	Grape production t/ha
Bican roz 80/10/6	2018	567	264	2.64	162.0	7.0	10.44	24
	2019	838	260	2.60	185.0	3,4	11.6	24
	2020	766	170	1.70	197.0	2.6	12.76	24
	2021	794	172	1.72	159.8	5.9	9.72	24
	2022	605	269	2.59	166.2	5.1	9.28	24
Average Bican roz 6 Mf		**714**	**225**	**2.25**	**174.0**	**4.8**	**10.76**	**24**
'Bican roz' initial variety		403	165	1.65	148.0	5.8	7.96	19

The grape is par excellence a noble fruit, which possesses a history and a culture. The elegance of its shape and appearance (visual and tactile sensory) together with the organoleptic properties (gustatory sensory) and the high energy value make it a prestigious and generous product, particularly appreciated by the consumer [14].

The beries of the clone Bican roz 6 Mf have a greater weight than those of the initial variety (Figure 3).

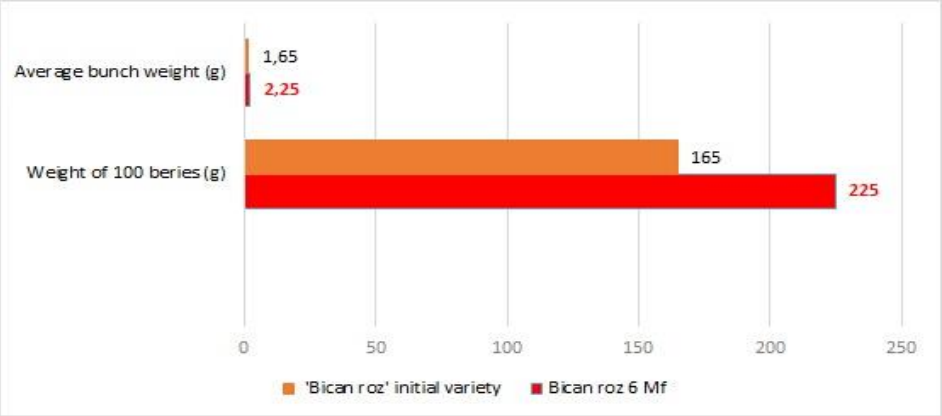


Figure 3. Average berry weights of the Bican roz 6 Mf clone, compared to the initial variety //
Figura nr. 3. Greutăți medii bob și strugure, ale clonei Bican roz 6 Mf, comparativ cu soiul inițial

Regarding the sugar content, the Bican roz 6 Mf clone differed significantly from the initial variety, having an average of 174 g/L compared to 148 g/L, the value of the initial variety (Figure 4).

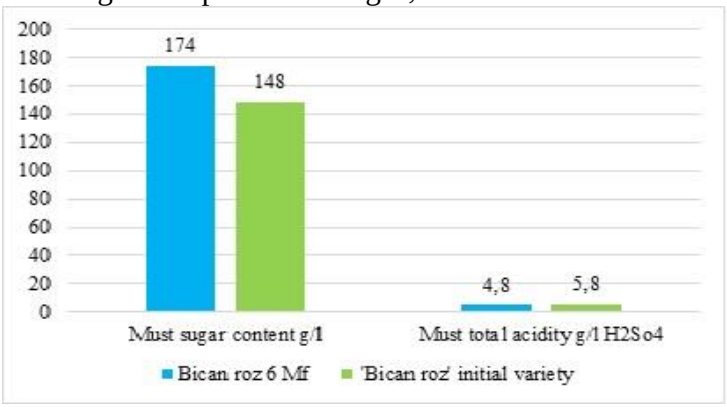


Figure 4. Total acidity and sugar content of the Bican roz 6 Mf clone compared to the initial variety //
Figura nr. 4. Aciditatea totală și concentrația în zaharuri a clonei Bican roz 6 Mf, comparativ cu soiul inițial

Grape yield raported as kg/trunk and t/ha is clearly higher for the clone (Figure 5).

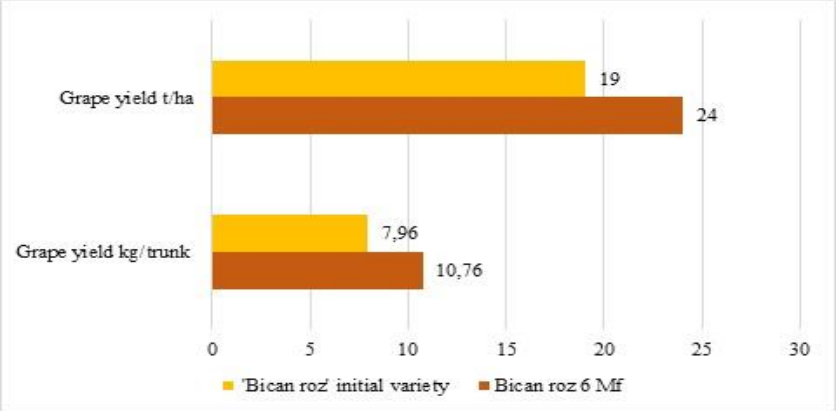


Figure 5. Average grape yields kg/trunk and t/ha of the Bican roz 6 Mf clone, compared to the initial variety // Figura nr. 5. Producții medii de struguri kg/butuc și t/ha ale clonei Bican roz 6 Mf, comparativ cu soiul inițial

The technological indices for the Bican roz 6 Mf clone are clearly higher, compared to the initial variety, the productive potential of the clone is highlighted and leads to the improvement of the assortment of varieties for table grapes in the Dobrogea area (Table 7).

Table 7. Bican roz 6 Mf clone technological indices compared to the initial variety // Indicii tehnologici ai clonei Bican roz 6 Mf, comparativ cu soiul inițial

Clones	Year	Berry index	Bunch structure index	Berry composition index	Yield index	No. berries/ bunch	Peduncule length (cm)
Bican roz 80/10/6	2018	31	5.1	6.6	4.6	50	3.5
	2019	38	5.4	6,7	5.2	56	2.8
	2020	35	5,6	6,7	4.0	44	3.3
	2021	22	5.8	6,7	4.1	52	3.04
	2022	29	5.1	6.8	2.1	33	2.86
Average Bican roz 6 Mf		**31**	**5.4**	**6,7**	**4.5**	**47**	**3.1**
'Bican roz' initial variety		28	5.4	6,7	4.3	35	3.03

The ripening is medium, the bunch has a cylindrical-conical shape, is long, with a pink color and of a very large size, the peduncle is long, with medium compactness and ripening. The berry is troncovoid, large, pink, with a fairly easy pedicel detachament. The berry is also characterized by high epidermal thickness, semi-crunchy pulp, moderately firm skin, absent or very weak anthocyanin pigmentation, with a different taste and fully formed seeds, it is recommended for consumption in fresh state (table 8).

Table 8. Technological characteristics regarding commercial value, suitability for transport and storage // Caracteristici tehnologice privind valoarea comercială, pretabilitatea la transport și păstrare

Clonal selection	Bunch size	Berry size and uniformity	Compactness	Shape and color	Pruine layer thickness	Skin adherence and elasticity	Pulp consistency	Presence of seeds	Taste	Aroma
Bican roz 6 Mf	Very Large	Large and uniform	Compact	Ovoid, Pink	Medium	Moderately Firm	Crisp	Fully Formed	Different taste	Unflavoured

CONCLUSIONS

The team of researchers from SCDVV Murfatlar during the period 2018-2022 carried out a study on 5 clonal elites of the 'Bican roz' variety, selected from existing trunks in the ampelographic collection of the research station. In this study, the ampelographic characters and agrobiological

properties were established, and the technological and agroeconomic characterization was also carried out.

During the 5 years of observations, the clonal elite 80/10/6 was remarked, which after being tested by ISTIS was approved as Bican roz 6 Mf in 2022.

The clonal elite has good fertility (79%) and great vigor. Concerning the active vegetation period Bican roz 6 Mf buds in the second half of April, blooms in early June, starsts the version in the second half of August and ripening in the second decade of September, having a vegetation period of 199 days, compared to the initial variety that has 206 days.

The grapes and berries of the clone Bican roz 6 Mf have a greater weight than those of the initial variety, and the grape yield raported as kg/trunk and t/ha is clearly higher for the clone.

The technological indices for the Bican roz 6 Mf clone are clearly higher, compared to the initial variety, the productive potential of the clone is highlighted and leads to the improvement of the assortment of varieties for table grapes in the Dobrogea area. It is recommended for fresh consumption.

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AGROBIOLOGICAL, TECHNOLOGICAL AND OENOLOGICAL POTENTIAL OF SOME HYBRID ELITE WITH BIOLOGICAL RESISTANCE OBTAINED AT RDSVO ODOBEŞTI

POTENȚIALUL AGROBIOLOGIC, TEHNOLOGIC ȘI OENOLOGIC AL UNOR ELITE HIBRIDE CU REZISTENȚĂ BIOLOGICĂ OBȚINUTE LA SCDVV ODOBEŞTI

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Abstract
In the context of current climate changes, the exploitation of viticultural biodiversity and the practice of a sustainable, environmentally friendly viticulture, with the reduction of the quantities of pesticides applied through phytosanitary treatments, requires the obtaining and promotion of vine varieties with complex biological resistance, with high potential of adaptation to the ecopedoclimatic conditions of each wine growing area, and valuable technological characteristics. Responding to this desire in the period 2020-2022, at RDSVO Odobeşti were studied in order to evaluate the agrobiological, technological and oenological potential of two hybrid elites with biological resistance: hybrid elite 2-5 (Galbenă de Odobeşti x Lydia) and hybrid elite 10-18 (Italian Riesling x Siebel 6720). This paper presents the agrobiological and technological potential of the two hybrid elites in the climatic conditions of the 2020, 2021 and 2022 wine years. The results obtained from this study showed that the two hybrid elites (E.H. 2-5 and E.H. 10-18) have a high agrobiological, technological and oenological potential, shows high biological resistance to the main diseases of the vine, and can be proposed for testing and approval at ISTIS, in order to improve the national assortment and practice a sustainable viticulture.
Keywords: biological resistance, vine variety, agrobiological and technological value

Rezumat
În contextul schimbărilor climatice actuale, valorificarea biodiversității viticole și practicarea unei viticulturi durabile, prietenoase cu mediul, cu reducerea cantităţilor de pesticide aplicate prin tratamentele fitosanitare, impune obținerea şi promovarea soiurilor de viță-de-vie cu rezistență biologică complexă, cu, potențial ridicat de adaptare la condițiile ecopedoclimatice ale fiecărui areal viticol, și caracteristici tehnologice valoroase. Răspunzând acestui deziderat în perioada 2020-2022, la SCDVV Odobeşti au fost studiate în vederea evaluării potențialului agrobiologic, tehnologic și oenologic, două elite hibride cu rezistență biologică: elita hibriă 2-5 (Galbenă de Odobeşti x Lydia) și elita hibridă 10-18 (Riesling italian x Siebel 6720). Această lucrare prezintă potențialul agrobiologic, tehnologic și oenologic al celor două elite hibride în condițiile climatice ale anilor viticoli 2020, 2021 și 2022. Rezultatele obținute în urma acestui studiu au arătat faptul că cele două elite hibride (E.H 2-5 și E.H. 10-18) au un potențial agrobiologic, tehnologic și oenologic ridicat, prezintă rezistență biologică ridicată la principalele boli ale viței de vie, putând fi propuse spre testare și aprobare la I.S.T.I.S., în vederea îmbunătățirii sortimentului național și practicarea unei viticulturi durabile.
Cuvinte cheie: rezistență biologică, soi vinifera, valoare agrobiologică și tehnologică

INTRODUCTION

Practicing sustainable viticulture in tandem with environmental and health concerns is currently one of the most important goals of the wine world. The diversification of viticultural genetic resources by creating varieties with tolerance to biotic stress factors has an important role in protecting the environment, it being known that native varieties have genes for tolerance and resistance to diseases and pests, ensuring its sustainable development at the national, regional level and local (Fregoni, 1998).

Community regulations only recognize grape varieties of the European species *Vitis vinifera* L. for the production of appellation wines, while disease-resistant grape varieties come from crosses between *Vitis vinifera* L. and other *Vitis* species (American and/or Asian, carrying resistance genes). The most promising option to reduce the need for fungicides in viticulture is the use of resistant varieties (Riaz et al., 2019, Bavaresco, 2019). Research programs at renowned viticultural research centers in Europe and America have resulted in the creation of new varieties to combat disease and weather challenges through crosses between so-called European vinifera and others native to North America and Asia, such as *V. riparia, V. labrusca* and *V. rotundifolia* (Ilnitskaya et al., 2019, Ollat et al., 2019). The research carried out in the last four decades in our country led to the creation of several grapevine genotypes with increased resistance to diseases, and the results were expressed in the creation of a base with valuable genetic material and the homologation of many varieties (Damian et al., 2012, Culcea et al., 2004; Puşcalău et al., 2018). In this context, the valorification of valuable genetic material with high productive and qualitative potential, with tolerance to diseases and resistance to stress factors, adapted to the ecopedoclimatic conditions of each wine-growing area, is one of the strategic objectives of research at RDSVO Odobeşti, where two varieties with biological resistance were approved only in the last ten years (Bosoi et al., 2020; Puşcalău et al., 2022).

MATERIALS AND METHODS

The study was carried out in a plantation over 30 years old, established on a leached chernozem type soil, located in the biological field of the Research and Development Station for Viticulture and Oenology (RDSVO) Odobești, with geographical coordinates 45º45′ north latitude, 27º06′ eastern length and an altitude of 150 m. The study was carried out during 2020-2022.

The biological material was represented by two hybrid elites with biological resistance: E.H. 2-5 (Galbenă de Odobeşti x Lydia) and E.H. 10-18 (Italian Riesling x Siebel 6720). The studied hybrid elites were grafted on Kobber 5 BB rootstock. The pruning system practiced was the Dr. Guyot system, with a fruit load between 38-44 buds/vine, distributed over 8-9 buds per fruit cane with 2 buds per spur, and a semi-high training form. To combat pathogens (*Plasmopara viticola, Uncinula necator, Botrytis cinerea*, etc.), six phytosanitary treatments were applied.

In order to establish the agrobiological potential, observations and determinations were made regarding: the phenological spectrum, the vigor of growth through measurements of the shoots during the period of intense growth; elements of fertility and productivity by calculating the percentage of fertile shoots (FS%), fertility coefficients (Cfa and Cfr) and productivity indices (Ipa and Ipr); behavior to the main vine diseases and stress factors (drought) by scoring from 1 to 9 according to the resistance scale developed by OIV (2009). To evaluate the technological potential, determinations were made regarding the quantity and quality of grape production (kg/but., t/ha; g/l sugars, g/l acidity tartaric acid). The oenological potential of the two hybrid elites was evaluated by determining the compositional profile of the obtained wines. Climate data for the study period were provided by the AgroExpert weather station and the multi-year climate database of RDSVO Odobești.

RESULTS AND DISCUSSIONS

Climate conditions. The research period (2020 - 2022) was characterized by high heliothermal availability, the thermal regime expressed by average annual temperatures (ºC) and the sum of useful temperature degrees (∑ºtu) registering much higher values than the multiannual values in the years 2020 and 2022, and values close to multi-year averages in 2021 year (Table 1). Compared to the multiannual values for these climatic elements (11.1 °C and 1630.8 °C, respectively), the average annual temperature recorded an excess of 2.3 °C in 2020 year and 1.6 °C in 2022, and for the sum of

useful temperature degrees (∑ºtu), a thermal increase of 359.8°C was recorded in 2020 and 403.3°C in 2022.

Table 1. The main climatic parameters from the study period (Odobești, 2020 - 2022) //
Principalii parametri climatici din perioada de studiu (Odobești, 2020 - 2022) *

Month	Climate parameter											
	Average temperature (°C)				The sum of the useful temperature degrees (°C)				Rainfall (mm)			
	Multi annual	2019/ 2020	2020/ 2021	2021/ 2022	Multi annual	2019/ 2020	2020/ 2021	2021/ 2022	Multi annnal	2019/ 2020	2020/ 2021	2021/ 2022
XI	5.2	9.0	5.3	7.6	9.9	49.4	0.0	11.7	46.8	68.2	12.2	12.0
XII	0.3	3.0	2.5	1.5	0.5	0.0	0.0	0.0	40.0	42.8	98.6	76.2
I	-1.5	1.7	0.6	1.9	0.1	0.0	0.0	0.0	32.5	0.4	47.0	6.4
II	0.0	5.6	1.6	4.9	0.9	2.3	2.0	0.8	32.4	9.0	4.4	4.8
III	4.5	8.6	4.6	4.9	12.3	30.8	0.4	23.3	33.0	20.2	46.8	1.2
IV	11.2	12.2	8.9	11.6	70.8	82.1	15,3	76.1	48.9	5.2	41.0	65.8
V	16.8	15.8	16.5	17.7	218.7	179,6	200.5	239.6	73.5	55.6	22.8	31.6
VI	20.2	21.8	20.3	22.7	312.5	353.3	309.0	380.6	84.6	85.2	134.6	48.2
VII	22.1	23.5	24.3	24.0	375.6	419,1	443.5	432.5	78.6	28.2	40.0	51.0
VIII	21.6	24.3	22.9	24.3	360.3	441.8	398.6	442.2	60.2	13.0	45.0	31.6
IX	17.0	20.6	16.7	17.2	212.1	317.5	199.8	214.6	45.6	31.4	6.2	26.8
X	11.0	14.2	10.6	13.8	66.1	123.7	35.1	221.7	45.2	66.0	9.4	1.2
Annual average/ amount	11.1	13.4	11.2	12.7	1639.8	1999.6	1604.2	2043.1	621.3	425.2	508.0	356.8
during the vegetation period	18.2	19.7	18.3	19.6	1550.0	1793.4	1568.7	1785.6	391.4	218.6	289.6	255.0

***Data provided by the weather station AgroExpert of RDSVO Odobesti //**
*Datele provin de la Stația meteo AgroExpert a SCDVV Odobesti

The precipitation regime during the three years of the study was deeply deficient, both during the year and during the vegetation period. Compared to the multiannual values recorded for the entire period of a wine-growing year and for the vegetation period (621.3 mm, respectively 391.4 mm), the amount of precipitation recorded represented only 68.4%, respectively 55.8% in 2020, 81.7%, respectively 74.0% in the year 2021 and 57.4%, respectively 65.1% in the year 2022. Another negative characteristic of precipitation in the three years of the study was represented by the defective quantitative distribution, more chosen during the vegetation period of the vine. From the point of view of the water regime, this situation shows the fact that the study period (years 2020-2022) was a dry one for the Odobești wine-growing area.

The phenological spectrum. In the climatic conditions specific to the study period (2020 - 2022), the processes of growth and development of the vine, respectively the development of the vegetation phases for the two hybrid elites taken in the study evolved relatively normally (Figure 1 and Figure 2).



Figure 1. Vegetation phases of the hybrid elite 2-5 // Fazele de vegetație la elita hibridă 2-5



Figure 2. Vegetation phases of the hybrid elite 10-18 // Fazele de vegetație la elita hibridă 10-18

Vegetation phases at the two hybrid elites were initiated and completed 5 - 15 days earlier in study years 2020 and 2022 compared to 2021 (Table 2).

Table 2. The phenological spectrum (Odobeşti, 2020 - 2022) // Desfășurarea principalelor faze de vegetație (Odobești, 2020 -2022)

The hybrid elite	Year	The phenological stages								Active vegetation period (days)
		Disbudding		Flowering		Veraison		Full ripening		
		∑tu (°C)	Date	∑tu (°C)	Date	∑tu (°C)	Date	∑tu (°C)	∑tu (°C)	
E.H. 2-5	2020	13.IV	60,3	05.VI	268,5	04.VIII	791,1	15.IX	582,8	156
	2021	02.V	38,3	15.VI	299,7	12.VIII	807,7	20.IX	387,4	142
	2022	16.IV	74,7	04.VI	318,0	29.VII	1051,0	12.IX	566,6	151
Average		22.IV	57.7	09.VI	295,4	05.VIII	883.3	16.IX	512.3	150
E.H. 10-18	2020	14.IV	60,3	03.VI	248,1	02.VIII.	785,1	10.IX	541,0	150
	2021	01.V	27,3	10.VI	272,1	09.VIII	804,5	16.IX	399,9	139
	2022	16.IV	74,7	31.V	265,1	26.VII	1064,4	09.IX	576,5	148
Average		22.IV	54.1	05.VI	261.7	02.VIII	884.7	12.IX	505,8	146

The calendarically, with the exception of disbudding, for the phenophases of flowering and ripening of grapes, an advance of 2-5 days was recorded in hybrid elite 10-18, compared to hybrid elite 2-5. The duration of the active vegetation period varied between 142-156 days in hybrid elite 2-5 and 139-150 days in hybrid elite 10-18. The average value calculated over the study period (2020-2022) shows that for the initiation and development of the budding phenophase, hybrid elite 2-5 has needed a ∑tu°C of 57.7°C, with 3.6°C more compared to elite hybrid 10-18 (54.1°C).

The flowering phenophase occurred under the conditions of the accumulation of a sum of useful temperature degrees of 295.4 °C for hybrid elite 2-5, a higher value by 33.7°C compared to that recorded for hybrid elite 10-18 (261.7 °C). For the initiation and progression of the veraison phase, the two studied hybrid elites required a ∑tu°C around 884°C, and the bunch ripening phenophase required a sum of 512.3°C useful temperature for the hybrid elite 2-5 and 505.8°C ∑tu for hybrid elite 10-18.

Fertility and productivity. The fertility and productivity characteristics of the studied hybrid elites were evaluated by the percentage of fertile shoots, fertility coefficients and productivity indices (Table 3).

Table 3. The fertility and productivity characteristics (Odobeşti, 2020 - 2022) //
Caracteristicile de fertilitate și productivitate (Odobești, 2020 – 2022)

The hybrid elite /year		Fertile shoots (%)	Fertility coefficients		Average weight grapes (g)	Productivity indices	
			Relative	Absolute		Relative	Absolute
E.H. 2-5	2020	66,5	1,07	1,60	172,4	184	276
	2021	81,5	0,97	1,18	211,7	205	250
	2022	61,7	0,71	1,16	211,0	150	245
Average		69,9	0,91	1,31	198,4	180	257
E.H. 10-18	2020	66,4	1,05	1,58	192,3	202	303
	2021	82,7	1,11	1,34	185,7	206	249
	2022	61,8	1,11	1,49	207,2	230	309
Average		70,3	1,09	1,47	195,1	213	287

The percentage of fertile shoots calculated during the study period recorded very close average values (69.9% for hybrid elite 2-5, respectively 70.3% for hybrid elite 10-18). Fertility coefficients (absolute and relative) recorded higher average values in the hybrid elite 10-18 (Cfa - 1.09; Cfr - 1.47) and lower in the hybrid elite 2-5 (Cfa 0.91; Cfr 1 ,31). Since the average values calculated for the mass of a grape were close (198.4 g for hybrid elite 2-5, respectively 195.1 g for hybrid elite 10-18), and the average values obtained for productivity indices (relative and absolute) have were higher for the hybrid elite 10-18 (Ipr – 213; Ipa – 287).

Vegetative growth of shoots. The climatic conditions of the years 2020 and 2022 were less favorable, affecting the physiological and metabolic processes that condition the growth and development of the shoots (Figure 3).

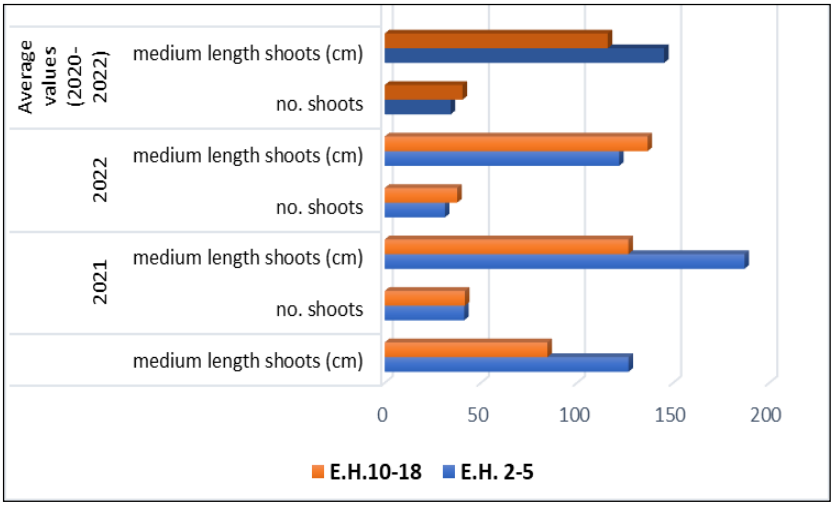


Figure 3. Vegetative growth - average values (Odobești, 2020-2022) //
Creșterile vegetative – valori medii (Odobești,2020-2022)

Compared to 2021, a year with climate parameter values close to multiannual values, vegetative growth in 2020 year recorded lower average values by 32.2% in hybrid elite 2-5 and by 33.2% in hybrid elite 10-18. Vegetative increases recorded in 2022 year had values 34.8% lower in hybrid elite 2-5 and relatively close to those of 2021 year in hybrid elite 10-18. The analysis of vegetative growth during the study period (2020–2022) shows that at an average number of 34.3 shoots/vine for hybrid elite 2-5, respectively 40.5 shoots/vine for hybrid elite 10-18, the average length of the main shoots was 145.3 cm in hybrid elite 2-5, respectively 116.0 cm at hybrid elite 10-18. Taking into account the average values obtained for the length of the shoots, the hybrid elites taken in the study were classified into vigor classes according to the OIV 351 descriptor, as follows: hybrid elite 2-5 with medium to high growth vigor (grade 5-7) and hybrid elite 10-18 with medium growth vigor (grade 5).

Behavior to cryptogamic diseases. Biological resistance to the main cryptogamic diseases was determined by calculating the degree of attack on leaves and grapes (Table 7). Under the influence of the climatic conditions during the vegetation period recorded during the study period (2020-2022), the values obtained for the degree of attack were sub-unit for the main pathogens of the vine. Determined by the different climatic conditions of the three years of the study, higher values were recorded in the year 2021.

Based on the data obtained, the resistance of the hybrid elites studied to the main diseases of the grapevine was assessed by the OIV ampelographic descriptors method, the scoring being done by

assigning numbers according to the level of expression, the two elites being marked with grade 7 (high resistance) and grade 9 (very high resistance).

Table 4 .Behavior at the main diseases of the vine (Odobeşti, 2020 - 2022) //
Comportarea la principalele boli criptogamice ale viței-de-vie (Odobești, 2020 – 2022)

The hybrid elite	The plant organ	The degree of attack of cryptogamic diseases (DA)								
		Downy mildew (*Plasmopara viticola*)			Powdery mildew (*Uncinula necator*)			Gray rot (*Botrytis cinerea*)		
		2020	2021	2022	2020	2021	2022	2020	2021	2022
E.H. 2-5	Leaf	0.00	0.26	0,10	0.00	0.07	0,02	0.00	0.00	0,00
	Grape	0.03	0.19	0,12	0.00	0.19	0,13	0.27	0.03	0,13
E.H. 10-18	Leaf	0.00	0.23	0,13	0.00	0.10	0,04	0.00	0.00	0,00
	Grape	0.10	0.25	0,17	0.07	0.22	0,11	0.22	0.15	0,15

Drought resistance (OIV 403) - According to the *list of OIV descriptors for grape varieties and Vitis species*, 2nd edition, 2009. In the climatic conditions of the study period with two years 2020 and 2022, considered on the basis of recorded climatic data, some of the driest years in the Odobești vineyard, hybrid elite 2-5 showed high to very high tolerance (grade 7-9), and hybrid elite 10-18 medium tolerance to the phenomenon of atmospheric and pedological drought. The hybrid elites studied did not show specific manifestations of thermal and water stress (withering of shoot tips, yellowing leaves at the base of the trunk, wilting of grapes, etc.).

Technological potential. The study of the technological characteristics of grape production completed the elements of knowledge for the studied hybrid elites (Table 5 and Figure 4).

Table 5. The quantitative and qualitative characteristics of grape production (Odobești, 2020 - 2022) //
Caracteristicile cantitative și calitative ale producției de struguri (Odobești, 2020 - 2022)

The hybrid elite		Weight grape (g)	Production grape		The juice		
			kg/vine	t/ha	Sugars g/l	Total acidity g/l acid tartric	Gluco acidimetric index
E.H. 2-5	2020	172.4	5.17	19.58	183	6.90	27
	2021	211.7	6.08	23.03	193	6.96	28
	2022	211,0	4,24	16,06	200	5,55	36
Average		198,4	5,16	19,55	192	6,47	30
E.H. 10-18	2020	192.3	6.73	25.49	206	6.33	33
	2021	185.7	6.85	25.95	222	7.42	30
	2022	207,0	6,76	25,60	211	6,15	34
Average		195,0	6,78	25,68	213	6,63	32

The main quantitative and qualitative characteristics of grape production refer to average grape weight, average grape yield (kg/vine; t/hectare), sugar content (g/l), total acidity (g/l tartaric acid) and the glucoacidimetric index of the juice.

The average value of the mass of grapes during the three years of the study was 198.4 g in hybrid elite 2-5 and 195.0 g in hybrid elite 10-18. The two hybrid elites recorded higher values of the average production of grapes per vine in the year 2021 compared to the years 2020 and 2022, the average over the three years of the study being 5.16 kg/vine in the hybrid elite 2-5 and 6.78 kg/vine for the elite 10-18.



a) **b)**

Figure 4. a) Hybrid Elite 2-5; b) Hybrid Elite 10-18 // a) Elita hibridă 2-5; b) Elita hibridă 10-18

The lack of precipitation and maximum temperatures higher than 30°C recorded for 55 days during the ripening period of the grapes influenced in 2020 the optimal development of the biochemical processes of sugar accumulation in the grains. Under these conditions, the two genotypes accumulated between 183 g/l sugars (E.H. 2-5) and 206 g/l sugars (E.H. 10-18), under conditions of a total acidity of 6.90 g/l tartaric acid, respectively 6.33 g/l tartaric acid. The average value of the amount of sugars accumulated in grapes during the three years of the study was 192 g/l in the elite 2-5 and 213 g/l in the hybrid elite 10-18, under the conditions of a total acidity content of 6, 47 g/l tartaric acid, respectively 6.63 g/l tartaric acid (Figure 5). Average values for the glucoacidimetric index were recorded between 30 for the 2-5 hybrid elite and 32 for the 10-18 hybrid elite.

The oenological potential of the two hybrid elites was evaluated by determining the compositional profile of the obtained wines, the harvests of 2020 and 2021 (Table 6).

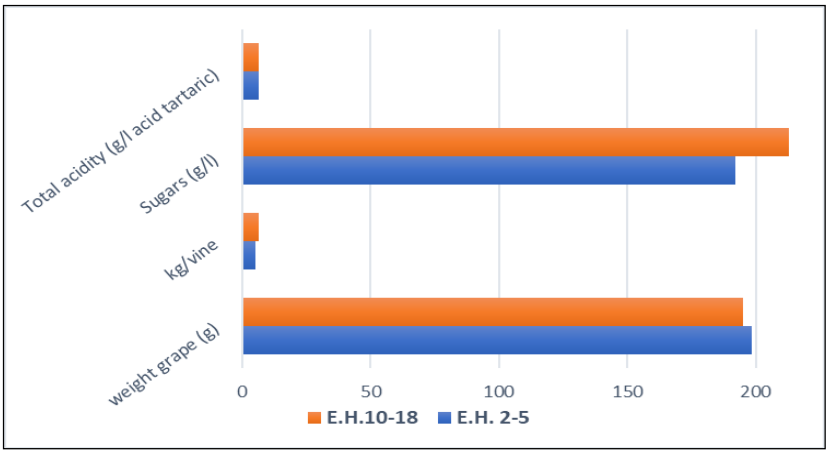


Figure 5. The quantitative and qualitative characteristics of grape production (Odobeşti, 2020-2022) //

Carateristicile cantitative și calitative ale producției de struguri (Odobești,2020-2022)

Table 6. The compositional profile of wines - general composition analysis (Odobești, 2021, 2022) //
Profilul compoziţional al vinurilor - analize de compoziţie generală (Odobeşti, 2021,2022)

Elita hibridă		The general composition parameters								
		Free SO_2 (mg/l)	**Total SO_2** (mg/l)	**Density** (g/cm^3)	**Alc. conc.** (% vol.)	**Total acidity** (g/l ac. tartric)	**Volatile acidity** (g/l ac acetic)	**Sugar reducing** (g/l)	**Total dry extract** (g/l)	**Non-reducer extract** (g/l)
E.H. 2-5	2021	38,24	117,28	0,9910	12,30	5,72	0,29	1,24	18,50	17,26
	2022	9,72	63,68	0,9908	12,15	5,66	0,26	3,81	20,85	16,99
Average		23,75	90,48	0,9909	12,22	5,69	0,27	2,52	19,67	17,04
E.H. 10-18	2021	40,79	122,64	0,9912	14,00	5,35	0,29	0,93	24,20	23,27
	2022	33,25	100,76	0,9910	12,86	5,35	0,36	0,61	24,88	24,27
Average		37,02	111,70	0,9911	13,43	5,35	0,32	0,77	24,54	23,77

The average values for the main compositional characteristics of the obtained wines, namely alcohol concentration (% vol.), total acidity (g/l tartaric acid) and total dry extract (g/l) demonstrate that from the hybrid elites 2-5 and 10-18 it is possible to obtain high quality wines with an alcoholic potential of over 12% vol alc. (12.22 g/l at hybrid elite 2-5, respectively 13.43% vol. alc. at hybrid elite 10-18), with a total acidity that varied between 5.35 g/l tartaric acid at E.H. 10-18 and 5.69 g/l tartaric acid at E.H. 2-5. The total dry extract (g/l) recorded average values that varied between 19.67 g/l in hybrid elite 2-5 and 23.77 g/l in hybrid elite 10-18. For wines obtained from varieties that have interspecific hybrids in their composition, especially red wines, a parameter of interest is the qualitative/quantitative presence of malvidin diglycoside. The qualitative analysis regarding the presence of this compound demonstrated the absence of malvidin in the wines obtained from the two hybrid elites.

The agrobiological, technological and oenological potential demonstrated by the hybrid elites 2-5 and 10-18 recommends their enrollment in the homologation process with a view to introduction into culture, to complete the assortment of varieties with biological resistance to the main cryptogamic vine diseases and with tolerance to abiotic stress factors (drought, frost).

CONCLUSIONS

1. Except for the 2021 wine year which recorded values close to the multiannual averages, the 2020 and 2022 wine years were some of the driest years recorded in the Odobești vineyard.
2. The hybrid elites studied showed a high fertility potential, the percentage of fertile shoots varying between 69.9% in hybrid elite 2-5 and 70.3% in hybrid elite 10-18.
3. The analysis of the growth of the main shoots during the vegetation period (2020–2022), shows that the hybrid elite 2-5 shows medium to high growth vigor (grade 5-7), compared to the hybrid elite 10-18 which presented growth vigor average (grade 5).
4. Hybrid elite 2-5 and hybrid elite 10-8 demonstrated during the three years of study high and very high resistance to the main vine diseases.
5. Under the conditions of some dry years (2020 and 2022), hybrid elites 2-5 and 10-18 showed a high to very high tolerance to the phenomenon of atmospheric and pedological drought.
6. Elite hybrids 2-5 and 10-18 demonstrated superior technological potential, achieving average grape yields of 5.16 kg/vine (E.H. 2-5) and 6.78 kg/vine (E.H. 10-18), with average sugar accumulations of 192 g/l (E.H. 2-5) and 213 g/l (E.H. 10-18), supported by good total acidity values (6.47 g/l tartaric acid at E.H. 2-5 and 6 .63 g/l tartaric acid at E.H. 10-18).
7. The hybrid elites 2-5 and 10-18 demonstrated a superior oenological potential, the obtained wines presenting an alcoholic concentration of over 12% vol., a total acidity of 5.69 g/l tartaric acid (EH 2-5) respectively 5, 35 g/l tartaric acid (E.H.10-18) and extractivity (17.04 g/l non-reducing dry extract at hybrid elite 2-5, respectively 23.77 g/l at hybrid elite 10-18).

8. The agrobiological, technological and oenological potential demonstrated by hybrid elites 2-5 and 10-18 recommends their promotion in culture in order to diversify the assortment of varieties with biological resistance to the main vine diseases and with tolerance to climate change.

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ANALYSIS OF SOME ECOLOGICAL INDICATORS OF INSECT SPECIES FROM A VINEYARD PLANTATION, CĂLINEȘTI – ARGEȘ

ANALIZA UNOR INDICATORI ECOLOGICI AI SPECIILOR DE INSECTE DINTR-O PLANTAȚIE VITICOLĂ, CĂLINEȘTI - ARGEȘ

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Abstract

The field research was carried out in a vineyard belonging to INCDBH, located in the south of Romania (Călinești, Argeș). Between June - August (2021-2022) Barber traps were placed to assess the ecological indicators of insects from this region with a temperate climate (species abundance, Simpson, Shannon, SHE analysis). The identified insect species were classified into 9 orders and 18 families, respectively: Araneae (Gnaphosidae, Sparassidae and Philodromidae), Coleoptera (Carabidae, Cerambycidae, Coccinellidae, Dermestidae, Mordellidae), Dermaptera (Forficulidae), Hemiptera (Anthocoridae, Rhyparochromidae), Homoptera (Cicadellidae), Hymenoptera (Formicidae), Neuroptera (Chrysopidae), Opiliones (Sironidae, Phalangiidae) and Orthoptera (Acrididae, Gryllidae). Thus, 29 species of insects were identified with 148 individuals in 2021 and 303 in 2022. The largest number of individuals identified throughout the study period in the Barber traps belong to the genera Lasius and Melanogryllus.

Keywords*: *Biodiversity, indexes Simpson and Shannon, SHE analysis, Bray-Curtis analysis

Rezumat

Cercetările în teren s-au efectuat într-o plantație viticolă ce aparține INCDBH, amplasată în sudul României (Călinești, Argeș). În perioada iunie – august (2021-2022) s-au amplasat capcane de tip Barber pentru a evalua indicatorii ecologici ai insectelor din această regiune cu climat temperat (abundența speciilor, Simpson, Shannon, analiza SHE). Speciile de insecte identificate au fost clasificate în 9 ordine și 18 familii, respectiv: Araneae (Gnaphosidae, Sparassidae și Philodromidae), Coleoptera (Carabidae, Cerambycidae, Coccinellidae, Dermestidae, Mordellidae), Dermaptera (Forficulidae), Hemiptera (Anthocoridae, Rhyparochromidae), Homoptera (Cicadellidae), Hymenoptera (Formicidae), Neuroptera (Chrysopidae), Opiliones (Sironidae, Phalangiidae) și Orthoptera (Acrididae, Gryllidae). Astfel, s-au identificat 29 de specii de insecte cu 148 de indivizi în anul 2021 și 303 în anul 2022. Cel mai mare număr de indivizi identificați pe toată perioada de studiu în capcanele de tip Barber aparțin genurilor Lasius și Melanogryllus.

Cuvinte cheie*: *Biodiversitate, indicii Simpson și Shannon, analiza SHE, analiza Bray-Curtis

INTRODUCTION

Agriculture is an important human activity that affects the ecosystems sustainability. The intensification of land use changes the function of agroecosystems by diminishing biodiversity, causing transformations in the functional composition (Cardinale B. J. *et al*., 2012).

Biological pest control is considered an important ecosystem service and a valuable alternative to chemical control, contributing to sustainable viticulture (Samways M. J., 2005). Insects are an important component of biodiversity in agroecosystems, being essential for maintaining soil structure and fertility, organic matter decomposition, seed dispersal, crop fertilization and pest control (Provost C. and Pedneault K., 2016). The presence of insects is usually positively correlated with vegetation richness and diversity (Soliveres S. *et al*., 2016). Among them, predatory insects represent a very important group of natural enemies of pests, and their community structure and composition have a substantial effect on the effectiveness of biological control (Rusch A. *et al*., 2015). Measures to combat

pathogens and pests are essential for increasing production and profits because they can negatively affect biodiversity from the viticultural ecosystem (Tabaranu G. *et al*., 2018).

For knowing the diversity of insect species in a wine-growing area, Barber-type traps are mounted at ground level. They are made to be light, reliable (resistant), cheap and of different volumes. Barber traps are some of the oldest types of traps and most commonly are used to capture invertebrates (Woodcock B. A., 2008). In specialized literature, the most used solutions for fixing and preserving biological samples are considered: formaldehyde solution 4 - 5% (formalin); ethyl alcohol 80% (the concentration varies according to the group and the purpose of the investigation: 80% for genetic analyses; glycerinated liquid (95-96% ethyl alcohol - 1/3 + pure glycerin - 1/3 + distilled water - 1/3); Lugol's solution (2 g KI + 1 g I + 200 ml distilled water, with the possibility of adding approximately 20 ml glacial acetic acid) (Sîrbu I. and Benedek A. M., 2012).

Different trap characteristics have been reviewed and researched in recent decades to improve and standardize trap designs (Buchholz S. et al., 2010), the presence and colour of the rain cover (Buchholz S. and Hannig K., 2009; Császár P. *et al.*, 2018), sampling intervals (Schirmel J. et al., 2010), spatial distribution (Ward D. F. *et al.*, 2001), different preservatives (Schmidt M. H. *et al.*, 2006; Skvarla M. J. *et al.*, 2014), as well as trap diameter and the use of funnels (Császár P. *et al.*, 2018; Lange M. *et al.*, 2011). A meta-analysis by Brown G. R. and Matthews I. M., (2016) makes suggestions for standardizing different trap parameters (diameter, depth, colour, rain cover, preservative and funnel use). Different families of insects can be captured in these traps, such as: *Carabidae, Staphylinidae, Silphidae, Scarabaeidae, Rhysodidae, Nitidulidae, Lucanidae, Leiodidae, Geotrupidae, Elateridae, Curculionida, Cerambycidae, Chrysomelidae, Cicadidae, Anthicidae, Tettigoniidae, Gryllidae, Gryllotalipdae, Tetrigidae, Acrididae* (Beye H. *et al.*, 2023; Isaia G. *et al.*, 2023; Preda C. *et al.*, 2020).

MATERIALS AND METHOD

In the vineyard located on the territory of Călinești (located in the Ștefănești viticultural center), Barber traps were placed to evaluate the main ecological indicators of insects from this region with a temperate climate (species abundance, Simpson, Shannon indices, SHE analysis) and Bray-Curtis analysis. The determinations were made between June and August of 2021 compared to the same period of 2022. During the two years of the study, no treatments were carried out with substances that are part of the category of pesticides with use to combat insects in the vineyard. In recent years, it has been maintained as a black field, the soil is loosened and cleared of weeds by autumn and spring ploughing. At the same time, during the vegetation period, in the space between the rows, the soil was maintained with the help of the disk harrow. The entomological traps were set up so that they could retain insects in the monitored habitat for a period of two weeks. Thus, pits were made on the grapevine rows where jars (450 ml) were inserted, and subsequently filled with 2/3 formaldehyde solution in a 4% concentration. The jars were placed with the upper edge at ground level to facilitate the entry of insects easily into the collection jar (figure 1).

The traps were placed on the grapevine rows so as not to be damaged during the agrotechnical works carried out in the vineyard. The captured insects were removed from the collection jar of the trap together with the formaldehyde solution and with the help of the funnel they were emptied into plastic bottles (500 ml) to keep them intact and to facilitate their transport from the field to the laboratory. Each container was labelled with collected information (collection date, collection area, row, plot).

After collection, within the laboratory of Technologies - Plant Protection - Virology in Horticulture, samples of faunal material were analysed and operations were carried out to separate the biological material from plant residues and other impurities. The resulting insects were rinsed with water to remove the formaldehyde solution and identified using an IMP-scope digital microscope

(Spectrum Technologies, USA) and an Optika SZM-2 trinocular stereomicroscope equipped with an Optika CP 8 camera (Optika, Italy).

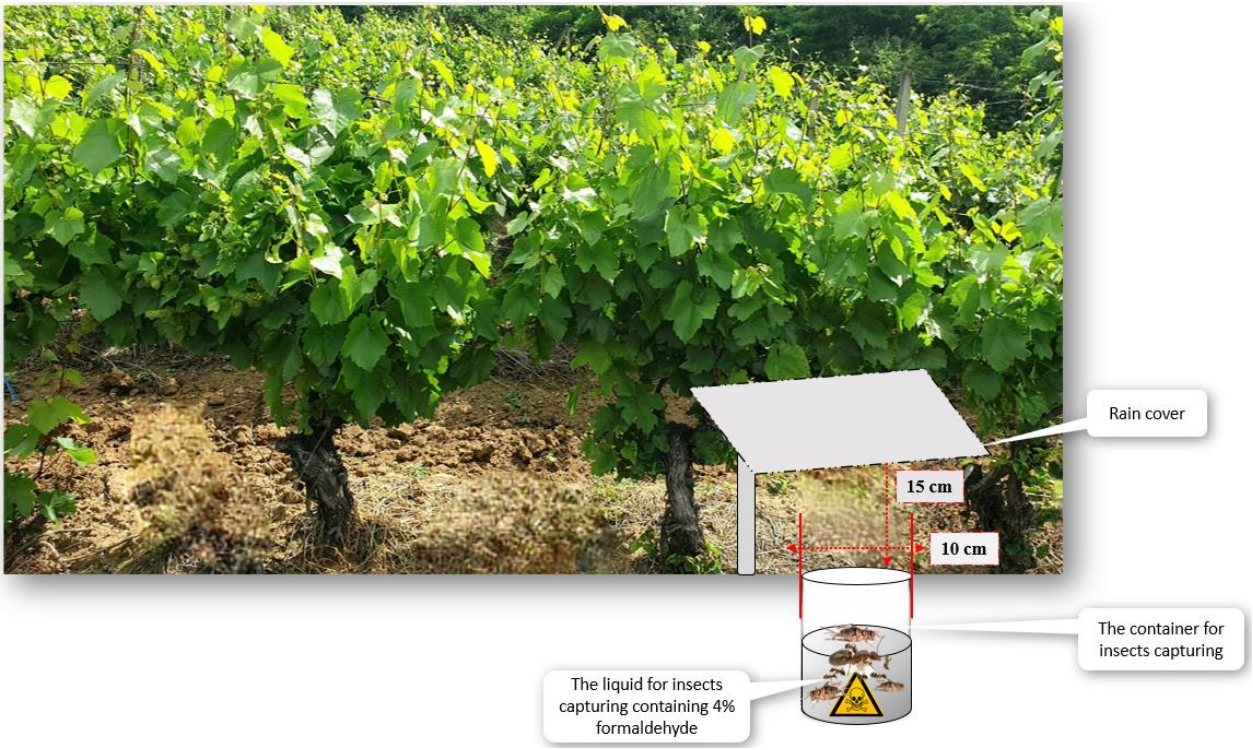


Figure 1. Installing the Barber trap on the grapevine rows // Instalarea capcanei Barber pe rândurile de viță-de-vie

RESULTS AND DISCUSSIONS

In the specialized literature, the total number of insect species on Earth is still not known exactly, but it is estimated that there are between 5 and 15 million species. Insects make up the most numerous groups of organisms on earth, approximately 66% of all animal species, by dispersing and exploiting all types of organic matter, they are found widespread in almost all habitats, forming an important part of every ecosystem (Jankielsohn A., 2018).

Between June and August 2021, 21 species with 148 individuals were captured: *Acheta domesticus*, *Calliptamus italicus*, *Camponotus* sp., *Chrysoperla carnea*, *Cyphophthalmus duricorius*, *Gnaphosa lucifuga*, *Gryllus campestris*, *Harmonia axyridis*, *Lasius* sp., *Melanogryllus desertus*, *Micrommata virescens*, *Modicogryllus frontalis*, *Mordella aculeata*, *Opilio* sp., *Opiliones* sp., *Pezotettix giornae*, *Phalangium opilio*, *Raglius alboacuminatus*, *Scaphoideus titanus*, *Scymnus* sp., *Thanatus arenarius*, *Scotophaeus blackwalli*. In the same period, but in 2022, a number of 14 species with a total of 303 individuals were recorded: *Attagenus* sp., *Camponotus* sp., *Dorcadion fulvum*, *Forficula auricularia*, *Harpalus* sp., *Lasius* sp., *Melanogryllus desertus*, *Modicogryllus frontalis*, *Orius sp.*, *Pezotettix giornae*. Among all the identified species, *Chrysoperla carnea* is the only beneficial species that feeds on the *Helicoverpa armigera* caterpillar, *Bemisia tabaci*, aphids (ex: *Aphis pomi*), mites, bedbugs, coccids, small hemipteran pests, as well as the grapevine pest *Jacobiasca lybica* (Rehman H. *et al.*, 2020; Usman M. *et al.*, 2012; Kumar A. *et al.*, 2019; Hagley E. A. C., 1989; Khfif K. *et al.*, 2023; Pinzón-Hamón D. A. *et al.*, 2018) and *Mordella aculeata* pollinates plants (Rather Z. A. *et al.*, 2023). During the study, the species *Harmonia axyridis* was also identified, which is a predator of aphids but also feeds on ripe fruits, grapes, pollen and nectar (Jovičić I. *et al.*, 2020).

In table 1 it can be seen that 29 species were captured and identified, of which 148 individuals in 2021 and 303 individuals in 2022. Among them, the largest number of individuals identified in Barber traps belongs to the *Lasius genus*. According to specialized studies, it has been proven that certain subgenera of *Lasius* such as *Chthonolasius* and *Dendrolasius* use fungi (ascomycetes) to bind masticated wood and soil to strengthen the walls of their nests (Brown B.E. *et al*., 2022).
Ants are widely distributed in both tropical and temperate regions, feeding on decaying plant material while also being predators (Sheikh A. H. *et al.*, 2018). The genus *Lasius* is a group of northern temperate ants with 107 existing species. The genus is ecologically important and often serves as a model for studying insect biology. *Lasius* ants are particularly known for their symbiotic relationships with dew-producing insects and for temporary social parasitism (Boudinot B. E. *et al.*, 2022). This means parasitic queens invading the nests of other *Lasius*, killing the host queen, and using the subordinate host ants to raise their own workers, leading to damage to the host, eventually leaving a colony of only parasitic conspecifics. At the same time, ants provide the fungi with nutrients and prevent the growth of competing fungal species (Boudinot B. E. *et al.*, 2022). Social parasitism is known in species currently classified in the subgenera *Austrolasius Faber*, *Acanthomyops Mayr*, *Chthonolasius Ruzsky* and *Dendrolasius Ruzsky* (Raczkowski J. M. and Luque G. M., 2011).

Tabel 1. Species richness collected in Barber traps // Bogăția speciilor colectate în capcanele Barber

No.	Order	Family	Species	June 2021	June 2022	July 2021	July 2022	August 2021	August 2022
1.	Araneae	Gnaphosidae	*Gnaphosa lucifuga*	1					
2.			*Scotophaeus blackwalli*		1				
3.		Sparassidae	*Micrommata virescens*			1			
4.		Philodromidae	*Thanatus arenarius*		1				
5.	Coleoptera	Carabidae	*Carabus coriaceus*		1			1	
6.			*Harpalus* sp.		2				
7.		Cerambycidae	*Dorcadion fulvum*		1				
8.		Coccinellidae	*Harmonia axyridis*	1					
9.			*Scymnus* sp.					4	
10.		Dermestidae	*Attagenus* sp.		1				
11.		Mordellidae	*Mordella aculeata*					1	
12.			*Tomoxia bucephala*					2	
13.	Dermaptera	Forficulidae	*Forficula auricularia*		1				1
14.	Hemiptera	Anthocoridae	*Orius* sp.		1		1		
15.		Rhyparochromidae	*Raglius alboacuminatus*			1			
16.	Homoptera	Cicadellidae	*Scaphoideus titanus*					1	
17.	Hymenoptera	Formicidae	*Camponotus* sp.	1		5	1	4	
18.			*Lasius* sp.	7	79	8	56	17	26
19.	Neuroptera	Chrysopidae	*Chrysoperla carnea*					1	
20.	Opiliones	Sironidae	*Cyphophthalmus duricorius*					5	
21.		Phalangiidae	*Opilio* sp.			1		6	
22.			*Phalangium opilio*	2					
23.	Orthoptera	Acrididae	*Calliptamus italicus*	2				2	
24.			*Chorthippus* sp.		1				
25.			*Pezotettix giornae*		1	1	3	10	1
26.		Gryllidae	*Gryllus campestris*	1				5	
27.			*Melanogryllus desertus*	10	74	5	4	18	2
28.			*Modicogryllus frontalis*	6	37	8	7	5	
29.			*Acheta domesticus*					5	
Total month/year				**31**	**201**	**30**	**72**	**87**	**30**
Total year 2021							**148**		
Total year 2022							**303**		

Another species that has recorded a large number of individuals belongs to the genus *Melanogryllus*, which has great economic importance in agriculture. Its mode of nutrition is zoophytophagous, with both larvae and adults partially or completely devour leguminous cereals, forages, as well as the shoots of industrial plants (cotton, hemp, sunflower, rapeseed, etc.). It is widespread in Europe, North America, and Central Asia (Stahi N., 2007; Kuralbaevich B. M., 2022).

Also, in Ștefănești vineyard center, but in a different location, between the years 2020-2021, 37 insect species were identified in Barber soil traps, with 8 more species than in the vineyard in the locality of Călinești. The only common species were: *Acheta domesticus*, *Microммata virescens*, *Carabus coriaceus*, *Scymnus* sp., *Tomoxia bucephala*, *Raglius alboacuminatus*, *Scaphoideus titanus*, *Camponotus* sp., *Lasius* sp., *Cyphophthalmus duricorius*, *Opilio* sp., *Calliptamus italicus*, *Pezotettix giornae*, *Gryllus campestris*, *Melanogryllus desertus*, *Modicogryllus frontalis*, and *Acheta domesticus* (Vizitiu D. et al., 2022).

The average air temperature in the year 2021 ranged from 1.06°C (January) to 25.18°C (July), while in the year 2022 it varied from 1.80°C (January) to 23.72°C (July). In the months of July and August of 2021 and 2022, the highest temperature increases compared to the multi-year average of 1979-2011 were recorded. However, these months showed the largest increases in the year 2021, with 3.5°C and 2.64°C higher than the multi-year average, respectively (figure 2).

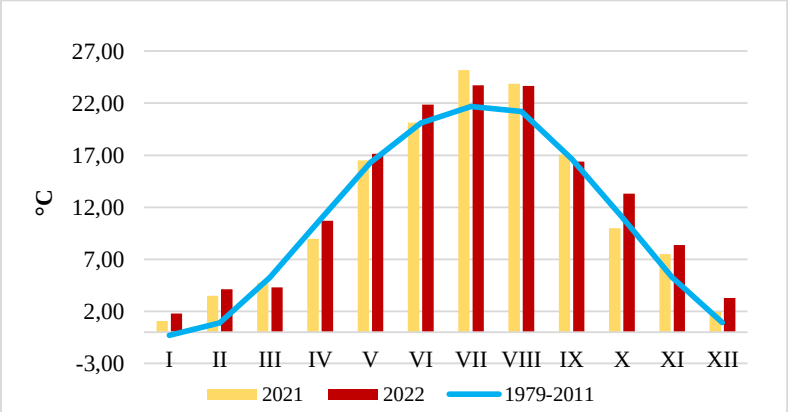


Figure 2. The evolution of daily average temperatures in the years 2021 and 2022 compared to multi-year temperatures (1979-2011) // Evoluția temperaturii medii zilnice în anii 2021 și 2022 comparativ cu temperaturile multianuale (1979-2011)

In the year 2021, 770.8 mm of precipitation were recorded, while in 2022, 680.4 mm were recorded, with 104.91 mm and 16.51 mm more than the multi-year average (1979-2011). The highest amount of precipitation was recorded in August of 2022, which represented a surplus of 111.34 mm compared to the multi-year average (figure 3).

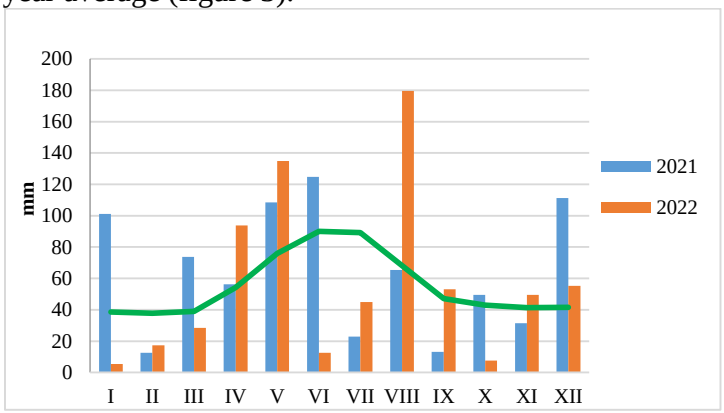


Figure 3. The evolution of precipitation in the years 2021 and 2022 compared to multi-year precipitation (1979-2011) // Evoluția precipitațiilor în anii 2021 și 2022 comparativ cu precipitațiile multianuale (1979-2011)

The Shannon diversity index is a common index used to characterize species diversity within a community and takes into account both the abundance and evenness of species distribution (Popov C. et al., 2009). The abundance of insect species was most evenly distributed in August 2021 and least evenly distributed in August 2022 (figure 4), possibly due to the abundant precipitation recorded in that month.

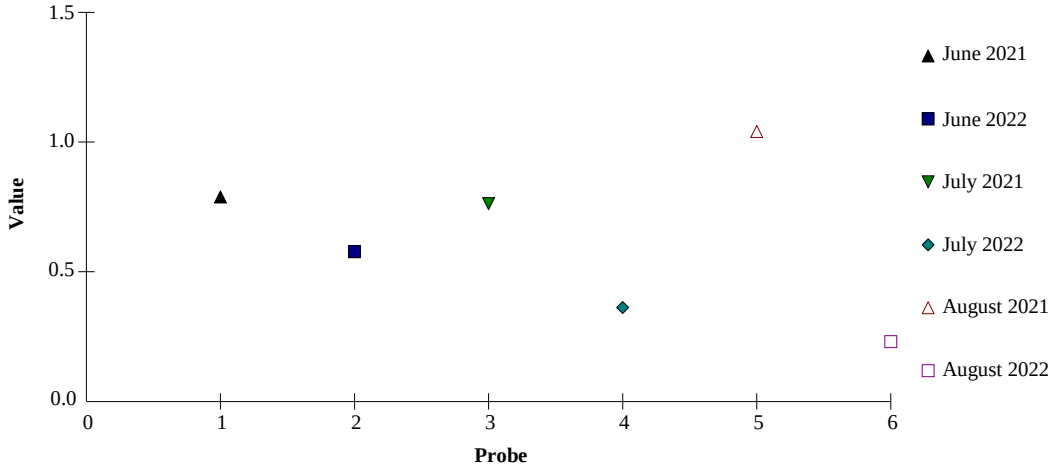


Figure 4. Shannon index calculated in the 2021 and 2022 years conditions //Indicele Shanon calculat în condițiile anilor 2021 și 2022

The Simpson diversity index is used in ecology to describe the diversity of a habitat and takes into account the number of species present and the abundance of each species (Popov C. et al., 2009). Regarding the Simpson index, the data in figure 5 show that the lowest diversity of insect species, throughout the study period, was recorded in August 2022, while the highest diversity of species was recorded in August of 2021.

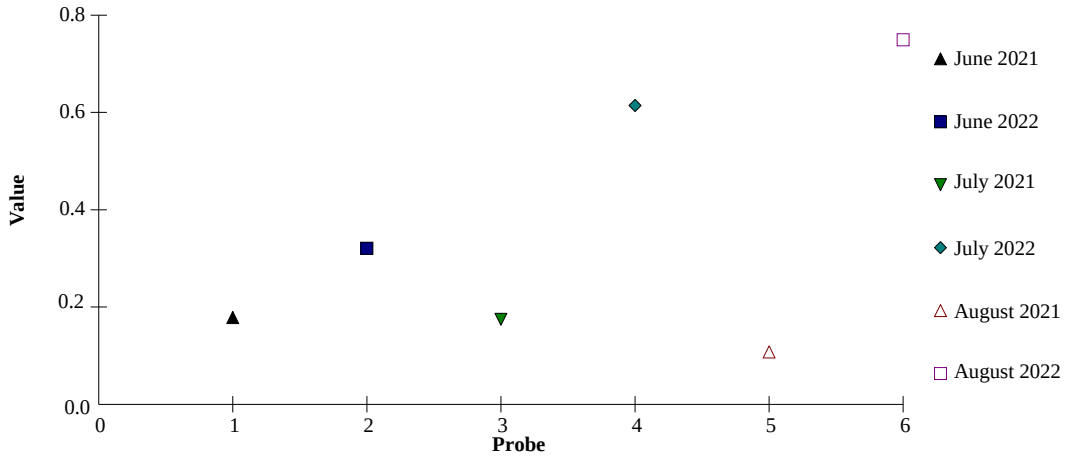


Figure 5. Simpson index calculated in the 2021 and 2022 years conditions // Indicele Simpson calculat în condițiile anilor 2021 și 2022

The SHE analysis (figure 6) has shown that there is a balance between species richness and evenness throughout the study period, except for the month of June 2021.

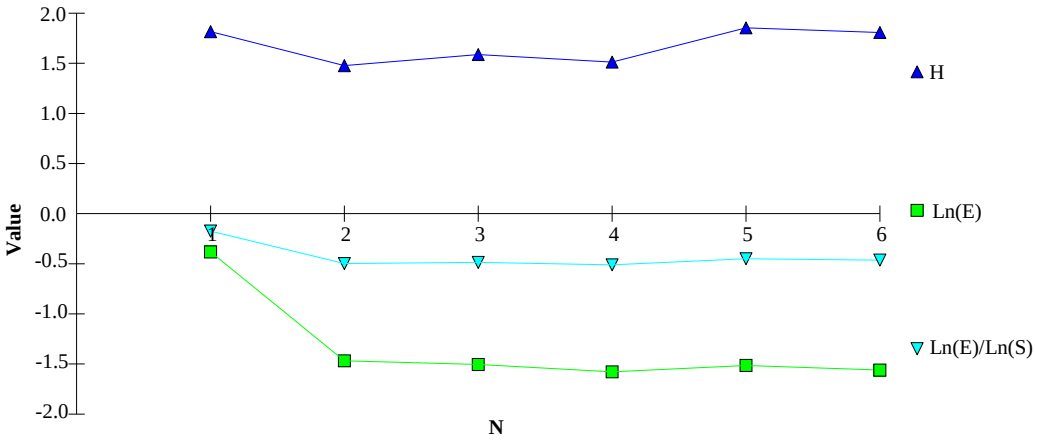


Figure 6. SHE analysis // Analiza SHE

To determine the degree of similarity in species composition, a Bray-Curtis analysis was performed. Thus, it can be observed in figure 7 that there is no similarity between the two years, but only between the months of the same year. The highest similarity is between the months of July and August of 2022, followed by June - July 2021.

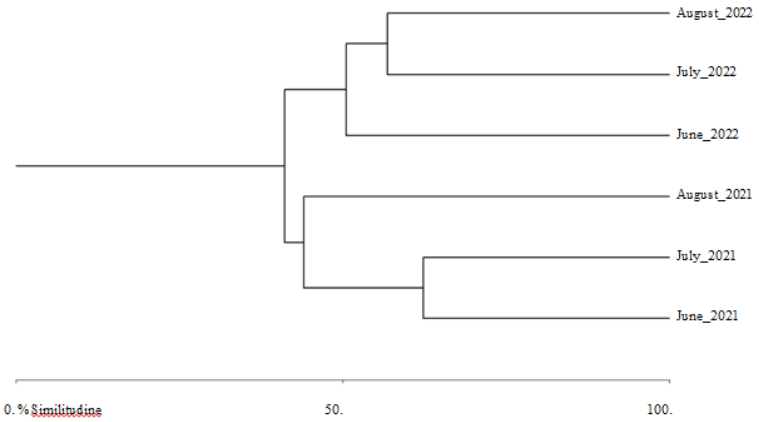


Figure 7. Bray-Curtis analysis // Analiza Bray-Curtis

Shannon and Simpson indices were used in this paper, some of the most widely used biodiversity indicators (Kim B. R. *et al*., 2017).

CONCLUSIONS

Throughout the two-year monitoring period (2021-2022), the insect species with the highest number of individuals captured using Barber traps were representatives of the *Lasius* and *Melanogryllus* genera. During the two-year study, a total of 148 individuals were recorded in 2021 and 303 individuals in 2022.

The abundance of insect species was distributed most evenly in August 2021 and most unevenly in August 2022. The lowest diversity of insect species was recorded in August 2022 due to the high amount of precipitation (179.6 mm), while the highest diversity was observed in August 2021 when the

precipitation amount was lower (65.4 mm). Therefore, the abundance of dominant species is closely related to the precipitation levels recorded in 2021-2022. The species with the highest number of individuals did not meet optimal conditions for their habitat in August of both study years. The two predominant species, *Lasius* sp. and *Melanogryllus desertus*, because of their ecology prefer a habitat with high temperatures, habitats with sparse vegetation, and low precipitation amounts. The highest similarity in species composition was recorded between July and August in 2022, followed by June-July 2021.

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EVOLUTION OF THE PRECIPITATION REGIMEN AND HUMIDITY RESERVES IN THE SOIL OF VINEYARDS IN THE NORTH-EASTERN PART OF ROMANIA

EVOLUȚIA REGIMULUI DE PRECIPITAȚII ȘI A REZERVEI DE UMIDITATE DIN SOL ÎN PLANTAȚIILE VITICOLE DIN ZONA DE NORD-EST A ROMÂNIEI

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Abstract

Insufficient precipitations and their poor distribution associated to other meteorological elements generate critical periods for the vine from physiological point of view that are reflected in grape production. The paper presents the evolution of precipitations from the vineyard ecosystem of Iasi vineyard between 1989 – 2022, based on which a series of biochemical coefficients, deviations from multiannual average values, as well as their influence on the dynamics of soil humidity (accessible humidity and water deficit) in the 2000 – 2022 period, were calculated. Based on the analysis of registered data we observed a lowering of the precipitation regimen usually unevenly distributed, represented by torrential rains that alternate with long draught periods. The increasingly lower precipitation quantities and high temperatures have caused a sharp decrease of accessible humidity in the soil, considerably below the optimal values for vine and an increase of deficit.

Key words: *vineyard, precipitation, accessible humidity, water deficit*

Rezumat

Insuficiența precipitațiilor şi distribuția lor necorespunzătoare asociată altor elemente meteorologice generează pentru vița-de-vie perioade critice din punct de vedere fiziologic, care se reflectă în producția de struguri. În lucrare este prezentată evoluția precipitațiilor din ecosistemul viticol al podgoriei Iaşi înregistrate în perioada 1989 – 2022, pe baza cărora s-au calculat o serie de coeficienți bioclimatici, abaterile față de valorile medii multianuale, precum şi influența acestora asupra dinamicii umidității solului (umiditatea accesibilă şi deficitul de apă) din perioada 2000 – 2022. Din analiza datelor înregistrate s-a remarcat o diminuare a regimului de precipitații distribuit frecvent neuniform, reprezentat prin ploi torențiale, care alternează cu perioade lungi de secetă. Cantitățile tot mai mici de precipitații şi temperaturile mari au determinat scăderea accentuată a valorilor umidității accesibile din sol, cu mult sub valorile optime pentru viță-de-vie şi creşterea deficitului.

Cuvinte cheie: *plantații viticole, precipitații, umiditatea accesibilă, deficit apă*

INTRODUCTION

The effects of climate change in Romania have been felt more and over the last decades through the increase of the incidence of freeze, draught and flood phenomena, generating a negative effect on the yield of cultures and reduction of fauna and flora biodiversity. Analyzing the monthly and seasonal averages of precipitations between 1901 – 2000 we noticed a decrease tendency of annual precipitation quantities and a modification of their distribution, especially in the vegetation period (Dumitrescu A. *et al.*, 2015, Sandu I. *et al*., 2010).

For the climate area in the north-east of the country we have registered certain periods (years) with normal pluviometric characteristics, but certain excess and deficit periods deviating from the norm stand out. The time distribution of the excess/deficit precipitation periods has a certain regularity. The contrasting periods follow each other in such a way that after a rainy period there is no normal period, nut several years of draught (Berbecel *et al*., 1970).

During the agricultural year, the autumn and winter seasons are the period when the humidity reserves of the soil is built, due to the relatively abundant rainfall, and mainly due to low water loss through evaporation. The increase of the draught pfenomenen frequency may have destructive effects for vineyards when in the autumn and winter of the previous years there is a precipitation deficit ant the quantities registered in the spring are not sufficient to replenisch the water rezerves in the deep layers of the soil from which the vines take their water (Zaldea *et al*., 2017; Zaldea *et al.*, 2021). In the context, the paper has the purpose of underlining the climate modifications over the last decades in Copou ecosistem, Iaşi vineyard, which in the decrease of precipitation regimen, increase of dry years and their impact on the humidity reserve of the soil.

MATERIAL AND METHOD

The study was based on a series of daily meteorological parameters recorded at the SCDVV Iaşi weather station, located in the northern part of the municipality of Iaşi, at 191 m altitude, 47°12`18`` north latitude and 27°32`04`` east longitude. The analysed period covered a series of homogeneous data for the last 34 years (1989-2022), based on which were calculated precipitation coefficient (Constantinescu Gh., 1964), hydrothermal coefficient (Selyanianov, 1928) and Martonne aridity index (De Martonne, 1926).

In order to establish soil moisture, the oven drying method was used (Canarache, 1964). Thus, for each month in the growing season, sampling by layers was performed every 10 cm, up to a depth of 150 cm; the results were first expressed in percentage compared to the weight of dry soil, then in volume percentage. Based on the hydrophysical indices values, the soil moisture available at a given time (Macc), expressed in mm and %. In order to establish the degree of available plant water supply, the available moisture (Macc) was compared to the useful water capacity (UWC), previously calculated for the Copou Iaşi wine centre.

RESULTS AND DISCUSSIONS

In the Copou-Iaşi wine centre, the precipitation multiannual average (1981-2010) is 579.6 mm, with 398.1 mm during the growing season (April-September).

The analysis of annual precipitations for the interval 1989 - 2022 highlights a wetter period between 1991 and 2005, with values above average, followed by a period of pluviometric deficit, between 2006 and 2022, with values below the period average (Figure 1). The lowest amount of annual precipitation was recorded in 2015 of 365.5 mm, and the highest in 1991 of 829.5 mm.

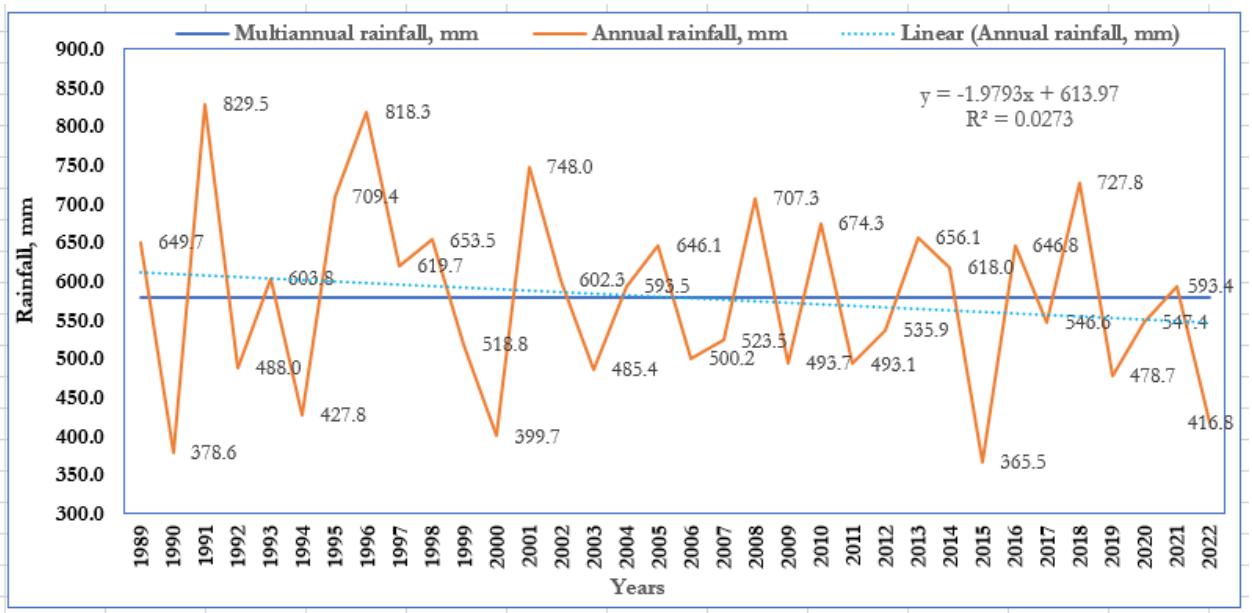


Fig. 1. Evolution of annual precipitations recorded in the Copou-Iaşi wine centre (1989 - 2022) /*Evoluţia precipitaţiilor anuale înregistrate în centrul viticol Copou Iaşi (1989 - 2022)*

In order to highlight the variations in annual precipitations, the deviations from the average were calculated; this allowed for their characterisation according to the Hellman criterion. The graphical representation shows a decrease in quantities towards the end of the analysed interval (Figure 2).

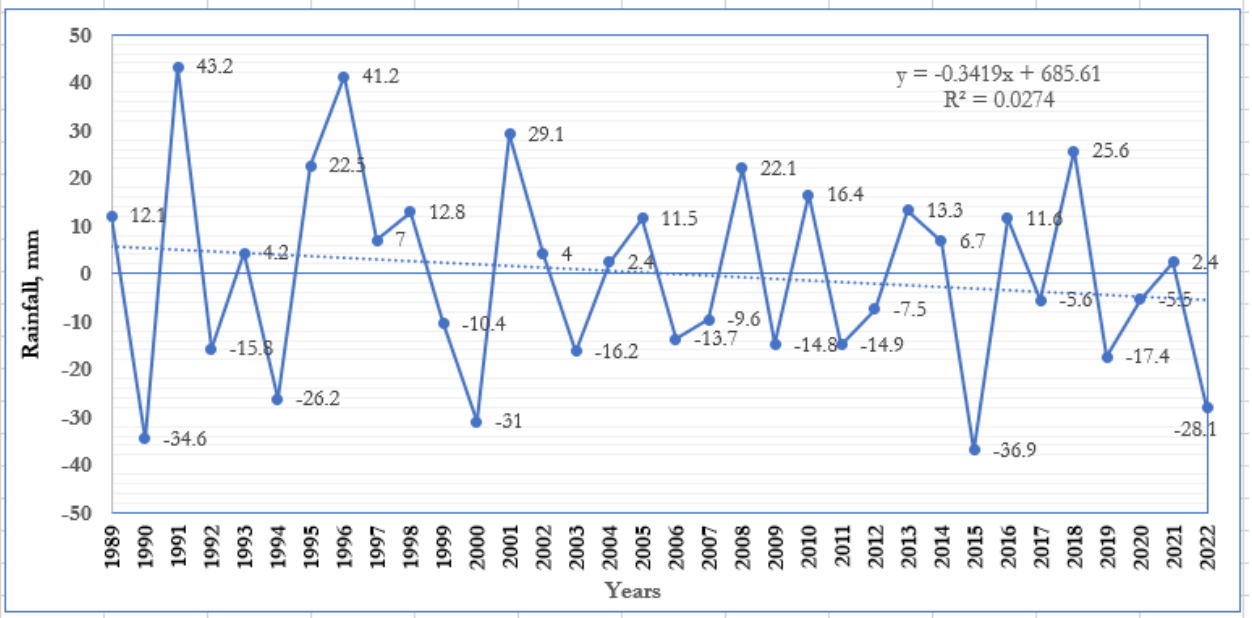


Fig. 2. Deviations of annual precipitations compared to the multiannual average (1989 -2022)/*Abaterea precipitaţiilor anuale faţă de media multianuală (1989 -2022)*

As regards the annual deviations from the average, it can be noticed that in 47.06% of the analysed years, the total precipitation was below average, while in 52.94% the values were higher than the multiannual average.

By calculating the frequency of annual precipitations recorded in the Copou-Iaşi wine centre, their probability was established (Table 1). Thus, it was observed that the probability of annual precipitation with values between 601-650 mm is 20,59%, and between 401-450 mm of 5.88%.

Table 1. **Frequency of annual precipitation and its probability*/Frecvenţa precipitaţiilor anuale şi probabilitatea acestora***

Precipitation, mm	No. of years	Probability, %	Precipitation, mm	No. of years	Probability, %
351-400	3	8.82	601-650	7	20.59
401-450	2	5.88	651-700	3	8.82
451-500	6	17.65	701-750	4	11.76
501-550	5	14.71	751-800	0	0.00
551-600	2	5.88	801-850	2	5.88
Total nr. ani	**34**				

Depending on the deviations from the average rainfall, it was noted that of the 34 years included in the study (1989-2022), only five are considered normal in terms of rainfall, and most of them - 15 years - had little precipitation, characterised from "moderately dry" (4 years), to "dry" (3year), "very dry" (3 years) and 5 "extremely dry" years (Table 2).

Table 2. Precipitations regime and characterisation of the analysed interval 1989 – 2022/*Regimul precipitaţiilor şi caracterizarea anilor în intervalul 1989 – 2022*

Deviation against average %	Hellman criterion	No. of cases	Years
<-20.0	exceedingly droughty	5	1990, 1994, 2000, 2015, 2022
-20.0….-15.1	very droughty	3	1992, 2003, 2019
-15.0….-10.1	droughty	3	2006, 2009, 2011
-10.0….-5.1	moderately droughty	4	2007, 2012, 2017, 2020
-5.0….5.0	normal	5	1993, 1999, 2002, 2004, 2021
5.1….10.0	moderately rainy	3	1997, 2014, 2016
10.1….15.0	rainy	4	1989, 1998, 2005, 2013
15.1….20.0	very rainy	1	2010
>20.0	exceedingly rainy	6	1991, 1995, 1996, 2001, 2008, 2018

High temperatures and drought caused by climate change also led to significant changes in multiannual bioclimatic coefficients and indicators, directly correlated with the average annual temperature, the active heat balance (Σ t°a), the amount of annual precipitations and precipitation in the growing season (Table 3).

Table 3. **The values of the main synthetic bioclimatic indicators from in the Copou Iaşi wine centre (1989 – 2022)*/Valorile principalilor indicatori bioclimatici cu caracter sintetic din centrul viticol Copou Iaşi (1989 – 2022)***

Year	The hydrothermal coefficient	The precipitation coefficient	The "de Martonne" aridity index	Year	The hydrothermal coefficient	The precipitation coefficient	The "de Martonne" aridity index
1989	1.9	3.4	31	**2006**	1.1	1.9	25
1990	0.8	1.5	18	**2007**	0.8	1.7	24
1991	2.5	4.3	43	**2008**	1.7	3.2	34
1992	1.2	2.1	25	**2009**	0.6	1.2	24
1993	1.4	2.3	31	**2010**	1.3	2.5	34
1994	1.2	1.6	20	**2011**	1.2	2.3	25
1995	1.8	3.2	36	**2012**	0.8	1.6	26
1996	1.9	3.4	43	**2013**	1.6	3.0	32
1997	1.5	2.6	33	**2014**	1.2	2.0	31
1998	1.1	2.0	33	**2015**	0.5	1.0	17
1999	1.0	2.0	28	**2016**	1.0	1.9	31
2000	0.8	1.5	19	**2017**	0.9	1.6	26
2001	1.8	2.9	38	**2018**	1.3	2.6	35
2002	1.4	2.3	30	**2019**	1.0	2.0	22
2003	0.9	1.7	25	**2020**	0.9	1.8	25
2004	1.3	2.2	30	**2021**	1.4	2.6	30
2005	1.4	2.6	33	**2022**	0.9	1.8	20

The hydrothermal coefficient, which represents the ratio between the sum of precipitations during the growing season and the active heat balance, reached minimum values of 0.5 in 2015 and maximum values of 2.5 in 1991. The trend of this coefficient is decreasing, as a result of the decreasing amounts of precipitations during the growing season. Values of the hydrothermal coefficient below 0.8 substantiate the need for vineyards irrigation.

The precipitation coefficient, established by the ratio between the amount of precipitation during the growing season and the duration of the bioactive period, registered lower values in the dry years and higher values in the rainy years (from 1.0 in 2015 and 4.3 in 1991).

The "de Martonne" aridity index, which expresses the ratio between the sum of annual precipitation and the useful temperature, registered a minimum value of 17 in 2015 and a maximum of 43 in 1991 and 1996. These values indicate that during 1989 - 2022, in the Copou – Iaşi wine centre there were both years that were part of a semi-arid climate, and years with a semi-humid and humid climate.

The values of these synthetic indicators confirm that during the analyzed period the driest year was 2015 and the rainiest was 1991.

The small amounts of precipitation corroborated with the high temperatures, led to a marked decrease in the values of the soil accessible moisture (Macc) during certain periods, far below the optimal ones for the vineyard culture. It is known that the optimal soil moisture for vineyard culture is between 50-80% of the useful water capacity (UWC), higher values being favourable for growing shoots, and the lowest for grain maturation (Moţoc M., 1968). In addition, during the long periods of drought (lasting for 2-3 years), the water reserve in the deep layers of the soil of 100-150 cm decreases.

Monitoring the monthly distribution of accessible soil humidity during 2000-2022 has highlighted the low values in the dry and very dry years: 2000, 2007, 2009, 2011, 2012, 2015, 2017, 2019, 2020 and 2022 (Table 5). Among these, the year 2020 stand out with extremely low values registered in almost all the months of the growing season – between 23-39%, on the whole depth of the soil profile (0-150 cm). At the same time, in the 0-20 cm layer, the accessible soil humidity values were below the wilting coefficient. In these conditions, the water deficit was very high, even in the deep layers (100-150 cm), from 54% in May to 82% in August (Table 6). During the years when the drought caused significant damage, the precipitation deficit started from the previous year (summer or autumn), continued during the winter, as well as in the spring and summer of the following year. Such situations were recorded for the years 2006-2007, 2011-2012, 2019-2020, 2021 -2022 and had negative effects on the growth of the stocks (Figure 3).

Table 5. ***Monthly distribution of available soil humidity, average values by layers*** **/Distribuţia lunară a umidităţii accesibile din sol, valori medii pe straturi**

Year	Depht, cm	Available humidity (%) from the vegetation period						Year	Depht, cm	Available humidity (%) from the vegetation period					
		IV	V	VI	VII	VIII	IX			IV	V	VI	VII	VIII	IX
2000	0 - 20	8	18	10	2	36	35	**2012**	0 - 20	43	60	14	11	18	24
	20 - 50	55	61	53	45	55	48		20 - 50	82	55	19	14	15	22
	50 -100	79	71	67	63	61	45		50 -100	94	81	43	23	20	24
	100 - 150	108	100	92	85	96	54		100 - 150	88	125	74	41	45	46
2001	0 - 20	26	48	62	20	27	42	**2013**	0 - 20	39	58	62	33	24	39
	20 - 50	57	62	72	39	32	67		20 - 50	67	69	78	53	17	41
	50 -100	40	68	86	58	42	63		50 -100	74	76	83	71	46	48
	100 - 150	50	61	114	87	65	42		100 - 150	121	127	135	112	94	65
2002	0 - 20	48	45	34	55	59	74	**2014**	0 - 20	49	63	36	43	26	21
	20 - 50	61	61	42	80	68	71		20 - 50	62	72	57	49	28	31
	50 -100	74	67	48	77	70	51		50 -100	80	85	67	48	32	38
	100 - 150	114	107	92	92	105	71		100 - 150	141	118	100	70	41	54
2003	0 - 20	56	32	32	58	42	35	**2015**	0 - 20	53	33	33	10	9	66
	20 - 50	69	60	52	53	53	42		20 - 50	65	46	48	36	25	34
	50 -100	77	66	50	57	49	48		50 -100	93	75	56	37	30	28
	100 - 150	117	103	84	83	76	74		100 - 150	111	119	81	46	63	54
2004	0 - 20	40	32	45	55	73	71	**2016**	0 - 20	61	59	53	23	14	9
	20 - 50	77	67	60	56	63	70		20 - 50	71	77	63	30	24	26
	50 -100	80	76	67	54	64	65		50 -100	82	73	53	35	32	22
	100 - 150	126	120	101	82	76	56		100 - 150	63	57	76	40	33	42
2005	0 - 20	64	66	49	65	62	42	**2017**	0 - 20	62	32	39	30	17	8
	20 - 50	77	83	60	65	66	59		20 - 50	71	60	45	34	32	23
	50 -100	85	99	72	68	69	63		50 -100	82	79	55	32	36	23
	100 - 150	126	134	117	113	95	90		100 - 150	122	134	82	71	55	45

2006	0 - 20	64	62	59	52	52	45	**2018**	0 - 20	27	23	67	39	24	17
	20 - 50	77	76	71	49	54	53		20 - 50	61	36	73	55	35	38
	50 -100	85	75	76	57	55	55		50 -100	100	70	79	73	58	37
	100 - 150	126	126	117	87	79	70		100 - 150	156	104	87	93	97	56
2007	0 - 20	35	32	4	29	65	36	**2019**	0 - 20	51	72	61	31	32	1
	20 - 50	69	61	43	26	56	54		20 - 50	63	67	70	46	22	7
	50 -100	58	54	40	30	33	52		50 -100	88	86	89	68	41	26
	100 - 150	72	67	56	38	37	55		100 - 150	119	124	152	122	82	37
2008	0 - 20	65	61	58	63	50	23	**2020**	0 - 20	40	59	57	21	12	-22
	20 - 50	75	66	65	67	51	69		20 - 50	28	24	35	34	22	24
	50 -100	101	93	66	87	57	62		50 -100	29	32	37	32	18	28
	100 - 150	148	117	70	124	68	64		100 - 150	28	46	39	43	38	36
2009	0 - 20	35	70	49	11	9	6	**2021**	0 - 20	55	46	47	42	26	24
	20 - 50	70	69	63	34	26	9		20 - 50	56	82	70	52	31	32
	50 -100	83	73	72	33	22	18		50 -100	76	72	84	68	40	32
	100 - 150	118	100	91	75	42	38		100 - 150	80	114	119	98	78	62
2010	0 - 20	72	47	48	56	59	61	**2022**	0 - 20	69	48	6	13	17	24
	20 - 50	64	65	75	77	75	70		20 - 50	69	60	13	41	18	23
	50 -100	90	77	85	86	85	74		50 -100	66	47	22	40	28	32
	100 - 150	145	126	128	128	120	89		100 - 150	71	70	27	54	48	39
2011	0 - 20	59	40	70	52	29	32								
	20 - 50	72	66	84	64	33	36								
	50 -100	88	73	88	84	38	39								
	100 - 150	135	110	116	116	50	63								



Fig. 3. Aspects of vineyards affected by drought/*Aspecte din plantațiile viticole afectate de secetă*

Table 6. **Monthly distribution of soil humidity deficit, average values by layers/*Distribuţia lunară a deficitului de umiditate din sol, valori medii pe straturi***

Year	Depht, cm	Soil humidity deficit (%) from the vegetation period						Year	Depht, cm	Soil humidity deficit (%) from the vegetation period					
		IV	V	VI	VII	VIII	IX			IV	V	VI	VII	VIII	IX
2000	0 - 20	92	82	90	98	64	65	**2012**	0 - 20	57	40	86	89	82	76
	20 - 50	45	39	47	55	45	52		20 - 50	18	45	81	86	85	78
	50 -100	21	29	33	37	39	55		50 -100	6	19	57	77	80	76
	100 - 150	-	-	8	15	4	45		100 - 150	12	-	26	59	55	54
2001	0 - 20	74	52	38	80	73	58	**2013**	0 - 20	61	42	38	67	76	61
	20 - 50	43	38	28	61	68	33		20 - 50	33	31	22	47	83	59
	50 -100	60	32	14	42	58	37		50 -100	26	24	17	29	54	52
	100 - 150	50	39	-	13	35	58		100 - 150	-	-	-	-	6	35
2002	0 - 20	52	55	66	45	41	26	**2014**	0 - 20	51	37	64	57	74	79
	20 - 50	39	39	58	20	32	29		20 - 50	38	28	43	51	72	69
	50 -100	26	33	52	23	30	49		50 -100	20	15	33	52	68	62
	100 - 150	-	-	8	8	-	29		100 - 150	-	-	-	30	59	46
2003	0 - 20	44	68	68	42	58	65	**2015**	0 - 20	47	67	67	90	91	34
	20 - 50	31	40	48	47	47	58		20 - 50	35	54	52	64	75	66
	50 -100	23	34	50	43	51	52		50 -100	7	25	44	63	70	72
	100 - 150	-	-	16	17	24	26		100 - 150	-	-	19	54	37	46
2004	0 - 20	60	68	55	45	27	29	**2016**	0 - 20	39	41	47	77	86	91
	20 - 50	23	33	40	44	37	30		20 - 50	29	23	37	70	76	74
	50 -100	20	24	33	46	36	35		50 -100	18	27	47	65	68	78
	100 - 150	-	-	-	18	24	44		100 - 150	37	43	24	60	67	58

2005	0 - 20	36	34	51	35	38	58	**2017**	0 - 20	38	68	61	70	83	92
	20 - 50	23	17	40	35	34	41		20 - 50	29	40	55	66	68	77
	50 -100	15	1	28	32	31	37		50 -100	18	21	45	68	64	77
	100 - 150	-	-	-	-	5	10		100 - 150	-	-	18	29	45	55
2006	0 - 20	36	38	41	48	48	55	**2018**	0 - 20	73	77	33	61	76	83
	20 - 50	23	24	29	51	46	47		20 - 50	39	64	27	45	65	62
	50 -100	15	25	24	43	45	45		50 -100	-	30	21	27	42	63
	100 - 150	-	-	-	13	21	30		100 - 150	-	-	13	7	3	44
2007	0 - 20	65	68	96	71	35	64	**2019**	0 - 20	49	28	39	69	68	99
	20 - 50	31	39	57	74	44	46		20 - 50	37	33	30	54	78	93
	50 -100	42	46	60	70	67	48		50 -100	12	14	11	32	59	74
	100 - 150	28	33	44	62	63	45		100 - 150	-	-	-	-	18	63
2008	0 - 20	35	39	42	37	50	77	**2020**	0 - 20	60	41	43	79	88	122
	20 - 50	25	34	35	33	49	31		20 - 50	72	76	65	66	78	76
	50 -100	-	7	34	13	43	38		50 -100	71	68	63	68	82	72
	100 - 150	-	-	30	-	32	36		100 - 150	72	54	61	57	62	64
2009	0 - 20	65	30	51	89	91	94	**2021**	0 - 20	45	54	53	58	74	76
	20 - 50	30	31	37	66	74	91		20 - 50	44	18	30	48	69	68
	50 -100	17	27	28	67	78	82		50 -100	24	28	16	32	60	68
	100 - 150	-	-	9	25	58	62		100 - 150	20	-	-	2	22	38
2010	0 - 20	28	53	52	44	41	39	**2022**	0 - 20	31	52	94	87	83	76
	20 - 50	36	35	25	23	25	30		20 - 50	31	40	87	59	82	77
	50 -100	10	23	15	14	15	26		50 -100	34	53	78	60	72	68
	100 - 150	-	-	-	-	-	11		100 - 150	29	30	73	46	52	61
2011	0 - 20	41	60	30	48	71	68								
	20 - 50	28	34	16	36	67	64								
	50 -100	12	27	12	16	62	61								
	100 - 150	-	-	-	-	50	37								

CONCLUSIONS

Analyzing the annual precipitation in the interval 1989 and 2022 we notice a wetter period between 1991 and 2005 with values above the average, following by a deficient pluviometric deficit period between 2006 and 2022. Low quantities of precipitations, corroborated with high temperatures have caused a sharp decrease of the values of humidity accessible from the soil, in certain periods, considerably under the optimum for vine culture.These have usually been higher in the first two months of the vegetation period (April and May), and then there was a decrease of available humidity from one month to another and an increase of the deficit up to the deep layers (100 – 150 cm).

The evolution of precipitation quantities registered in the last 34 years in the Iaşi vineyard indicate an increase of the frequency of the drought phenomenon, especially after the year 2000, largely affecting vineyards. Under these circumstances, it is increasingly necessary to create Vinifera varieties and drought-resistant rootstocks and to generalization and expend the irrigation system at the national level.

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GENETIC VARIABILITY WITHIN THE BAZNA AND RED MANGALITSA BREEDS

DIVERSITATEA GENETICĂ ÎN CADRUL RASELOR DE SUINE BAZNA ŞI MANGALIŢA ROȘIE

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Abstract

*The objective of this study is to determine genetic variability, within two indigenous swine breeds, **Bazna** and **Red Mangalitsa**. The biological samples were collected under sterile conditions from a number of 12 pigs from the red **Mangalitsa** breed and 15 pigs from the **Bazna** breed. It seems that at the mitochondrial DNA level, the genetic background on the maternal line of the **Mangalitsa** breed is European, arguing the formation of this breed on the territory of Europe. At the level of mitochondrial DNA, genetic diversity is reduced, thus having a high degree of inbreeding both within the **Red Mangalitsa** variety breed and within the wild boar. A number of 10 analyzed samples from the **Bazna** breed were grouped in the European genetic cluster, the other 5 samples being grouped in the Asian cluster (divided into two subclusters), together with the **Vietnamese** breed. The results of the genetic variability analysis based on SNP polymorphisms indicate that the swine breeds studied form several clusters with composed group of breeds or groups of breeds. We could conclude that the **Mangaliţa** breed of swine, the red variety, raised in the mother nucleus at RDSA Turda, presents a reduced genetic diversity at the level of mitochondrial DNA. On the other hand, the **Bazna** swine breed bred in the maternal nucleus from RDSA Turda, presents a fairly high genetic diversity at the level of mitochondrial DNA, which confirms the existence of a large number of maternal lines in this population.*

Keywords: Bazna, Red Mangalitsa, mitochondrial DNA, SNP polymorphisms, genetic diversity

Rezumat

*Obiectivul acestui studiu este de a determina variabilitatea genetică, în cadrul a două rase de porcine indigene, **Bazna** și **Mangaliţa Roșie**. Probele biologice au fost recoltate în condiții sterile de la un număr de 12 porci din rasa **Mangaliţa roșie** și 15 porci din rasa **Bazna**. Cel puțin la nivelul ADN mitocondrial, fondul genetic pe linia maternă a rasei **Mangaliţa** este european, argumentând formarea acestei rase pe teritoriul Europei. La nivelul ADN-ului mitocondrial, diversitatea genetică este redusă, având astfel un grad ridicat de consangvinizare atât în cadrul rasei de suine **Mangaliţa Roşie**, cât și în cadrul mistreţului. Un număr de 10 probe analizate din rasa **Bazna** au fost grupate în clusterul genetic european, celelalte 5 probe analizate fiind grupate în clusterul asiatic (împărţite în două subclustere), alături de rasa vietnameză. Rezultatele analizei variabilității genetice pe baza polimorfismelor SNP, indică faptul că rasele de porci studiate formează grupuri compuse de rase sau grupuri de rase. Am putea concluziona că rasa de porc **Mangaliţa**, varietatea roșie, crescută în nucleul mamă la SCDA Turda, prezintă o diversitate genetică redusă la nivelul ADN-ului mitocondrial. Pe de altă parte, rasa porcină **Bazna** crescută în nucleul matern de la SCDA Turda, prezintă o diversitate genetică destul de mare la nivelul ADN-ului mitocondrial, ceea ce confirmă existența unui număr mare de linii materne în această populație.*

Cuvinte cheie: Bazna, Mangalita roșie, AND mitocondrial, polimorfisme SNP, variabilitate genetică

INTRODUCTION

The domestication of animals is a revolutionary event that has profoundly influenced human history (Huang et al., 2020). The swine (*Sus scrofa*) was independently domesticated in Europe and Asia about 10,000 years ago (Larson et al., 2005). On the European continent there are small populations belonging to the transboundary breed **Mangalitsa** (in Austria, Germany, Romania, Serbia and Hungary), or to the local breeds **Turopolje** (in Croatia) and **Bazna** (in Romania) (Zahan, 2017). The importance of local breeds as valuable genetic reservoirs is well established (Zorc et al., 2022). The **Red Mangalitsa** descends directly from the wild boar and is a rustic breed of swine from the Carpathian Basin (based in Hungary and Romania) (Oroian and Petrescu., 2014). The **Bazna** is a breed originating in Romania, formed in 1872 from a cross between **Mangalitsa** sows and **Berkshire** boars (Draganescu et al., 2008).

Genetic diversity of farm animals is necessary to meet current production needs in different environments, to enable sustained genetic improvement, and to facilitate rapid adaptation to changing breeding goals (Noter., 1999). Maintaining a sufficient diversity of animal genetic resources is necessary to ensure the sustainability of agricultural production, especially under conditions of climate change (Boettcher et al., 2015). The way to improve sustainability is a low-input system based on selecting animals that are more robust to climate change and are better adapted to convert low-quality feed (local feed, feed by-products and food waste) into meat, but it is feasible if this is combined with a reduction in food waste (Rauw et al., 2020).

Aspects of domestication have often been investigated by studying mitochondrial DNA, while genetic diversity was initially studied using simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) in intensively selected breeds but also in autochthonous populations with limited diffusion (SanCristobal et al., 2006).

Mitochondrial (mt) DNA is haploid, non-recombining and almost exclusively maternally inherited (Fajardo et al., 2008). The advantage of mitochondrial DNA analysis derives from the fact that there are many mitochondria per cell and many DNA molecules in each mitochondrion, making mtDNA a naturally amplified source of genetic variation (Girish et al., 2004). In addition, mitochondrial genes evolve much faster, thus, contain more sequence diversity, facilitating the identification of phylogenetically related species (Mortiz et al., 1987).

Microsatellites and SNP-type polymorphisms have been mainly used for traceability purposes, the latter currently predominates over the former, presenting many advantages such as easier manipulation in the laboratory, low mutation rate and better suitability for standardization (Fries and Durstewitz, 2001). SNP-type polymorphisms provide the opportunity for genome-wide comparisons between individuals and races (Dadousis et al., 2022). The development of SNP-type polymorphism panels with genome-wide distributed SNPs has provided new opportunities to investigate and decipher the complex relationship between indigenous pig breeds (Lukic et al., 2020).

Preserving the diversity of indigenous pig breeds (*Sus scrofa*) is a key factor for sustaining the pork chain (both locally and globally), including the production of high-quality branded products, the enrichment of animal biobanking and the progress of conservation policies. In addition, local pig farming is closely linked to high-quality niche products that contribute to the development and sustainability of the local economy (Picardy et al., 2019). No less important is the growing demand for organic and high welfare animal-based food products, which has led consumers to prefer local breed products that are considered more nutritious, tasty, healthy and safe, and because animals are usually grown freely and outdoors (Garcia-Gudino et al., 2021).

MATERIAL AND METHOD

Biological samples

The biological samples were collected under sterile conditions from a number of 12 pigs from the red **Mangalitsa** breed and 15 pigs from the **Bazna** breed. The Vietnamese breed was chosen as a swine breed with an Asian background, and hair samples were collected from 9 individuals. Wild boar hair samples were collected from 22 individuals during the hunting season. Genomic SNP data available in genebanks were used to perform preliminary studies on genetic differentiation from the **Mangalitsa** breed red variety from Hungary and other commercial pig breeds (**Landrace, Great White, Hampshire**).

DNA purification from the collected samples

DNA purification from hairs was performed using the ZR Genomic DNA II Miniprep kit (Zymo Research). The bulbs of the harvested hairs (10/individual) were transferred into sterile 1.5 ml Eppendorf tubes, appropriately marked. Tissues were lysed in 400 µl of lysis buffer and then incubated for 20 min at room temperature. The obtained lysate was transferred to a purification column, and the resulting eluate in the collection tube by centrifuging the columns for 1 minute at 10,000 rpm was removed. The DNA retained in the filters of the purification columns was washed twice with 400 µl wash buffer from the kit (wash buffer) and re-centrifuged. The purified DNA from the filter was eluted in 50 µl of elution buffer by centrifugation at 18,000 rpm. These DNA samples were later used for cytochrome B sequencing in the study subjects.

PCR amplification and sequencing of the cytochrome B (Cyt B) gene from mitochondrial DNA

A reference sequence available in GenBank (Acc. Number AJ002189) was used for the design of the primers required for the amplification and sequencing of the cytochrome B gene coding region from pig and wild boar mitochondrial DNA. The PCR amplification mixture (Taq polymerase, Buffer, MgCl2, dNTPs, primers) DNA was made using a commercial kit 2X PCR Master mix Bioline (table 1).

Table 1. Preparation of the reaction mix for PCR amplification / Prepararea mixului de reacţie pentru amplificarea PCR

Component	Volume for 1 sample (µl)
sterile milli Q water	8,5
master mix 2X	12,5
primer forward	1
primer revers	1
Total reaction mix	**25**

A 23 µl volume of the amplification mixture was transferred into 0.2 ml tubes labeled with the sample number. 2 µl of DNA from each sample was then added to each tube.

PCR amplification was performed in the thermal cycler according to the program:

- DNA predenaturation at 95°C/3 minutes → 1 cycle
- denaturation at 94°C/1 minute
- fixing the primers at 58°C/1 minute } 35 cycles
- extension (Elongation) at 72°C/1 minute
- final extension at 72°C/3 minutes → 1 cycle

Amplification quality was achieved electrophoretically by migrating 5 µl of PCR product in 2% agarose gel in 1X TBE buffer at 70 V for two hours.

Sequencing PCR amplification of purified PCR products was performed in separate reactions with PCR primers using the BigDyeTerminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, USA), according to the instructions.

Sequencing products were purified using the BigDyeXterminator kit (Thermo Scientific) and subsequently migrated to a SeqStudio sequencer (Thermo Scientific).

Analysis procedure using the DNA microarray technique

The analysis procedure followed a work protocol and steps established by the manufacturers of the genotyping arrays. In the case of pigs, an array of 60,000 SNPs was used.

Genotyping by the DNA microarray technique is based on the use of a matrix on which specific allele DNA probes are fixed, positioned in the vicinity of some predetermined SNPs from the pig genome. These SNPs can be located in exons, introns or intergenic regions. A certain amount of the purified DNA samples is injected onto the genotyping arrays. Amplification takes place simultaneously in the tens of thousands of positions (polymorphic loci) represented on the array, each with a fluorescently labeled nucleotide (A, T, G or C). These incorporated nucleotides are complementary to the SNPs in the template DNA hybridized to the probes on the array.

The detection of fluorescent nucleotides incorporated in each investigated position represented on the genotyping array and the identification of SNPs is done with the help of a high-resolution scanner.

Bioinformatics interpretation of data

The analysis of the obtained sequencing chromatograms was performed by visual inspection using the BioEdit program. The obtained sequences were aligned using the ClustalW2 program. The calculation of the differentiation / genetic diversity indices and the preparation of the phylogenetic trees was carried out with the help of the MEGA and DnaSP programs.

The bioinformatics analysis of the data (identification of the SNPs, respectively of the genotypes from the 60,000 investigated loci) was carried out with the help of specific software: e.g. Plink, Admixture etc.

RESULTS AND DISCUSSIONS

Analysis of genetic diversity and phylogenetic relationships based on mitochondrial DNA sequences.

Within the phylogenetic tree (figure 1) obtained through the bioinformatics analysis of the variability of the nucleotide sequence of the Cyt B gene obtained by sequencing this region in the three breeds of domestic pigs and wild boar, two main clusters were highlighted: the European and the Asiatic ones.

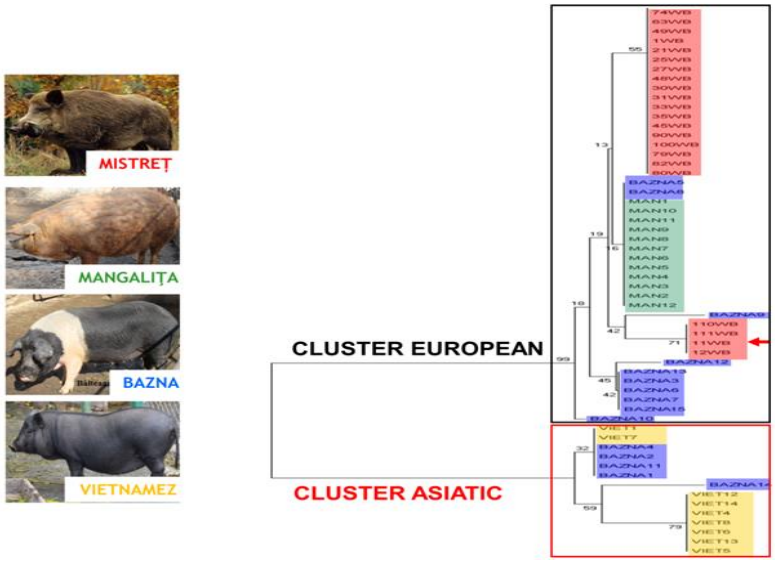


Figure 1. Phylogenetic tree obtained based on polymorphism of the Cyt B gene nucleotide sequence from mitochondrial DNA / Arbore filognetic obținut pe baza polimorfismului secvenței nucleotidice a genei Cyt B din ADN-ul mitocondrial

Looking at the European cluster, we notice that there are samples obtained from the **Mangalitsa** swine breed, the Red variety, and from wild boars. It follows from this that, at least at the mitochondrial level, the genetic background on the maternal line of the **Mangalitsa** breed is European, arguing the formation of this breed on the territory of Europe.

At the level of mitochondrial DNA, genetic diversity is reduced, thus having a high degree of inbreeding both within the **Red Mangalitsa** variety breed and within the wild boar (table 2).

Only one haplotype was identified, both in the case of the **Mangalitsa** breed and in the wild boar (table 2). In the case of the **Red Mangalitsa** breed, this can be explained by the lack of new individuals from other populations that can be used to increase genetic diversity and reduce inbreeding.

Table 2. Genetic diversity at the level of mitochondrial DNA / Diversitatea genetică la nivelul ADN-ului mitochondrial

Species / Breed	Cytochrome B (Cyt B) – mitochondrial DNA			
	Analyzed sequences (no.)	Nucleotide diversity	The diversity of haplotypes	Haplotypes identified (no.)
Wild boar	18*	0,0000	0,000	1
Red Mangalitsa	12	0,0000	0,000	1
Bazna	15	0,008	0,857	7
Vietnamese pork	9	0,004	0,511	2

Within the wild boars analyzed, 18 of the 22 were grouped in a subcluster resulting in a reduced genetic diversity and therefore a high inbreeding (figure 1). The other 4 individuals from whom samples were taken for analysis grouped in a subcluster different from the base population, being closer to the **Bazna** breed (figure1) (these 4 analyzed samples were excluded from the analysis due to the possibility of be hybrids with the domestic swine).

Considering the formation of the **Bazna** breed, the Cyt B analysis showed different results compared to those obtained in the **Red Mangalitsa** variety breed. Thus, within the **Bazna** breed, a number of 10 analyzed samples were grouped into the European genetic cluster (figure 1). Here we have a great genetic diversity due to the fact that 8 of the 10 analyzed samples formed 4 different subclusters. Two of the analyzed samples were grouped in the subcluster of the **Mangalitsa** breed, a fact due to the maternal line contribution of this breed in the formation of the **Bazna** breed. This positioning of the samples analyzed in the European cluster is not surprising if we look at the past where we observe a concordance between the formation of the breed and its first European origin on the maternal side.

However, a number of 5 analyzed samples coming from the **Bazna** breed were grouped in the Asian cluster, in two subclusters alongside the **Vietnamese** breed (figure 1). This is not surprising, thus confirming its Asian origin on the maternal line, this positioning being consistent with the formation of the **Bazna** breed and the majority of British swine breeds (**Wessex, Large White** and **Hampshire**) that were massively introgressed in the 19th century with swine breeds from the Far East, for reproductive maturity and to increase the amount of fat in the carcass.

Within the **Bazna** breed, a great diversity of nucleotides was found in the analyzed Cyt B sequence, corresponding to the 7 distinct haplotypes highlighted (table 2). This fact is correlated with the multiracial maternal origin of the **Bazna** breed.

Based on the mitochondrial sequences obtained, the genetic distances (Fst index) were also calculated between the three breeds of domestic swine analyzed, respectively the wild boar (table 3). The Fst index is calculated to highlight the degree of genetic differentiation between populations / breeds / species. The values of the Fst index can be between 1 and -1. Values below 0.05 indicate little genetic differentiation between populations. Values between 0 and -1 indicate weak or non-existent differentiation between populations. Populations show an average degree of differentiation when Fst

values are between 0.05 - 0.5. Values between 0.5 and +1 indicate significant genetic differentiation between populations/breeds.

Table 3. Genetic distances revealed by the Fst index calculated based on the nucleotide diversity of the CytB gene / Distanțele genetice evidențiate de indicele Fst calculat pe baza diversității nucleotidice a genei CytB

Species / Breed	**Bazna**	**Vietnamese pork**	**Red Mangalitsa**	**Wild boar**
Bazna	-	-	-	-
Vietnamese pork	0,410***	-	-	-
Red Mangalitsa	0,272***	0,873***	-	-
Wild boar	0,933***	0,970***	0,981***	-

*** - P < 0,001

A significant difference can be observed between the wild boar and the three domestic swine breeds with an Fst index above 0.9 (table 3). There is also a significant difference between the **Red Mangalitsa** breed and the **Vietnamese** breed with an Fst index of 0.873, a fact due to the lack of the gene pool of breeds of Asian origin when the **Red Mangalitsa** breed was formed. Due to the maternal line contribution of the **Mangalitsa** breed to the formation of the **Bazna** breed, we have an average genetic differentiation index (0.272). Also due to the Asian gene pool in the background of the **Bazna** breed, we have an average genetic differentiation between the **Bazna** breed and the **Vietnamese** breed with an Fst index of 0.410.

Analysis of genetic diversity and phylogenetic relationships based on SNP polymorphisms.

In order to investigate the phylogenetic relationships and genetic distances between the studied pig breeds and wild boar, a 3D MDS (multidimensional scaling plot) diagram was constructed with the help of the PLINK program (figure 2). A first analysis of the results indicates that the swine breeds analyzed form several clusters composed of breeds or groups of breeds (fig. 1). We observe in the MDS plot that the **Mangalitsa** swine breeds from Romania and **Mangalitsa** from Hungary were positioned at a relatively appropriate distance due to their common genetic background. This positioning indicates a degree of differentiation between the two populations, this differentiation being auspicious as it allows for the genetic survival of the breed and the reduction of inbreeding through the exchange of breeding stock between the two populations.

The **Bazna** breed clustered close to the **Hampshire breed**, which is not surprising given the significant contribution of this breed to the formation of the **Bazna** breed.

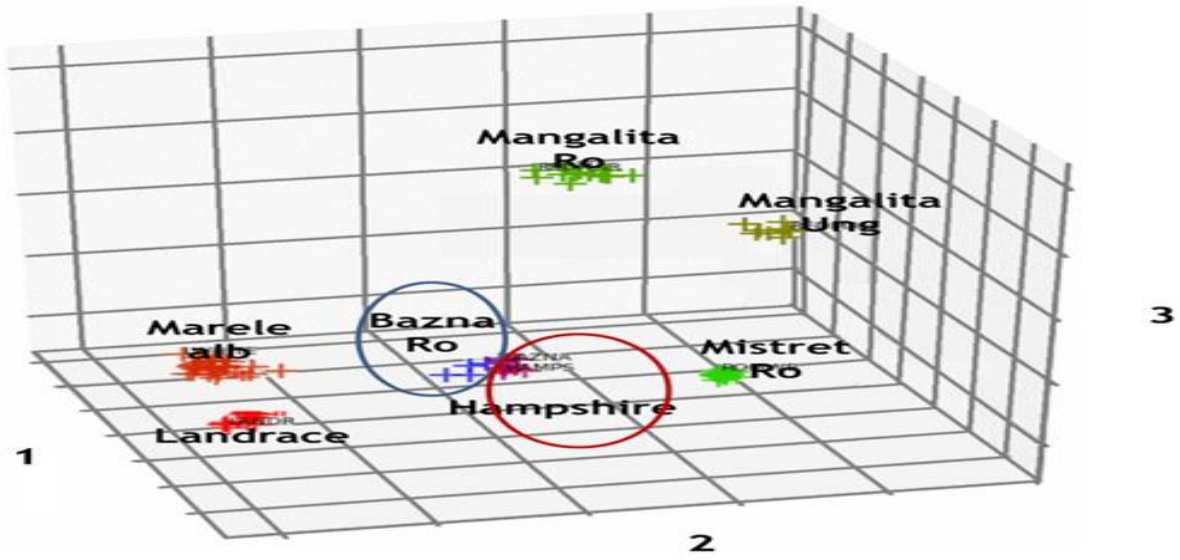


Figure 2. 3D MDS diagram of the relationships between the studied swine breeds and the wild boar / Diagramă 3D MDS privind relațiile dintre rasele de porcine studiate și porcul mistreț

The values of the Fst index, determined on the basis of the genotypes obtained at the loci analyzed, confirmed these results (table 4.). This index measures the degree of genetic differentiation (genetic distance) between breeds due to their genetic makeup.

The **Red Mangalitsa** breed population is genetically distant both from wild boar with a genetic differentiation index of 0.186, and from commercial breeds (table 4.).

Surprisingly, the **Bazna** breed shows a higher degree of differentiation compared to the **Mangalitsa** breed, although as was highlighted in the case of the mt DNA analysis, this breed contributed on the maternal line to the formation of the **Bazna** breed. This difference, however, is due to the polyracial origin of the **Bazna** breed. A significant genetic difference between the **Bazna** breed and the wild boar was also found.

Table 4. Indices of genetic differentiation (Fst) between the studied swine breeds and wild boar calculated from SNP genotyping data / Indicii de diferențiere genetică (Fst) dintre rasele de porcine studiate și porcul mistreț, calculați pe baza datelor de genotipare SNP

	Red Mangalitsa (H)	Red Mangalitsa (Ro)	Bazna	Hampshire	Landrace	The great White	Wild boar (Ro)
Red Mangalitsa (H)	-	-	-	-	-	-	-
Red Mangalitsa (Ro)	0,115	-	-	-	-	-	-
Bazna	0,259	0,234	-	-	-	-	-
Hampshire	0,279	0,259	0,329	-	-	-	-
Landrace	0,201	0,180	0,207	0,241	-	-	-
The great White	0,204	0,175	0,202	0,238	0,125	-	-
Wild boar (Ro)	0,185	0,186	0,243	0,267	0,196	0,198	-

CONCLUSIONS

We could conclude that the **Red Mangalitsa** breed, raised in the mother core from RDSA Turda, presents a reduced genetic diversity at the level of mitochondrial DNA. This indicates a high degree of maternal inbreeding, which could affect the future genetic stability of this population. However, the population of the Romanian **Red Mangalitsa** shows at the autosomal level a certain degree of genetic differentiation (Fst=0.115) compared to the population of the **Red Mangalitsa** from Hungary.

The **Bazna** swine breed, raised in the maternal nucleus from RDSA Turda, shows a fairly high genetic diversity at the level of mitochondrial DNA, which confirms the existence of a large number of maternal lines in this population. This finding is consistent with the admixed (polyracial) origin of the **Bazna** breed. The results of the DNA analyzes highlight the fact that a proper management of this population can lead to its numerical increase, without affect the genetic stability of the **Bazna** breed.

The effective management of the domestic swine breeds **Red Mangalitsa** and **Bazna**, breeds with a vulnerable status, currently represents one of the most important challenges for the breeders' associations that lead breeding programs, as well as for the authorities in the field. The use of DNA markers in the classical breeding programs of the two breeds, currently based only on phenotypic selection, can contribute substantially to their more efficient management and to their genetic stabilization.

The advantages of using the information provided by DNA markers in classical breeding programs can be multiple, among which we mention: the precise establishment of the inbreeding coefficient within the pig populations of the two breeds, which allows a more efficient matching of matings between individuals little related; tracking with greater precision the transmission of characters from parents to offspring by knowing the real parentage of the offspring; fixing in the population some mutations associated with morpho-productive characters of interest; determination of genetic distances to other pig breeds based on the calculation of genetic differentiation indices (e.g. Fst).

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THE PREVALENCE OF DIGESTIVE PARASITES OF PIGS IN SMALLHOLDERS FROM HÂRTIBACIU VALLEY, SIBIU COUNTY, ROMANIA

PREVALENŢA PARAZIŢILOR DIGESTIVI LA PORCI ÎN GOSPODĂRII DE PE VALEA HÂRTIBACIULUI, JUDEŢUL SIBIU, ROMÂNIA

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Abstract

Parasitic diseases cause significant economic losses in pigs, but are often overlooked because of the apparent lack of clinical signs. The current study aimed to identify the parasitic profile of swine raised in 14 smallholders from Hârtibaciu Valley, Sibiu. A number of 260 samples were collected, during winter and spring, from piglets, fatteners, and sows. The coproparasitological examination used usual methods. The number of eggs (EPG), oocysts (OPG), and cysts (CPG) were counted per gram of fecal matter.The examination revealed parasitic infections with B. coli, Eimeria spp., A. suum, T. suis, and Oesophagostomum spp. The prevalence (P) and average intensity (AI) of infections varied between farms, seasons and age group. The overall prevalence in all smallholders, according to the age category, was 60% for Eimeria spp., 22% - B. coli, 27% - Oesophagostomum spp., 29% - A. suum, and 8% - T. suis, in piglets. In fatteners, Eimeria spp. had a prevalence of 28%, B. coli - 49%, A. suum - 33%, T. suis - 8%, Oesophagostomum spp. – 29% and in sows Eimeria spp. - 23%, B. coli - 43%, A. suum - 27%, and Oesophagostomum spp. - 37%. Therefore, the present study revealed statistically significant differences between age groups, seasons, and smallholders for all diagnosed parasites. The presence of infections with B. coli, A. suum, and T. suis, parasites with zoonotic potential, justifies their supervision and control, in the vision of the "One health, one medicine" concept.

Keywords: digestive parasites, epidemiology, smallholders, pigs

Rezumat

Bolile parazitare provoacă pierderi economice semnificative la porci, dar sunt adesea trecute cu vederea din cauza lipsei aparente a semnelor clinice. Studiul actual a avut ca scop identificarea profilului parazitar al suinelor crescute in 14 gospodarii din Valea Hârtibaciu, Sibiu. Un număr de 260 de probe au fost colectate, în timpul iernii și primăverii, de la purcei, grăsuni și scroafe. Examenul coproparazitarologic a folosit metode uzuale. Numărul de ouă (OPG), oochisturi (OPG) și chisturi (CPG) au fost numărate per gram de materie fecală. Examinarea a relevat infecții parazitare cu B. coli, Eimeria spp., A. suum, T. suis și Oesophagostomum spp. Prevalența (P) și intensitatea medie (IM) a infecțiilor au variat între ferme, anotimpuri și grupa de vârstă. Prevalența totală la toate gospodăriile, în funcție de categoria de vârstă, a fost de 60% pentru Eimeria spp., 22% - B. coli, 27% - Oesophagostomum spp., 29% - A. suum și 8% - T. suis, la purcei. La grăsuni, Eimeria spp. a avut o prevalență de 28%, B. coli - 49%, A. suum - 33%, T. suis - 8%, Oesophagostomum spp. – 29% și la scroafe Eimeria spp. - 23%, B. coli - 43%, A. suum - 27%, și Oesophagostomum spp. - 37%. Prin urmare, studiul de față a evidențiat diferențe semnificative statistic între grupele de vârstă, anotimpuri și gospodării pentru toți paraziții diagnosticați. Prezența infecțiilor cu B. coli, A. suum și T. suis, paraziți cu potențial zoonotic, justifică supravegherea și controlul acestora, în viziunea conceptului "One health, one medicine".

Cuvinte cheie: paraziţi digestivi, epidemiologie, gospodării, suine

INTRODUCTION

In pig-rearing systems, parasitic diseases are responsible for substantial losses in both pig reproduction and productivity, which represents a significant obstacle to an efficient and profitable animal management (Kochanowski et al., 2017; Ózsvári et al., 2018). Parasitic infections can lead to intestinal malabsorption, compromised or delayed immunity following vaccination protocols, impaired fertility, increased susceptibility to bacterial and viral illnesses, as well as a decline in meat quality (Lai et al., 2011).

Factors such as age and sex of the animals, hygiene, management practices, and geographical location serve as risk factors that, in association with the digestive parasites of pigs, affect the welfare and health of the animals (Permin et al., 1999; Geresu et al., 2015; Roesel et al., 2017). Flooring, waste drainage facilities, humidity, and temperature provide optimal conditions for the propagation and survival of parasitic stages in feed and contaminated environments (Ali et al., 2018; Sharma et al., 2020). Animal overcrowding, the presence of rodents, access to non-potable water, open defecation, inadequate shelter, improper feeding, health status, and host immunity are other significant factors in the development of parasitic stages, creating optimal conditions for infection. This could be attributed to coprophagy in pigs, which makes them more susceptible to parasitic infections (Ali et al., 2018; Sharma et al., 2020).

Gastrointestinal parasitic diseases generally exhibit a relatively slow progression, and frequently, pigs do not show any noticeable symptoms (Băieș et al., 2021). Within animal populations, there are infected swine with a low number of parasites (intensity) and low pathogenicity, resulting in an unaffected clinical condition. However, these animals serve as carriers and eliminators of parasites, disseminating invasive elements into the external environment, thus contaminating the rest of the herd (Geresu et al., 2015; Roesel et al., 2017).

The most common mistakes in combating pig parasitoses include the lack of regular coproparasitological examinations, inappropriate administration and dosage of antiparasitic drugs, ineffective disinfection, or the incorrect choice of disinfectant agent (Selzer and Epe., 2021).

This study aimed to identify the parasitic profile of pigs from three age categories (piglets, fatteners, and sows) raised in smallholders in the Hârtibaciu Valley, Sibiu County.

MATERIAL AND METHODS

This study aims to ascertain the prevalence and intensity of parasitic infections in pigs of various breeds, raised in a low-input system (smallholder) in the Hârtibaciu Valley area.

The Hârtibaciu Valley, also known as the ”Green Valley”, offers a unique landscape with abundant pastures, forests, and agricultural fields. Iacobeni is a commune located in Sibiu County, Transylvania, consisting of villages such as Iacobeni (the administrative center), Movile, Netuș, Noiștat, and Stejărișu. Positionated 70 km from the city of Sibiu, 12 km from Agnita, and 32 km from Sighișoara, the commune is characterized by hilly terrain, traversed by the Hârtibaciu River. The fertile lands, cultivated without the use of chemicals, establish the Green Valley as one of the most suitable places for agriculture, especially for organic farming.

Within the commune, there is an estimated population of around 700 pigs. The main swine breeds found among the local smallholders include Pietrain, Large White, Duroc, Landrace, Bazna, and their crossbreeds. Pigs are raised in either individual or collective pens. However, the practice of free-range pig rearing is becoming less common due to restrictions aimed at preventing domestic pigs from encountering wild boars and some others potentially spreading African swine fever (ASF).

Faecal samples were collected from 14 smallholders residing in the villages of Iacobeni, Netuș și Stejarișu. The shelters are subject to a daily cleaning routine, and some of them have outdoor areas. Additionally, a few smallholders have adopted a free-range pig-rearing system. The sampling procedure encompassed three distinct age groups (piglets, fatteners, and sows). The collection of faecal took place during two seasons: winter and spring, spanning the years 2021-2022. A total of 135 animals were sampled during the winter season, followed by 125 animals during the spring season. Specifically, in the winter, 35 faecal samples were procured from piglets, 50 from fatteners, and 50 from sows. In the subsequent spring season, 25 faecal samples were obtained from piglets, while 50 samples each were collected from fatteners and sows. Age-wise, the piglets ranged between 6 to 12 weeks, fatteners were between 4 and 8 months, and sows ranged from 2 to 5 years in age.

Faecal samples were individually collected from the rectum of the animals. The samples were examined macroscopically (color, consistency, and presence of any visible parasites). Subsequently, the sterile containers were labeled and stored at 2-8 °C until testing. Each faecal sample underwent an individual coproparasitological examination.

Throughout the study, the animals did not exhibit any clinical signs attributed to parasitic infections. Certain smallholders adhered to an established deworming protocol, while a limited number of animals were not dewormed. The deworming procedure was overseen by the local veterinarian upon the owners' s request. Sows were dewormed with injectable ivermectin (0.3 mg/kg body weight), fatteners received injectable levamisole solution (5 mg/ kg body weight) and piglets were orally administered toltrazuril (20 mg/ kg body weight).

The collected samples were processed and examined at the Department of Parasitology and Parasitic Diseases of the Faculty of Veterinary Medicine from Cluj-Napoca. The coproparasitological methods used for processing the faecal samples included flotation (Willis, McMaster), active sedimentation, modified Ziehl-Neelsen method, modified Blagg method, and the cultivation of strongyle eggs and coccidian oocysts. The number of eggs/oocysts/cysts (EPG/OPG/CPG) was counted per gram of faecal matter using established protocols (Mircean et al., 2011).

RESULTS AND DISCUSSION

The coproprasitological examination revealed parasitic infections with *Balantioides coli, Eimeria* spp*., Ascaris suum, Oesophagostomum* spp*.*, and *Trichuris suis* (Figure 1). The prevalence (P) and mean intensity (MI) of the infection varied according to the age and category of investigated pigs (Table 1 and Table 2). It is noteworthy that all samples subjected to active sedimentation, modified Ziehl-Neelsen, and modified Blagg methods, were negative. Furthermore, all identified oocysts belonged to the *Eimeria* genus, and all the strongyle larvae were attributed *Oesophagostomum genus***.**

Among piglets, in both seasons, the most prevalent parasite was *Eimeria* spp. (52%; 68%), followed in the winter season by *Oesophagostomum* spp. (34%), *A. suum* (17%), *T. suis* (12%), and *B. coli* (3%), and in the spring season *A. suum* (40%), *B. coli* (40%), *Oesophagostomum* spp. (20%), and *T. suis* (4%) (Tables 1, 2 and 3).

In the case of fatteners, the most prevalent parasite during both seasons was *B. coli* (44%; 54%). In the winter season, this was followed by *Oesophagostomum* spp. (34%), *Eimeria* spp. (28%), *A. suum* (24%), and *T. suis* (4%), and during the spring season, by *A. suum* (42%), *Eimeria* spp. (28%), *Oesophagostomum* spp. (24%), and *T. suis* (4%) (Tables 1, 2, and 3).

Among sows, the highest prevalence, during the winter season was observed for *A. suum* (38%), followed *by B. coli* (36%), *Oesophagostomum* spp. (26%), and *Eimeria* spp. (16%). In the spring season, *B. coli* (50%) exhibited the highest prevalence, followed by *Oesophagostomum* spp. (48%), *Eimeria* spp. (30%), and *A. suum* (16%) (Tables 1, 2, and 3).

In the current study, *Trichuris suis* was identified only in piglets and fatteners displaying a prevalence of 8% (Table 3). In Europe, the prevalence of this nematode ranged from 2.9 to 11.2% among fatteners in Poland and reached up to 14.3% in the Netherlands (Eijck et al., 2005; Popiołek et al., 2009; Kochanowski et al., 2017). Notably, the prevalence of *T. suis* infection in India was lower at 6.43%, similar to other reports from Ghana (4.6%) and Zimbabwe (4.2%) (Sharma et al., 2020).

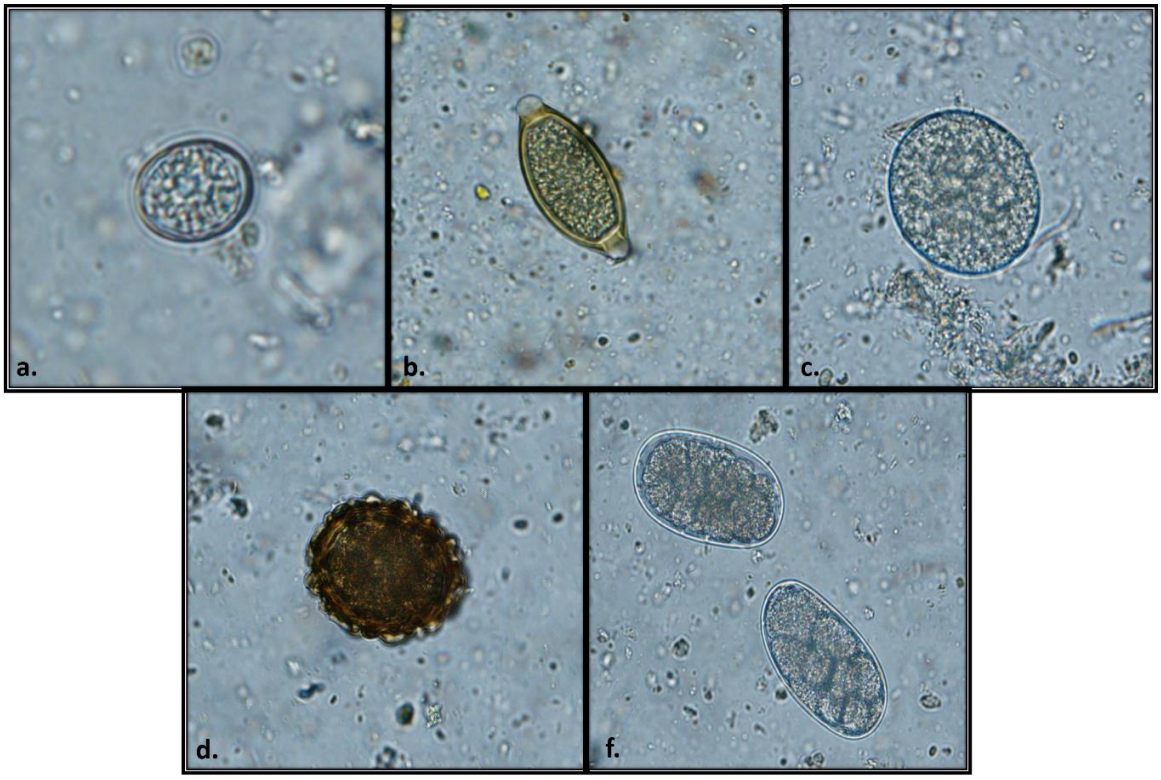


Figure 1. The results of coproparasitological examination: a. *Eimeria* spp. oocyst; b. *T. suis* egg; c. *B. coli* cyst; d. *A. suum* egg; f. *Oesophagostomum* spp. egg (400x) / Rezultatele examenului coproparazitologic: a. oochist de *Eimeria* spp.; b. ou de *T. suis*; c. chist de *B. coli*; d. ou de *A. suum*; f. ou de Oe*sophagostomum* spp. (400x).

Table 1. The prevalence (P%) and mean intensity (MI) in the 3 age groups in the winter season / Prevalenţa (P%) şi intensitatea medie (IM) la cele 3 categorii de vârstă în sezonul de iarnă

	Piglets		Fatteners		Sows	
Parasite	P %	MI	P %	MI	P %	MI
Eimeria spp.	52	881	28	1311	16	288
B. coli	4	400	44	173	36	160
A. suum	17	3508	24	667	38	727
Oesophagostomum spp.	34	246	34	433	26	131
T. suis	12	175	4	125	-	-

Table 2. The prevalence (P%) and mean intensity (MI) in the 3 age groups in the spring season / Prevalenţa (P%) şi intensitatea medie (IM) la cele 3 categorii de vârstă în sezonul de primăvară

	Piglets		Fatteners		Sows	
Parasite	P %	MI	P %	MI	P %	MI
Eimeria spp.	68	454	28	6519	30	600
B. coli	40	182	54	129	50	62
A. suum	40	1485	42	1384	16	994
Oesophagostomum spp.	20	140	24	480	48	958
T. suis	4	200	12	309	-	-

Table 3. The prevalence (P%) and mean intensity (MI) in the 3 age groups in the both season / Prevalența (P%) şi intensitatea medie (IM) la cele 3 categorii de vârstă în ambele sezoane

	Piglets		Fatteners		Sows	
Parasite	P %	MI	P %	MI	P %	MI
Eimeria spp.	60	667	28	3915	23	444
B. coli	22	291	49	151	43	111
A. suum	29	2496	33	1025	27	860
Esophagostomum spp.	27	193	29	456	37	544
T. suis	8	187	8	217	-	-

Table 4. The overall prevalence (P%) and mean intensity (MI) / Prevalența (P%) şi intensitatea medie (IM) totală

	Total	
Parasite	P %	MI
Eimeria spp.	37	1675
B. coli	38	184
A. suum	30	1460
Oesophagostomum spp.	31	398
T. suis	8	202

In the current study, *Ascaris suum* was identified in piglets, fatteners, and sows with a prevalence of 29%, 33%, and 27%, respectively (Table 3). Sharma et al. (2020) demonstrated that *A. suum* was the most prevalent digestive parasite in pigs (11.1%), within subtropical regions of India. In another study conducted on pigs of various ages, in Nordic countries, varying prevalences of *A. suum* were reported: 3.5% in Finland, 16% in Norway, and 15% in Sweden (Roepstorff et al., 1998). In South Africa, a study conducted in 2019 revealed a prevalence of 63.9% among fatteners (Nwafor et al., 2019). Several studies in China and Greece concluded that the prevalence of *A. suum* infection is lower in commercial farms (ranging from 0.9 to 16.5%), compared to free-range farms (Lin et al., 2013; Symeonidou et al., 2020).

The prevalence of *B. coli* infection in Italy was 36.66%, 40% in Bangladesh, and 22% in Malaysia, which concurs with our study's findings (38%), and contrary to the observations in Korea, where it was notably lower (4.5%) (Tan et al., 2014; Sharma et al., 2020). *B. coli* has been diagnosed in piglets from Bangladesh, Greece, and Germany, with prevalences of 28.6%, 2.6%, and 0.7%, respectively (Wieler et al., 2001; Dey et al., 2014; Symeonidou et al., 2020), while our study recorded a prevalence of 22% (Table 4).

In a study carried out in Azerbaijan involving pigs of different ages, a prevalence of *Eimeria* spp. ranging from 21.4% to 32.2% was identified (Ali et al., 2018). In contrast, Roepstorff et al. (1998) reported *Eimeria* spp. prevalence rates of 7% in Finland, 1% in Norway, and 8% in Sweden. Our study, however, found a higher prevalence of 37% (Table 4). Likewise, a study conducted in Poland on piglets revealed a coccidiosis prevalence of 31.4% (Kochanowski et al., 2017), while our study recorded a higher prevalence of 60% (Table 4). An investigation spanning African countries such as South Africa, Ethiopia, and Rwanda, recorded a prevalence of *Eimeria* spp. infection ranging from 5.6% to 88% across all age groups, with the highest prevalence in piglets, followed by fatteners and sows (Abdu and Gashaw, 2010; Nwafor et al., 2019; Tumusiime et al., 2020).

Oesophagostomum spp. was identified in all three age categories, with the highest prevalence in sows (37%), followed by fatteners (29%), and piglets (27%) (Table 3). Similar findings were observed in other countries such as Greece (sows-5.2%), Netherlands (piglets-12.5%, sows-37.5%), Poland (3.44-20.8%), South Africa (piglets-8%, sows-43.8%), and Ethiopia (6.75%) (Eijck et al., 2005; Abdu and Gashaw, 2010; Knecht et al., 2011; Nwafor et al., 2019; Symeonidou et al., 2020).

The presence of infections with potentially zoonotic parasites such as *B. coli*, *A. suum*, and *T. suis*, justifies their surveillance and control under the vision of the "One health, one medicine" concept. Nevertheless, further research is needed for a better understanding of the epidemiology of these infections in pigs raised in low-input, occasionally free-range systems, from our country.

CONCLUSIONS

The present study was conducted during the winter and spring seasons of 2021-2022, and involved a total of 135 pigs from 14 smalholders of the local population, located in the villages of Iacobeni commune, in the Hartibaciu Valley area, Sibiu County. The animals were raised in low-input systems, and occasionally had access to the outdoor environment (free-range).

In piglets, the most prevalent parasite was *Eimeria* spp. (60%), followed by *A. suum*, *Oesophagostomum spp*., *B. coli*, and *T. suis.* Among fatteners, the highest prevalence was recorded for *B. coli* (49%), followed by *A. suum*, *Oesophagostomum* spp., *Eimeria* spp., and *T. suis*. In sows, the highest prevalence was for *B. coli* (43%) infection, followed by *Oesophagostomum* spp., *A. suum*, and *Eimeria* spp.

Despite the significant prevalence and intensity levels of parasitic infections, all pigs were clinically asymptomatic, and no correlation was observed between parasitic infections and clinical manifestation.

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STUDY ON THE REPRODUCTIVE PERFORMANCE AND MILK PRODUCTION OF DAIRY BUFFALOES FROM THE CENTRAL AREA OF ROMANIA

STUDIU PRIVIND PERFORMANȚELE DE REPRODUCȚIE ȘI ALE PRODUCȚIEI DE LAPTE LA BIVOLIȚELE DIN ZONA DE CENTRU A ROMÂNIEI

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Abstract

Buffaloes raised in Romania have mixed productive abilities, milk-meat, the main ability is for milk production. In the center of the country, buffalo breeding has a long tradition of over a century. Buffalo milk has great nutritional value, lends itself very well to processing into specific products. On December 1, 2021, there were 18,328 buffaloes, of which 97% of the herds were in the center and northwest of Transylvania. In the center of the country, 5,388 heads are raised, in 592 farms of different sizes, which represents 29.40% of the total herd. The research carried out during six years, in ten farms, on a herd of 396 dairy buffaloes raised in the central area of Romania, revealed that the average interval between calvings is 438.04 days, with a high variability of the character in the population. This result is influenced by the average duration of the service period of 123.93 days, with V=89.06%, the average duration of gestation being relatively constant. The duration of lactation was influenced by the age of the animals and technological factors. The average amount of milk, fat and protein per total lactation, as well as the average content in fat and protein recorded different average values, being influenced by the rank of lactation. From the analysis of the milk production data recalculated at 270 days, it follows that there are no significant differences compared to the milk production per total lactation. The explanation lies in the fact that the total duration of lactation is not much longer than 270 days.

Key words: buffalo, milk production, farms, milking, fat, protein, reproduction

Rezumat

Bivolii crescuți în România au aptitudini productive mixte, de lapte-carne, aptitudinea principală este pentru producerea laptelui. În centrul țării creșterea bivolilor are o tradiție îndelungată, de peste un veac. Laptele de bivoliță are valoare nutritivă deosebită, se pretează foarte bine la procesare în produse specifice. La 01 decembrie 2021 existau 18328 bivoli, din care 97% din efective fiind în centrul şi nord-vestul Transilvaniei.. În centrul țării se cresc 5388 capete, în 592 ferme de diferite dimensiuni, ceea ce reprezintă 29,40% din efectivul total. Cercetările derulate pe parcursul a șase ani, în zece ferme, pe un efectiv de 396 bivolițe crescute în zona de centru a României, a relevat că intervalul mediu între fătări este de 438,04 zile, cu o variabilitate mare a caracterului în populație. Acest rezultat este influențat de durata medie a service-periodului de 123,93 zile, cu V=89,06%, durata medie a gestației fiind relativ constantă. Durata lactației a fost influențată de vârsta animalelor și de factorii tehnologici. Cantitatea medie de lapte, grăsime și proteină pe lactație totală, cât și conținutul mediu în grăsime și proteină a înregistrat valori medii diferite, fiind influențate de rangul lactației. Din analiza datelor producției de lapte recalculată la 270 zile, rezultă că nu sunt diferențe semnificative față de producția de lapte pe lactație totală. Explicația constă în faptul că durata totală a lactației nu este cu mult mai mare de 270 zile.

Cuvinte cheie: bivoli, producție de lapte, ferme, muls, grăsime, proteine, reproducție

INTRODUCTION

At the origin of the Romanian Buffalo is the wild Indian buffalo (*Bubalus arni*), but also the domestic one - the common buffalo (*Bubalus microceros*). From a genetic and geographical point of view, the Romanian buffalo is part of the river type, considered the common riparian buffalo. Regarding the period of entry of buffaloes into Romania, several assumptions are made. Information about buffaloes in Romania, since ancient times, is scarce. In Porumbacu (Sibiu county), 35 buffaloes and female buffaloes are recorded in the live inventory of the estate from 1632 (Mateș, 1974). Over time, since entering the country, the Romanian buffalo has been isolated from a reproductive point of view, through "geographical enclavization", determined by state administrative borders, acclimatizing in Transylvania where the climate is harsher and in the Danube Plain. Until 1990, the buffalo herds in Romania were maintained at an approximately constant level, they being considered a safe population, raised in Agricultural Production Cooperatives, State Agricultural Enterprises and households of the population. Thus, the animals were registered, infusion crosses were made with mixed-breed Murrah bulls imported from Bulgaria, the breed was approved in 1987, with the name Romanian Bubaline Breed, presenting distinct morphological and productive characteristics, for which the breeding program was also developed and the genealogical register was opened. The highest number of buffaloes in the country was in 1980 (228,000 buffaloes). After 1990, Romania's buffalo population decreased, as did most species of domestic animals, reaching 18,826 heads in 2014. The main causes of the numerical reduction of buffalo herds, after 1990, were multiple, of which we specify the massive slaughters, the abusive trade in animals, the retention of a small number of replacement female youth and the advanced age of the buffalo breeders in the rural area. Buffaloes raised in our country have mixed productive skills, milk-meat, the main skill is for milk production and it is spread in two large areas: Transylvania and the Danube Plain. In the center of the country, buffalo breeding has a long tradition of over a century. In the period 1899-1908, the average milk production of buffaloes in the area of Făgăraș, in the town of Sâmbăta was 1300 kg of milk/year. From the ranking of the best buffaloes, it can be seen that the total duration of lactation varied between 256 and 303 days, the amount of milk recorded values between 1398-1484 kg milk/lactation, with 7.28-11.21% fat (Rostafinski, 1912). On December 1, 2021, there were 18,328 buffalo heads in Romania, of which 14,060 were females for reproduction, and 4,268 youth heads (INS), 97% of the herd being in the center and north-west of Transylvania, mainly in the counties of Brasov, Sibiu, Cluj and Salaj. In the center of the country (Brașov and Sibiu counties) 5388 head are raised, in 592 farms of different sizes, which represents 29.40% of the total herd raised in the country (Genealogical Register of the Romanian Buffalo breed).

Considering the aspects specified above, the purpose of this work was to know thoroughly and realistically the parameters of reproduction and production of buffaloes in the central area of Romania.

MATERIAL AND METHOD

The existence in the central area of Romania of some buffalo breeding farms with herds of more than 20 heads made the research in the present study possible. At the same time, the grouping of large herds of buffaloes in a small area also offered the opportunity for research to be carried out in all farms in a short period of time, eliminating the errors given by the general and special environmental conditions.

The research took place over six years, in ten farms, on a herd of 396 buffaloes. The study of the reproductive parameters was carried out by analyzing the data from the passports of the females, from the individual animal reports (SNIIA), the breeding and calving register of the farms and from the Genealogical Register of the **Romanian Buffalo** breed. The study of the parameters of milk production in buffaloes was carried out by performing the control of milk production at intervals between 38-46 days, method (B6), by recording the amount of milk (kg). The quality parameters of the milk (fat and

protein content) were determined using the portable milkoscanner. For this purpose, 1483 completed lactations were analyzed. The rank of lactations was from lactation 1 to lactation 13. Milk production, in the case of each lactation, was recalculated as equivalent to 270 days (ICAR, 2017). At the same time, the data from the breeding and calving register of the farms and from the Genealogical Register for the Romanian Buffalo breed were analyzed. The obtained data were statistically processed and interpreted. In order to determine the main statistical indicators (mean, mean error, standard deviation and variability) a simple variance analysis was used.

RESULTS AND DISCUSSION

The analysis of reproductive rhythmicity in buffaloes takes into account the following indicators of interest, such as the gestation period (DG), the calving interval (CI) and the service period (SP). Calving interval is the interval in days between two successive calvings. The duration of the calving interval can be calculated according to the formula CI = SP+DG. For buffaloes, the service-period represents the interval from calving to the fertile mount and can be calculated according to the formula SP = dif-df, in which, dif is the interval from calving-insemination to the fertile mount, expressed in days and df is the calving date. Gestation duration represents the interval between fecund mounting/insemination and calving, expressed in days. The duration of gestation in buffaloes ranges between 300-330 days, with an average of 315 days.

From the analysis of reproduction parameters with implications on rhythmicity, in the farms studied, the values presented in table 1 resulted.

Table 1. Reproduction indices for dairy buffaloes in the analyzed farms / Indici de reproducție a bivolilor de lapte în fermele analizate

Specification	Total farms		
	n	$\overline{X} \pm S^2_{\overline{X}}$	V%
Gestation duration – DG (days)	396	314.10±0.22	1.50
Calving interval - CI (days)	396	438.04±6.30	30.88
Service period – SP (days)	396	123.93±6.30	89.06

An average calving interval on 396 buffaloes of 438.04±6.30 days is noted, with a high variability (V=30.88%), which indicates a heterogeneous population. This result is influenced by the average duration of the service period (123.93±6.30 days) and the very high variability (89.06%), the average duration of gestation being relatively constant (314.10±0.22 days), with a very small variability (1.50%). In order to have a more accurate picture of the duration of the interval between calvings and the service period calculated in the present study, we make the comparison with the average values determined in other buffaloes. Thus, in other studies (Vidu, 2008), on 686 buffaloes from other areas of the country, the average calving interval was 486.33 days and the service period was 171.33 days.

The study shows that the duration of the service period is influenced by the heat detection rate. For a very good reproduction activity of the buffaloes, it is necessary that the service period has a duration of up to 60 days.

The characterization of buffaloes in terms of milk production was carried out by studying three parameters, namely: the duration of lactation, the amount of milk milked, and the quality of the milk in terms of fat and protein content. The results of the milk production study are shown in table 2 (n = heads analyzed; ± = mean and standard error of the character; DL = duration of lactation; CL = average amount of milk in kg; CG = amount of fat in kg; CP = amount of protein; % G = fat content %; % P = protein content in %).

The research reveals that the average duration of lactations in buffaloes did not show significant differences between farms even from one rank to another, the variability of the character being below 10% (table 2, chart 1). The longest average duration of lactation was recorded in the 6th lactation, and

the lowest in the 8th - 13th lactations. An increase in the duration of lactation is observed from lactation I to lactation VI, followed by a decrease until lactation 13. The duration of lactation in buffaloes from the studied farms was influenced by the age of the animals as well as by technological factors.

The average amount of milk per total lactation in the buffaloes from the farms analyzed, recorded different average values, being influenced by the rank of lactation (table 2, chart 2). At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant.

Table 2. Productive parameters for lactations 1 - 13, recorded in dairy buffaloes from 10 farms / Parametri productivi pentru lactațiile 1 - 13, înregistrați la bivolițele de lapte din 10 ferme

Specification			First lactation			Second lactation			Third lactation			Fourth lactation		
			n	$X \pm S^2_x$	V	n	$X \pm S^2_x$	V%	n	$X \pm S^2_x$	V	n	$X \pm S^2_x$	V
Centralizer 10 farms	Total milk production	DL	225	275.68 ±1.03	5.58	201	280.81 ±1.24	6.27	211	288.86 ±1.42	7.12	172	294.17 ±1.81	8.07
		CL	225	1615.11 ±9.68	8.99	201	1725.95 ±10.78	8.85	211	1837.18 ±11.99	9.48	172	1939.18 ±12.17	8.23
		CG	225	120.28 ±0.70	8.79	201	128.79 ±0.81	8.90	211	137.49 ±0.91	9.64	172	145.55 ±0.93	8.37
		%G	225	7.49 ±0.01	1.34	201	7.50 ±0.01	1.24	211	7.51 ±0.01	1.12	172	7.51 ±0.01	1.14
		CP	225	67.97 ±0.40	8.72	201	72.22 ±0.44	8.56	211	77.12 ±0.52	9.83	172	81.55 ±0.53	8.56
		%P	225	4.23 ±0.01	1.80	201	4.22 ±0.00	1.62	211	4.21 ±0.00	1.48	172	4.20 ±0.00	1.55
	Milk production for 270 days	CL	225	1578.42 ±7.26	6.90	201	1680.87 ±8.95	7.55	211	1763.42 ±9.53	7.85	172	1850.18 ±9.84	6.97
		CG	225	118.19 ±0.55	7.01	201	125.96 ±0.68	7.66	211	132.37 ±0.73	8.00	172	138.97 ±0.76	7.14
		%G	225	7.48 ±0.01	1.34	201	7.49 ±0.01	1.31	211	7.50 ±0.01	1.15	172	7.50 ±0.01	1.16
		CP	225	66.80 ±0.30	6.66	201	70.71 ±0.36	7.23	211	74.32 ±0.42	8.18	172	77.90 ±0.43	7.31
		%P	225	4.23 ±0.01	1.80	201	4.21 ±0.00	1.64	211	4.21 ±0.00	1.46	172	4.20 ±0.00	1.53
			Lactation 5			Lactation 6			Lactation 7			Lactations 8 - 13		
	Total milk production	DL	130	299.46 ±2.19	8.35	112	295.29 ±2.11	7.56	90	279.23 ±1.62	5.50	342	272.41 ±0.83	5.62
		CL	130	1953.58 ±14.53	8.48	112	2024.47 ±14.80	7.74	90	1722.94 ±19.41	10.69	342	1552.12 ±8.15	9.72
		CG	130	146.15 ±1.13	8.84	112	151.97 ±1.12	7.80	90	128.82 ±1.49	10.94	342	116.55 ±0.60	9.58
		%G	130	7.50 ±0.01	1.25	112	7.51 ±0.01	1.14	90	7.49 ±0.01	1.21	342	7.50 ±0.01	1.34
		CP	130	82.04 ±0.66	9.22	112	85.26 ±0.63	7.85	90	72.63 ±0.86	11.20	342	65.88 ±0.32	8.98
		%P	130	4.19 ±0.00	0.95	112	4.20 ±0.01	1.64	90	4.22 ±0.01	1.42	342	4.24 ±0.00	1.80
	Milk production for 270 days	CL	130	1841.35 ±11.21	6.94	112	1922.57 ±12.71	7.00	90	1678.88 ±16.18	9.14	342	1534.80 ±6.81	8.22
		CG	130	137.84 ±0.88	7.26	112	144.44 ±0.95	6.96	90	125.95 ±1.26	9.50	342	115.38 ±0.50	8,10
		%G	130	7.49 ±0.01	1.27	112	7.50 ±0.01	1.09	90	7.49 ±0.01	1.17	342	7.50 ±0.01	1.34
		CP	130	77.35 ±0.51	7.45	112	80.96 ±0.55	7.20	90	71.04 ±0.73	9.72	342	65.22 ±0.26	7.40
		%P	130	4.19 ±0.00	0.97	112	4.19 ±0.01	1.63	90	4.21 ±0.01	1.43	342	4.24 ±0.00	1.80

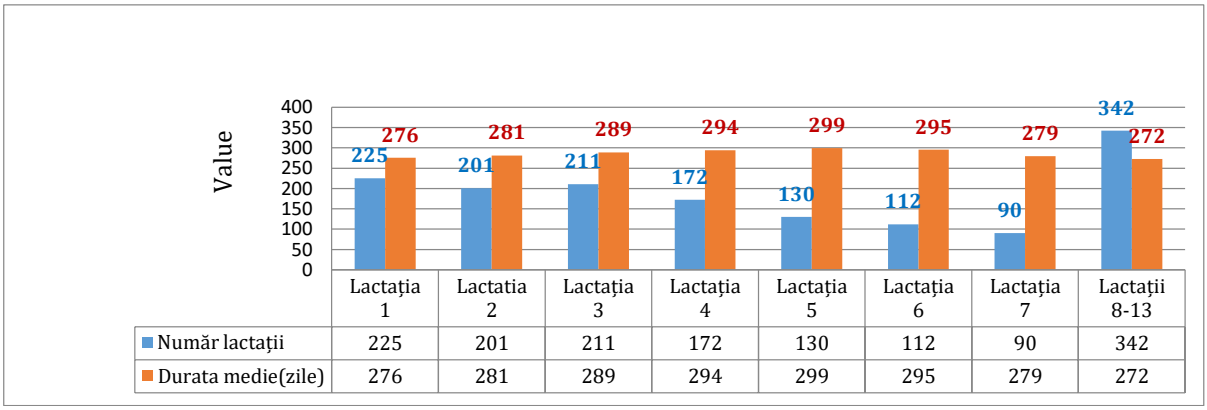
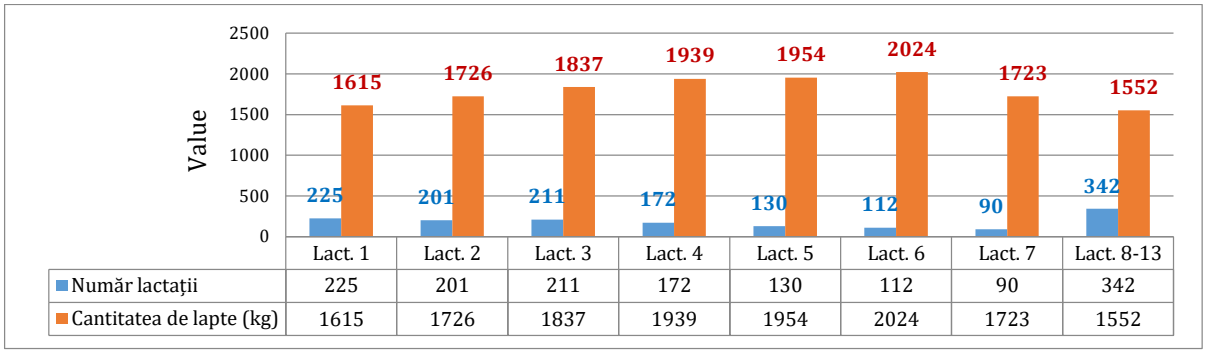


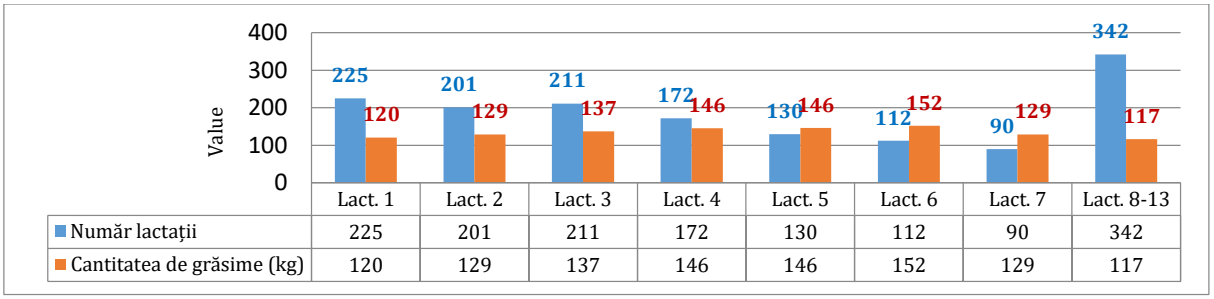
Chart 1. Evolution of lactation duration in dairy buffaloes / Evoluția duratei lactației la bivolițele de lapte



	Lact. 1	Lact. 2	Lact. 3	Lact. 4	Lact. 5	Lact. 6	Lact. 7	Lact. 8-13
Număr lactații	225	201	211	172	130	112	90	342
Cantitatea de lapte (kg)	1615	1726	1837	1939	1954	2024	1723	1552

Chart 2. The average amount of milk per total lactation in dairy buffaloes from the analyzed farms / Cantitatea medie de lapte per lactație totală la bivolițele de lapte din fermele analizate

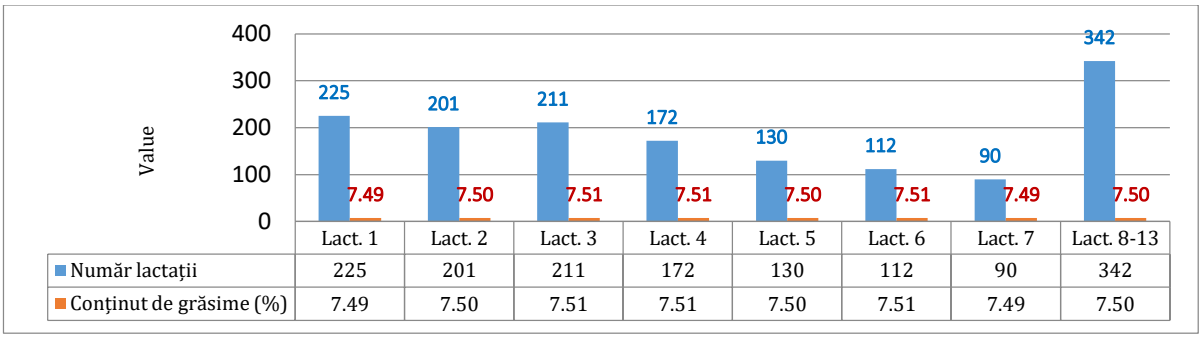
The average amount of fat per total lactation in buffaloes from the farms analyzed, recorded different average values, being influenced by the rank of lactation (table 2, chart 3).



	Lact. 1	Lact. 2	Lact. 3	Lact. 4	Lact. 5	Lact. 6	Lact. 7	Lact. 8-13
Număr lactații	225	201	211	172	130	112	90	342
Cantitatea de grăsime (kg)	120	129	137	146	146	152	129	117

Chart 3. The average amount of total lactation fat in dairy buffaloes from the farms analyzed / Cantitatea medie de grăsime per lactație totală la bivolițele de lapte din fermele analizate

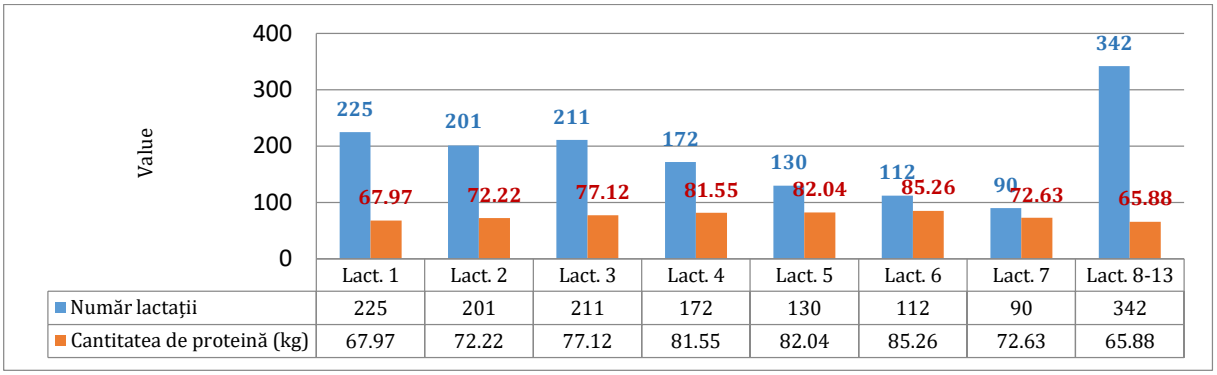
At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant. We specify the same conclusion for the fat content per total lactation (table 2, chart 4). At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant.



	Lact. 1	Lact. 2	Lact. 3	Lact. 4	Lact. 5	Lact. 6	Lact. 7	Lact. 8-13
Număr lactații	225	201	211	172	130	112	90	342
Conținut de grăsime (%)	7.49	7.50	7.51	7.51	7.50	7.51	7.49	7.50

Chart 4. The average milk fat content per total lactation in dairy buffaloes from the analyzed farms / Conținutul mediu de grăsime din lapte pe lactație totală la bivolițele de lapte din fermele analizate

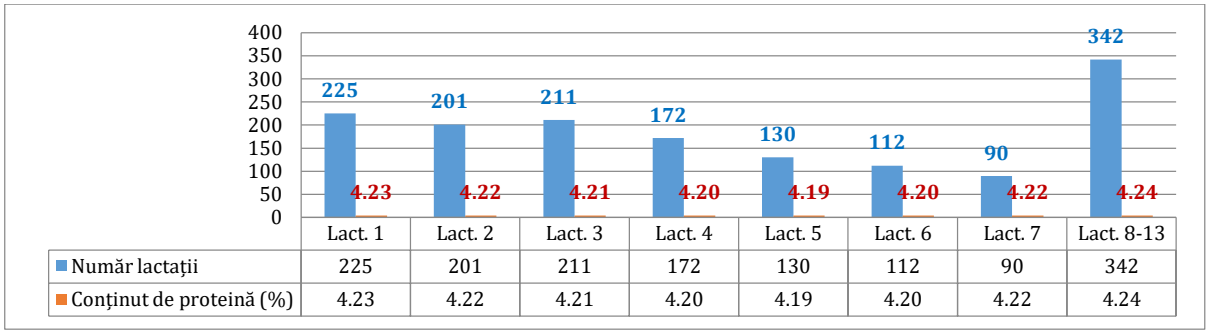
The average amount of protein per total lactation in buffaloes from the farms analyzed, recorded different average values, being influenced by the rank of lactation (table 2, chart 5). At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant.



	Lact. 1	Lact. 2	Lact. 3	Lact. 4	Lact. 5	Lact. 6	Lact. 7	Lact. 8-13
Număr lactații	225	201	211	172	130	112	90	342
Cantitatea de proteină (kg)	67.97	72.22	77.12	81.55	82.04	85.26	72.63	65.88

Chart 5. The average amount of protein per total lactation in dairy buffaloes from the analyzed farms / Cantitatea medie de proteine pe lactație totală la bivolițele de lapte din fermele analizate

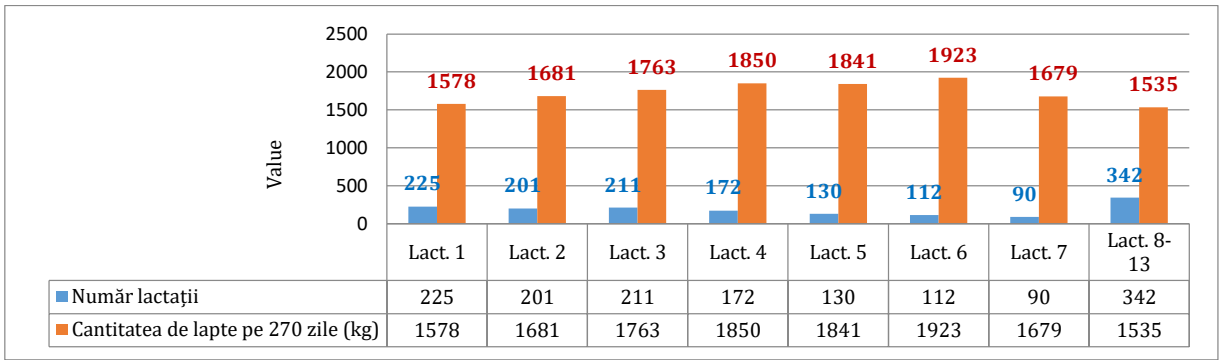
The results for the average protein content of milk per total lactation (table 2, chart 6). At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant.



	Lact. 1	Lact. 2	Lact. 3	Lact. 4	Lact. 5	Lact. 6	Lact. 7	Lact. 8-13
Număr lactații	225	201	211	172	130	112	90	342
Conținut de proteină (%)	4.23	4.22	4.21	4.20	4.19	4.20	4.22	4.24

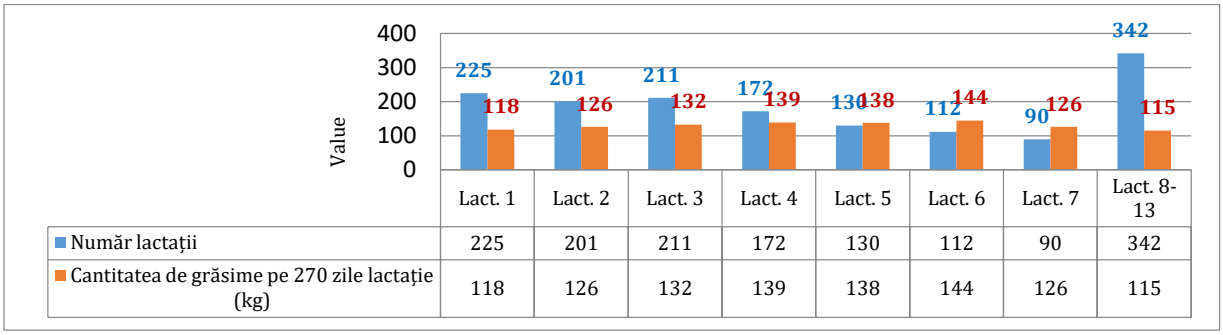
Chart 6. The average milk protein content per total lactation in dairy buffaloes from the analyzed farms / Conținutul mediu de proteine din lapte pe lactație totală la bivolițele de lapte din fermele analizate

From the analysis of milk production data on lactations 1-13, recalculated at 270 days (table 2, charts 7-11), it follows that there are no significant differences compared to milk production on total lactation. The explanation lies in the fact that the total duration of lactation is not much longer than 270 days, the highest average duration of lactation being recorded in the 6th lactation.



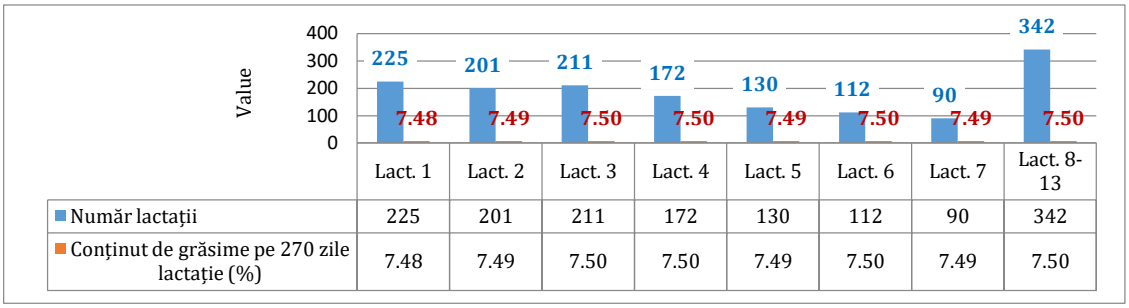
	Lact. 1	Lact. 2	Lact. 3	Lact. 4	Lact. 5	Lact. 6	Lact. 7	Lact. 8-13
Număr lactații	225	201	211	172	130	112	90	342
Cantitatea de lapte pe 270 zile (kg)	1578	1681	1763	1850	1841	1923	1679	1535

Chart 7. The average amount of milk per 270 days lactation in dairy buffaloes from the farms analyzed / Cantitatea medie de lapte la 270 de zile de lactație la bivolițele din fermele analizate



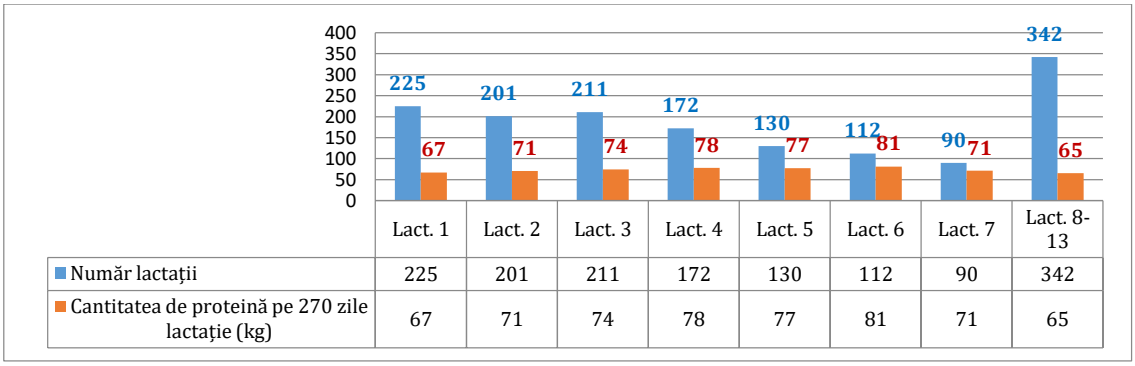
	Lact. 1	Lact. 2	Lact. 3	Lact. 4	Lact. 5	Lact. 6	Lact. 7	Lact. 8-13
Număr lactații	225	201	211	172	130	112	90	342
Cantitatea de grăsime pe 270 zile lactație (kg)	118	126	132	139	138	144	126	115

Chart 8. The average amount of fat per 270 days of lactation in buffaloes from the analyzed farms / Cantitatea medie de grăsime la 270 de zile de lactație la bivolițele din fermele analizate



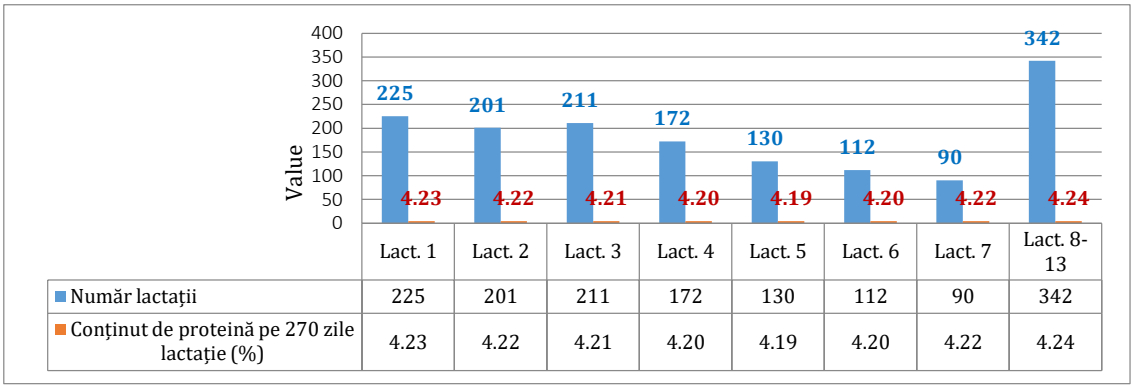
	Lact. 1	Lact. 2	Lact. 3	Lact. 4	Lact. 5	Lact. 6	Lact. 7	Lact. 8-13
Număr lactații	225	201	211	172	130	112	90	342
Conținut de grăsime pe 270 zile lactație (%)	7.48	7.49	7.50	7.50	7.49	7.50	7.49	7.50

Chart 9. The average milk fat content per 270 days lactation in dairy buffaloes from the analyzed farms / Conținutul mediu de grăsime din lapte pe 270 de zile de lactație în lactatele de bivoli din fermele analizate



	Lact. 1	Lact. 2	Lact. 3	Lact. 4	Lact. 5	Lact. 6	Lact. 7	Lact. 8-13
Număr lactații	225	201	211	172	130	112	90	342
Cantitatea de proteină pe 270 zile lactație (kg)	67	71	74	78	77	81	71	65

Chart 10. The average amount of protein per 270 days lactation in dairy buffaloes from the farms analyzed / Cantitatea medie de proteine pe 270 de zile de lactație în lactatele de bivoli din fermele analizate



	Lact. 1	Lact. 2	Lact. 3	Lact. 4	Lact. 5	Lact. 6	Lact. 7	Lact. 8-13
Număr lactații	225	201	211	172	130	112	90	342
Conținut de proteină pe 270 zile lactație (%)	4.23	4.22	4.21	4.20	4.19	4.20	4.22	4.24

Chart 11. The average milk protein content per 270 days lactation in buffaloes from the analyzed farms / Conținutul mediu de proteine din lapte pe 270 de zile de lactație la bivolițele din fermele analizate

CONCLUSIONS

The average interval between calvings in 396 buffaloes dairy raised in the central area of Romania was 438.04±6.30 days, with a high character variability in the analyzed population (V=30.88%). This result is influenced by the average duration of the service period (123.93±6.30 days, with V=89.06%), the average duration of gestation being relatively constant (314.10±0.22 days), with a very low variability (1.50%). The research undertaken reveals that the average duration of lactations in buffaloes did not show significant differences between farms even from one rank to another, the variability of the character being below 10%. The longest average duration of lactation was recorded in the 6th lactation, and the lowest in the 8th-13th lactations. An increase in lactation duration is observed from lactation I to lactation VI, followed by a decrease until lactation XIII. The duration of lactation in the buffaloes from the studied farms was influenced by the age of the animals as well as by the technological factors. The average amount of milk per total lactation in buffaloes recorded different average values, being influenced by the rank of lactation. At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant. The average amount of fat per total lactation recorded different average values, being influenced by the rank of lactation. At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant. We specify the same conclusion for the content in fat and protein per total lactation. At the same lactation rank, the variability of the character is below 10%, the differences between the average amounts of milk per lactation between farms being insignificant. The results for the average fat and protein content of milk, at the same stage of lactation, have a variability of less than 10%, the differences between the average amounts of milk per lactation between farms being insignificant. From the analysis of milk production data on lactations 1-13, recalculated at 270 days, it follows that there are no significant differences compared to milk production on total lactation. The explanation lies in the fact that the total duration of lactation is not much longer than 270 days, the highest average duration of lactation being recorded in the 6th lactation.

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STUDY ON BUFFALO BREEDING FOR MILK PRODUCTION IN THE CENTRAL REGION OF ROMANIA

STUDIU PRIVIND CREȘTEREA BIVOLILOR PENTRU PRODUCȚIA DE LAPTE ÎN REGIUNEA CENTRALĂ A ROMÂNIEI

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Abstract

Raising buffaloes for milk production is a tradition in many areas of the world, but also in Romania. Buffalo milk has great nutritional value, lends itself very well to processing into specific products. From a hygienic point of view, the number of somatic cells is lower than in taurines. Although the world population of buffalo showed a general upward trend, in Romania it decreased significantly in number during the same period. On December 1, 2021, there were 18,328 buffaloes, of which 14,060 females for reproduction, and 4,268 youth, 97% of the herd being in the center and northwest of Transylvania.. In the center of the country, 5,388 heads are raised, in 592 farms of different sizes, which represents 29.40% of the total workforce. In small farms, the focus should be on increasing individual milk production and increasing marketable production. In medium-sized farms, the specific recommended measures are to increase the herd of buffaloes, respectively from medium to large farms. For farms specialized in raising buffaloes cows, it is recommended to increase the individual milk production through selective reform and the retention of plus variants from the own pen, the application of optimized rations, artificial insemination, the use of MSC from bulls with superior breeding value for milk production, monitoring reproductive activity and increasing udder skills for mechanical milking.

Key words: buffaloes, milk production, farms, milking, feeding, breeding

Rezumat

Creșterea bivolilor pentru producția de lapte este o tradiție în multe zone ale lumii, dar și în România. Laptele de bivoliță are o valoare nutritivă mare și se pretează foarte bine procesării în produse specifice. Din punct de vedere igienic, numărul de celule somatice este mai scăzut decât la taurine. Deși populația mondială de bivoli a înregistrat o tendință generală ascendentă, în România aceasta a scăzut semnificativ ca număr în aceeași perioadă. La 1 decembrie 2021 erau 18.328 de bivoli, din care 14.060 femele pentru reproducție și 4.268 capete tineret, 97% din efective fiind localizate în centrul și nord-vestul Transilvaniei. În centrul țării sunt crescute 5.388 capete, în 592 ferme de diferite dimensiuni, ceea ce reprezintă 29,40% din totalul activității. În fermele mici, accentul ar trebui să fie pe creșterea producției individuale de lapte și pe creșterea producției comercializabile. În fermele de mărime medie, măsurile specifice recomandate sunt creșterea efectivului de bivoli, respectiv de la ferme medii spre mari. Pentru fermele specializate în creșterea bivolilor, se recomandă creșterea producției individuale de lapte prin reforma selectivă și reținerea plus variantelor din propriul efectiv, aplicarea de rații optimizate, inseminarea artificială, utilizarea MSC de la tauri cu valoare de reproducție superioară pentru producția de lapte, monitorizarea activității reproductive și creșterea abilităților ugerului pentru mulsul mecanic.

Cuvinte cheie: bivoli, producție de lapte, ferme, muls, hrănire, creștere

INTRODUCTION

Buffalo milk production is a tradition in many areas of the world such as the Caucasus, Asia, where sour milk, butter and yogurt from buffalo milk are very popular. In Italy, the buffalo milk industry is flourishing due to the popularity of mozzarella cheese. The large market for mozzarella cheese transforms buffalo farms into profitable enterprises and they can rely on modern exploitation techniques

and technologies in an organized manner. In South America, in countries such as Brazil and Argentina, buffaloes are raised for both milk and meat production, and more recently, buffalo milk and buffalo milk products have become very popular, and buffalo milk production has also reached countries that previously had no tradition in this production, such as Germany, Great Britain, and even the USA. Buffalo milk has a special nutritional value, being 58% richer in calcium than cow's milk, and the cholesterol content is 43% lower. It lends itself very well to processing into specific products (yoghurt, sour cream, Mozzarella, Camembert, Telemea cheeses). From a hygienic point of view, the number of somatic cells is lower, as a result of the lower risk of mastitis. In a study carried out in India, regarding buffalo milk compared to cow's milk, published in the Indian Dairy Industry magazine, the following was specified: from the point of view of cholesterol, its content in buffalo milk is 0.65 mg/g milk compared to cow's, which contains 3.14 mg/g milk. Much more buffalo milk is produced and marketed in India, and a number of its qualities make it more favorable for consumption. The analyzes carried out on buffalo milk showed that the value of the protein efficiency rate (Protein Efficiency Ratio) is 2.74 compared to that of cow's milk, which is 2.49. Thus, buffalo milk has approximately 11.42% more protein than cow milk. Buffalo milk is superior to cow's milk also in terms of mineral content, especially calcium, iron, potassium, which are approximately 92%, 37.7% and 118% higher than the content in cow's milk. The buffalo metabolizes carotene-rich products much better, which it completely metabolized into vitamin A, which will then pass into the milk, resulting in a greater amount of vitamin A in the buffalo's milk. Commercialization of buffalo milk is more viable than cow's milk in terms of manufactured products such as butter, a special product of buffalo milk, due to its low water content and high fat content. Very important is the low cholesterol content, which makes it much more popular on the market of healthy food products.

On the territory of Romania, buffaloes have been bred since the 4th-5th centuries, entering in two ways: from the Balkan Peninsula, being brought by the Turks and spread in the Romanian Plain and Dobrogea, and from the west, being brought by the Huns and spread by the Avars, in especially in the 9th and 12th centuries. The first written information was found in the town of Porumbarul de Jos and referred to the buffaloes from the Făgăraș region, and the first selection nucleus was formed in Șercaia (1870-1879). The chronicler Miron Costin reveals the importance given to buffaloes, especially in armed confrontations, when they were used to tow massive cannons, as a result of the impressive strength they developed. Although the total world population of buffalo showed a general upward trend, the population of buffalo in Romania decreased significantly in number during the same period. The strongest decrease occurred after 1990, being caused by several factors: the decrease in the importance of buffaloes in milk production (the demand for milk with a high fat percentage decreased); the decrease in the importance of buffaloes in labor production (mainly due to the degree of mechanization of work); the lack of support policies in the field of buffalo breeding; holsteinization of milk production.

On December 1, 2021, there were 18,328 buffalo heads in Romania, of which 14,060 were females for reproduction, and 4,268 youth heads (INS), 97% of the herd being in the center and north-west of Transylvania, mainly in the counties of Brasov, Sibiu, Cluj and Salaj. In the center of the country (Brașov and Sibiu counties) 5388 head are raised, in 592 farms of different sizes, which represents 29.40% of the total herd raised in the country (Genealogical Register of the Romanian Buffalo breed). Studies related to the economic efficiency of buffalo breeding, by type of farm, in Romania are insufficient. As a result, through this study, we aimed to analyze milk production, its destination, the feeding and milking technology used, as well as the way to ensure animal welfare, in farms of different sizes, from the most representative growing area in country, to formulate recommendations to increase milk production.

MATERIAL AND METHOD

This scientific study was carried out in 48 buffalo farms, in 10 localities in Brașov and Sibiu counties. In order to make relevant comparisons between holdings, three groups of farms were created, namely the group of small farms (1-5 heads), medium farms (6-19 heads) and large farms (over 20 heads). Of the 48 farms analyzed, 50% were medium-sized farms, 29% large-sized farms and 21% small-sized farms or households.

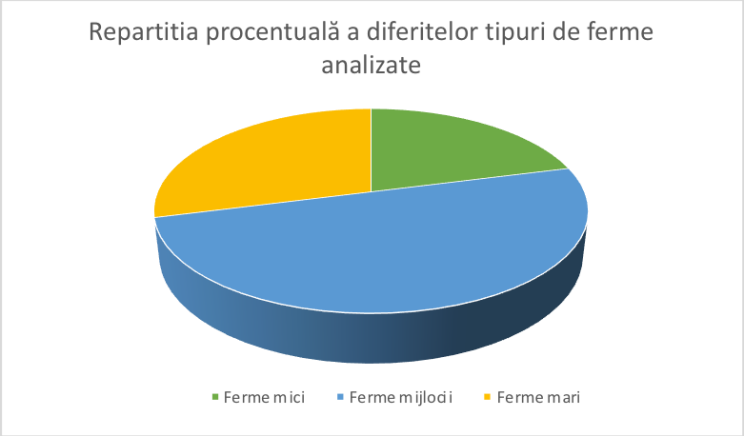


Chart 1. Breakdown of farm types analyzed / Repartiția tipurilor de ferme analizate

The working method applied was based on the application of questionnaires to all farmers participating in the study. The questions in the content of the questionnaire mainly related to the buffalo herd, broken down by age category, the amount of milk recorded daily and how the milk obtained on the farm is used. Thus, information was obtained regarding the consumption of buffalo milk at the farm level (own consumption and consumption for calves) and the amount of milk processed on the farm, delivered as raw material or as fresh milk and distributed through milk vending machines. Also, direct analyzes were made regarding the feeding technology used in the farm, the types of shelters and the way to ensure well-being at the farm level, as well as the applied milking technology. For the statistical evaluation of the indicators in the analyzed farms, the average differences between the results of the collected samples were expressed as a function of the standard deviation (SD) and were interpreted using the analysis of variance.

RESULTS AND DISCUSSION

Analysis of buffalo rearing in small-scale farms. In this first group, of small farms, there are 5 analyzed households. The total number of buffaloes in these farms varies between 6 and 10 heads, because along with the category of milk buffaloes there are also young animals. The average number of buffaloes was 4.6 heads, with ranges between 3 and 5 heads. The average amount of milk obtained per day is 8.4 liters of milk, with variations between 7 and 10 liters (table 1).

Table 1. Synthetic indicators in small farms / Indicatorii sintetici în fermele mici

Specification	Number of farms	$X \pm S_x$	MAX	Min	S	V%
Number buffaloes on exploitation	10	4.6±0.4	5	3	0.89	10
Amount of milk per head of dairy buffalo per day	10	8.4±0.5	10	7	1.14	13
The total amount of milk obtained on the farm per day	10	38±2.54	45	30	5.7	15
Own consumption of milk in the farm or household	10	2±0.03	-	-	0.2	3
Milk consumption for calves	10	18.8±5.9	40	4	5.9	15
The amount of milk processed	10	22.5±0.8	26	0	1.48	14

On average, in the analyzed farms, a quantity of 38 liters of buffalo milk is obtained. It is found that farmers do not consume large amounts of milk for their own food, but a significant amount is used to feed calves (18.8 kg). Regarding the category of milk intended for processing, we note that the milk is capitalized as such by selling it at vending machines located in cities. The selling price is 6 lei/liter (chart 2).

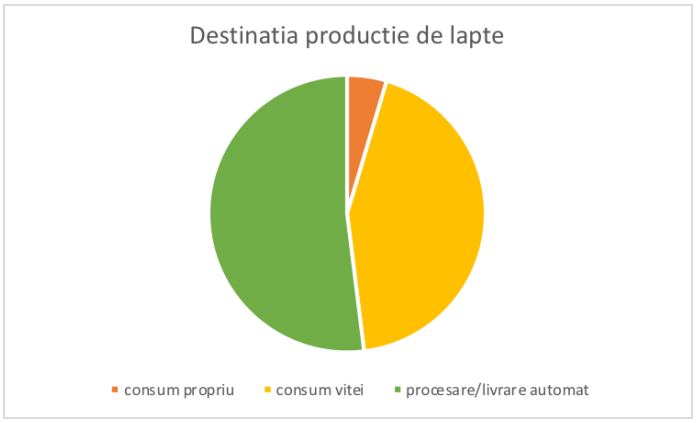


Chart 2. Distribution of milk production in small farms / Distribuția producției de lapte în fermele mici

From the analysis of the 10 holdings, a household was distinguished with 7 buffalo heads, respectively 3 lactating dairy buffaloes, which have an average daily production of 10 liters of milk, the amount of milk intended for the calves is very small, and the largest amount is delivered to vending machines sale of milk (chart 3).

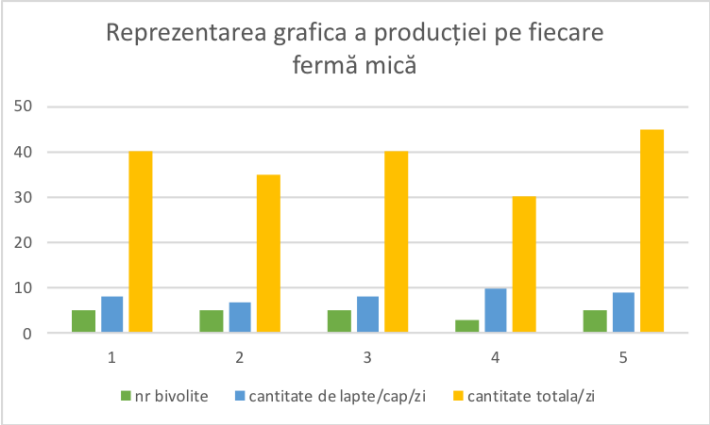


Chart 3. Distribution of milk production on 5 analyzed farms / Distribuția producției de lapte în 5 ferme analizate

In these small holdings, the work is done by family members. Buffalo breeding is carried on in a traditional system, the animals being kept in common stables with other animals (cows, sheep, pigs). The shelters have very limited interior arrangements, namely stands for rest and mangers for the administration of fodder during the winter period. Animals are kept in tethered system. As for feeding, it is carried out seasonally. During the summer season, the animals are kept on pasture and consume green mass ad libitum . During the winter, the buffaloes receive hay (8 kg), corn or beet silage (20 kg), roughage (4 kg) and, in some cases, concentrates (2 kg). The dairy buffaloes are milked manually, with the exception of one holding where the mobile milking group is used, equipped with a milking machine.

Analysis of buffalo rearing in medium-sized farms. Table 2 shows that 24 farms from Brașov and Sibiu counties were analyzed, with buffalo herds that varied between 50 and 20 total herds. The average number of dairy buffaloes per holding was 12.58, with variations between 24 and 9 heads.

Table 2. Synthetic indicators in medium-sized farms / Indicatorii sintetici din fermele de dimensiune medie

Specification	Number of farms	X ± S x	MAX	Min	S	V%
Number buffaloes on exploitation	24	12.58 ± 1.26	24	9	4.37	30
Amount of milk per head of dairy buffalo per day	24	7.65 ± 0.26	10	6.5	0.91	12
The total amount of milk obtained on the farm per day	24	94.3 ± 7.92	168	75	2.7	20
Own consumption of milk in the farm or household	24	2 ± 0.01	-	-	0.2	2
Milk consumption _ for calves	24	43.91 ± 6.5	102	24	2.7	19
The amount of milk processed	24	53.18 ± 5.35	94	33	1.78	18

The total amount of milk in the farm is twice as much as in the small farms, but it is observed that the same trend of giving a significant amount of milk to the calves is maintained.

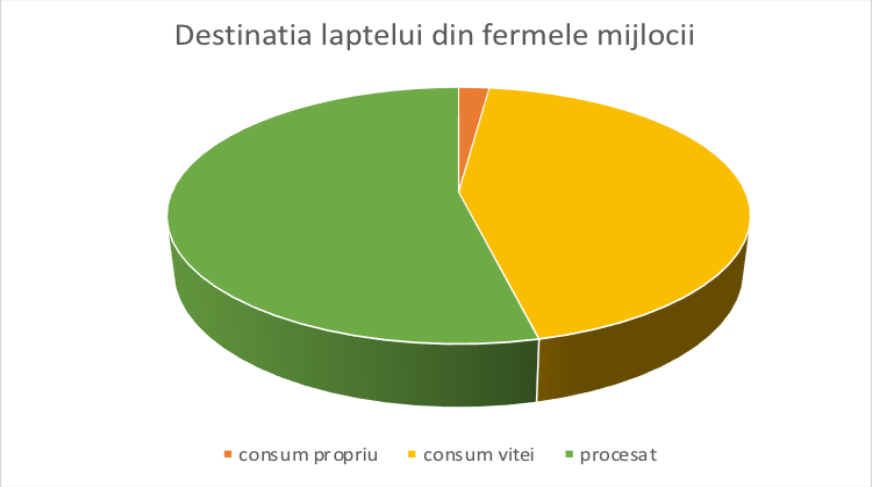


Chart 4. Distribution of milk use at the average farm level / Destinația laptelui din fermele de dimensiune medie

An interesting feature of medium-sized farms is that 85% of market milk is processed at the farm level. Milk processing at the farm level involves turning it into buffalo milk. The consumption to obtain one kilogram of Telemea cheese is between 3.5 kg of milk/kg of cheese and 4 kg of milk/kg of cheese. Regarding the selling price of cheese, it varies between 13 lei/kg and 20 lei/kg, which shows us a large variation depending on the ability to negotiate with customers. From chart 5, a great variability of the average milk production can be observed between the analyzed farms. A negative correlation is generally observed between the number of animals and the average amount of milk per head of dairy buffalo. As the number of buffaloes on the farm increases, the average amount of milk per dairy buffalo decreases, which is an indicator of the farmers concern for the quality of biological material.

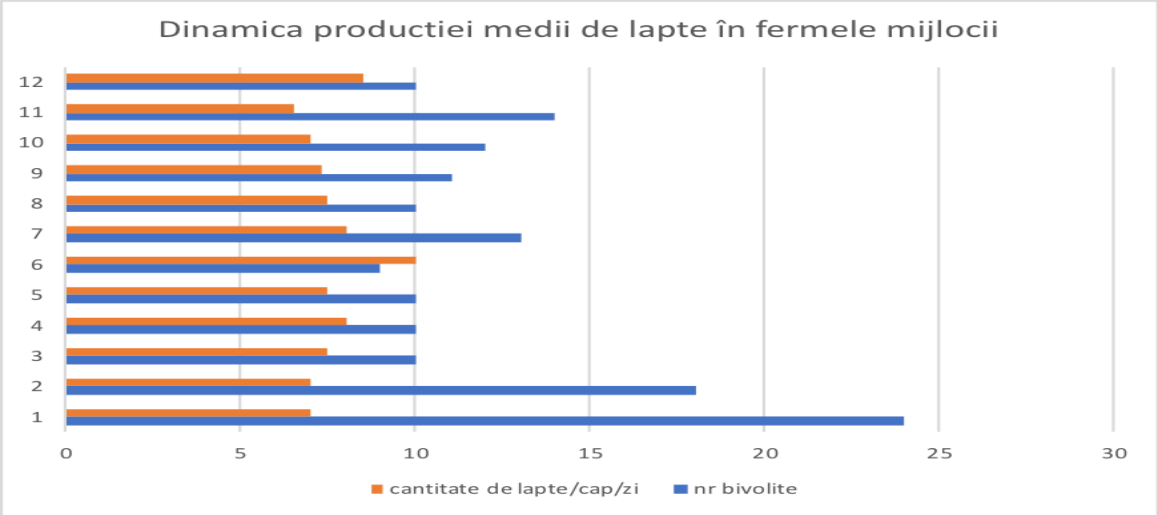


Chart 5. Production indicators in medium-sized farms / Indicatorii pentru producție în fermele de dimensiune medie

In medium-sized farms, dairy buffaloes are reared in the traditional system, with stable maintenance involved in more than 80% of cases. Buffaloes are milked mechanized in 35% of cases, and in the rest of the farms it is done manually. The feeding technology is seasonally differentiated, in the summer the animals stay on the pasture and the feed consists exclusively of green mass, and in the winter the feeding is done from the stock, with canned fodder.

Analysis of dairy buffaloes growth in large-scale farms. In this category we have grouped the farms that have a queen herd of more than 20 heads. 14 farms were analyzed, with herds of lactating buffaloes dairy between 20 and 200 heads. It can be seen from Table 3 that although the rearing system is intensive or semi-intensive, the average milk production is lower (6.01 liters of milk/dairy buffalo /day) compared to medium and small farms.

Table 3. Synthetic indicators in large farms / Indicatorii sintetici în fermele mari

Specification	Number of farms	$X \pm S_x$	MAX	Min	S	V%
Number buffaloes on exploitation	14	63.85 ± 0.26	200	20	0.68	30
Amount of milk per head of dairy buffalo per day	14	6.01 ± 0.33	7,6	5	0.89	29
The total amount of milk obtained on the farm per day	14	386.85 ± 0.54	1200	108	20.9	25
Own consumption of milk in the farm or household	14	2 ± 0.02	-	-	0.31	3
Milk consumption _ for calves	14	113.16 ± 0.98	323	44	4.7	21
The amount of milk processed	14	332 ± 5.41	1200	58	4.22	22

In large farms, significant daily quantities of milk are obtained, respectively between 108 and 1200 liters. Milk intended for feeding calves takes up about 1/3 of the total production, which means that a much larger amount is used (chart 6).

As far as the way of capitalizing the milk is concerned, we found a much greater variability. For the safety of the distribution, several beneficiaries are kept, who purchase the milk at different prices. A significant part is delivered to the processing factory, with different prices, depending on the supplier, between 2.5 and 4 lei/liter. There are farmers who also deliver to milk vending machines at the price of 5-6 lei/liter, but they also retain a certain amount of milk on the farm, which they transform into Telemea cheese (3.5 liters/kg of cheese) and on which uses it at the price of 20 lei/kg.

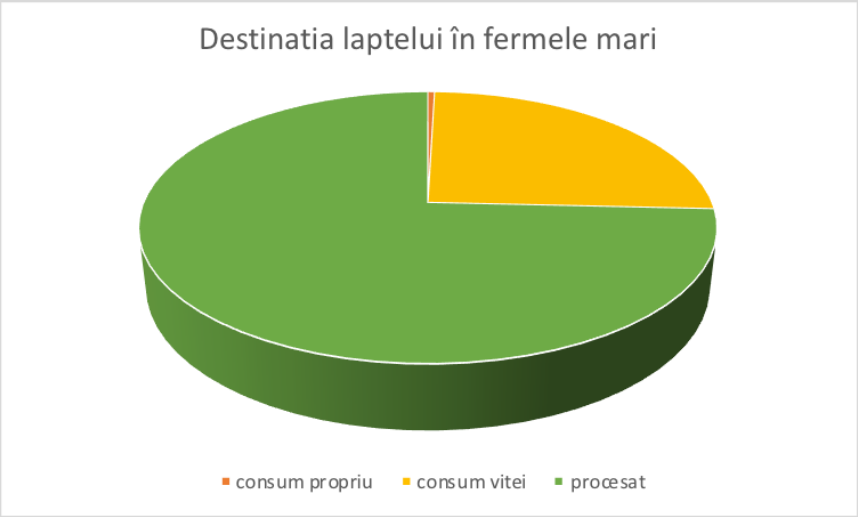


Chart 6. Distribution of milk production in large farms / Distribuția producției de lapte în fermele mari

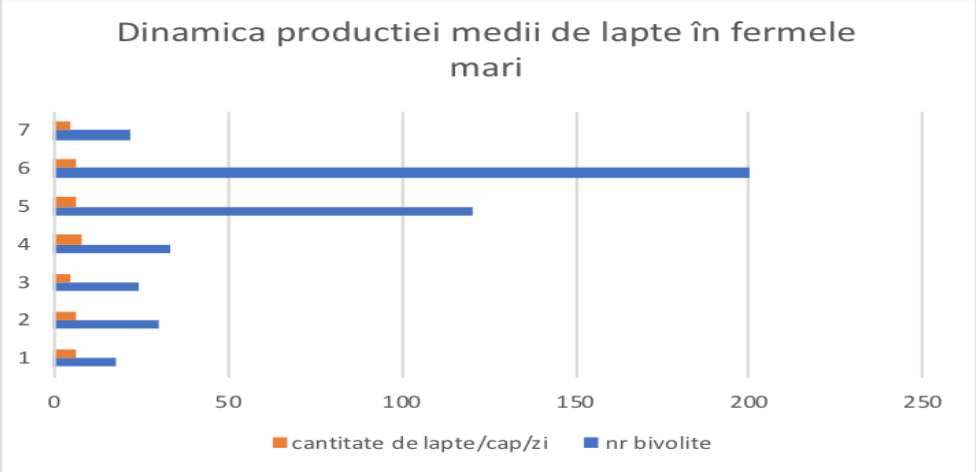


Chart 7. Correlation between livestock and milk production per head of buffalo cow per day in large farms / Corelația dintre efectivul de animale și producția zilnică pe cap de bivoliță în fermele mari

In large dairy buffaloes farms, a seasonally differentiated feeding technology is practiced, without concentrate supplements in the summer season. The maintenance of dairy buffaloes is carried on in large shelters, in tied or free housing. The applied milking technology is different, from manual milking in a farm of 22 milk dairy buffaloes, to milking in special rooms (barrel-type milking room). We have also identified mobile milking facilities (individual milking group) and pipe lines, with centralized milk transport in the milk cooling and storage tank.

CONCLUSIONS

In small farms, the focus should be on increasing individual milk production and increasing marketable production (commodity production). The main recommended measures are the following: the gradual increase of the queen stock, respectively of the size of the farm from a small farm to a medium farm; judicious selection of biological material by increasing the percentage of selective reform; replacement of reformed animals with heifers from own breeding, with high biological value, but also by purchasing heifers with high genetic potential, from medium and large farms; avoiding inbreeding by introducing artificial insemination; the introduction of official performance control; reducing the

consumption of whole milk for feeding calves by introducing feeding schemes in which the share of milk substitute increases; separating calves from buffaloes immediately after calving, so that the maternal reflexes specific to buffaloes do not develop and they no longer yield milk without the presence of the calf; keeping buffaloes with a high milk fat content on the farm; the development of balanced fodder rations, based on the calculation of what is needed for the maintenance of vital functions and for production, so that the animals benefit from all the nutrients necessary for the expression of genetic potential; the construction or modernization of shelters for the maintenance of dairy buffaloes taking into account the principles of welfare, which do not contradict the traditional breeding system; the gradual transition from the tied maintenance of the dairy buffaloes to the free maintenance, by increasing the accommodation surface provided per animal; increasing the degree of luminosity (increasing the glazed surface) and air volume (realizing higher shelters) in the shelters, so that the well-being principles regarding the microclimate to be respected; the introduction of mechanical milking both in shelter and on pasture, in summer camps - individual milking groups up to the size of 20 heads.

In medium-sized farms, the specific recommended measures are the following: increasing the herd of dairy buffaloes, respectively the size of the farm from a medium-sized farm to a large farm; setting a productivity threshold for buffaloes and keeping only those with average daily production above this threshold on the farm; avoiding inbreeding by using artificial insemination, with semen from bulls with superior breeding value; the development of fodder rations differentiated by physiological states and production levels, as well as the introduction of concentrate supplements during the summer period, simultaneously with the careful supervision of green mass production; the application of periodic care measures and overseeding of pastures, so that there is sufficient and constant production of green mass throughout the grazing period; the application of rational grazing in the summer period and the creation of a sufficient fodder base for the winter period; the development of modern shelters, which allow the herd to increase, without affecting the welfare of the animals; training or continuous training of farmers so that they know all the opportunities for farm development and improvement of milk production; for the efficiency of milk production, it is recommended to consult the farmers within the association, so that the price of the products sold does not fluctuate so much (between 13 and 20 lei/kg of Telemea) and to ensure the profitability of all farmers.

For farms specialized in raising milk buffaloes, the following is recommended: increase individual milk production by practicing a selective reform of up to 30% per year and retaining the plus variants from the own herd; the application of optimized rations for each technological group, respectively productive level and physiological state; applying artificial insemination to the entire herd and using semen from bulls with superior breeding value for milk production; monitoring the reproductive activity, namely the interval between calvings and the age of the first calving, so as to increase the production of goods per productive life; increasing the skills of the udder for mechanical milking by monitoring the morphology and function of the udder, the milking speed and the degree of milk yield; disseminating genetic progress by selling heifers to medium and small farms; assuming the role of a regional hub to engage with small and medium farmers to disseminate genetic progress and scientific advice; concluding firm and advantageous contracts with the processors, so that the buffalo milk brings a real profit for the farm; close welfare monitoring for all categories of buffaloes on the farm.

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STUDY ON LACTATION CURVE FOR MILK PRODUCTION INDICES IN MONTBELIARDE DAIRY COWS

STUDIU ASUPRA CURBEI DE LACTAȚIE PENTRU INDICII PRODUCȚIEI DE LAPTE LA VACILE DIN RASA MONTBELIARDE

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Abstract

*The aim of this paper was to highlight the effect of parity on the shape of the lactation curve for milk yield and composition in **Montbeliarde** cows. Researches were carried out on 367 lactations obtained from 115 **Montbeliarde** dairy cows from Hunedoara County. Data was obtained using the standard method of the official milk recording system. Only those lactations were used that had at least 11 controls. Test-day data was modelled using Wood's incomplete gamma function. Lactations were divided into three classes according to the parity of the cow: primiparous, secundiparous and multiparous. Parity had a significant influence ($p<0.001$) on the milk production. Milk production of multiparous cows (third lactation or higher) was 16-18% higher than in primiparous cows and 10-14% higher than in secundiparous cows. There were no significant ($p>0.05$) differences for milk production between primiparous and secundiparous cows. The shape of the lactation curve was significantly influenced ($p<0.05$) by parity in **Montbeliarde** dairy cows for milk yield, fat percentage, protein percentage and lactose percentage. Generally, primiparous cows had flatter lactations curves for all indices and lower test-day values, compared to multiparous cows that had sharper lactation curve with higher test-day values.*

Key words: dairy cows, lactation curve, milk production, Montbeliarde, parity

Rezumat

*Scopul acestei lucrări a fost acela de a evidenția efectul ordinii lactației asupra formei curbei de lactație pentru cantitatea și compoziția laptelui la vacile din rasa **Montbeliarde**. Cercetările s-au efectuat pe 367 lactații obținute de la 115 vaci de lapte crescute în județul Hunedoara. Datele s-au obținut din controlul oficial al performanțelor. S-au utilizat doar informațiile de la acele lactații care au avut cel puțin 11 controale. Datele de producție din ziua de control au fost modelate folosind funcția gamma incompletă a lui Wood. Lactațiile au fost împărțite în raport cu ordinea lactației, astfel: primipare, secundipare și multipare. Producția de lapte a vacilor multipare (lactația a treia sau mai mare) a fost cu 16-18% mai mare ($p<0,001$) decât cea a vacilor primipare și cu 10-14% mai mare ($p<0,001$) decât cea a vacilor secundipare. Nu au existat diferențe semnificative ($p>0,05$) în ceea ce privește producția de lapte între vacile primipare și secundipare. La vacile din rasa **Montbeliarde**, forma curbei de lactație pentru cantitatea de lapte, precum și pentru procentul de grăsime, proteină și lactoză din lapte a fost influențată în mod semnificativ de ordinea lactației ($p<0,05$). În general, vacile primipare au avut curbe de lactație mai plate pentru toți indicii productivi, precum și valori mai mici în ziua de control, comparativ cu vacile multipare, care au avut curbe de lactație mai ascuțite cu valori mai mari în ziua de control.*

Cuvinte cheie: vaci de lapte, curba de lactație, producția de lapte, Montbeliarde, ordinea lactației

INTRODUCTION

The lactation curve represents the evolution over time of the daily milk production during a lactation. The lactation curve allows the evaluation of important characteristics of milk production, such as the maximum daily yield, the time of reaching the maximum yield or the persistence of lactation.

These curves are also used to predict the total production of a cow or a farm when the quantities produced in a certain period are known (Matinez Lopez et al., 2019).

The lactation curve provides useful information to selection programs, as well as to the development of appropriate management decisions and setting up the production strategies at the farm level (Bouallegue and M'Hamdi, 2020). Several types of mathematical models are used to study lactation curves, each of them having advantages and disadvantages. They can be divided into empirical models, parametric models, exponential functions, linear fitting models, as well as other unclassified mathematical models.

Most studies on lactation curves have considered milk yield and described a standard curve in dairy cows, showing that peak milk yield occurs between the 4th and 8th week after calving, followed by a daily decrease in the amount of milk produced until the cow is weaned (Silvestre et al., 2009). The most common model used to describe the lactation curve is the model of Wood, 1967. Research carried out by Silvestre et al., 2009 showed that the milk production traits (milk yield, fat yield, protein yield, fat percentage, and protein percentage), as well as parity have an influence on the degree of confidence of the Wood model. Jingar et al., 2014 argue that the incomplete gamma function can be successfully used to model the lactation curve for hybrid cows with or without mastitis.

Somatic cell count (SCC) is an indicator of mastitis, but at the same time it is also influenced by other factors, such as: parity, breed and days in milk. SCC shows greater variability than the daily milk yield. The shape of the lactation curve for SCC varies markedly between dairy farms. The variability of lactation curves for milk yield and SCC between farms highlights the importance of addressing them at the farm level (Græsbøll et al., 2016).

MATERIAL AND METHOD

In order to understand the milk production from a quantitative and qualitative views in the **Montbeliarde** breed, periodic milk production controls were carried on, determining the following quantitative and qualitative indices of milk production: the daily milk yield, the fat percentage, the protein percentage, and the lactose percentage. The milk fat, protein and lactose yields were used for the analysis of variance.

Three hundred sixty-seven lactations obtained from 115 **Montbeliarde** cows, originating from a dairy farm of a commercial company in Hunedoara County, were studied, on cows that completed a normal lactation until September 30, 2021.

The experimental data were analysed using the National Agency for Animal Husbandry (ANZ) technique. The analysis of the chemical composition of the milk and the measurement of the milk yield obtained were carried on with built-in lactometers, at the milking parlour on the farm. The results were automatically collected with the help of a computer in the designed software of the milking parlour.

Only those cows that completed a normal lactation with at least 11 controls during lactation were considered. The data from the measurements made on each control day were used in the calculations. Thus, a number of 8,094 milk samples were used. The data from each control day were divided into three classes according to the parity of the cows, namely: primiparous, secondiparous and multiparous cows (third lactation and beyond third lactation).

The data were processed statistically with the help of the STATISTICA software (Hill, T. & Lewicki, P., 2006). Means and indices of dispersion on normal lactation were calculated for each parity, and by using ANOVA/MANOVA analysis of variance, the effect of this factor on the following characteristics was determined: milk yield per control day, fat yield, protein yield and lactose yield.

Based on the averages obtained, the parameters of the lactation curves were calculated using the mathematical model first proposed by Wood, 1967, called the "incomplete gamma function", having the following equation:

$$y_n = an^b e^{-cn}$$

where:

- e natural logarithm base
- y_n is the milk yield (fat percentage, protein percentage or lactose percentage) at the *n* moment from calving;
- a is the initial milk yield (fat, protein or lactose percentage), in the first day of lactation;
- b is the increase rate of milk yield (decrease rate of fat, protein or lactose percentage) up to the peak yield (nadir percentage);
- c is the decrease rate of milk yield (increase rate of fat, protein or lactose percentage) after the peak (nadir) value.

The incomplete gamma function model estimates peak milk yield to be equal to $y_{max} = a(b/c)be^{-b}$, production which is achieved at $n = b/c$ days from calving. The total milk yield can be estimated using the formula $Y = a\Sigma n^b e^{-cn}$, and the persistence (e.g. the length of time maximum production is maintained) can be estimated using the formula $S = c^{-(b+1)}$.

Initially, Wood used this model to highlight the effects of different factors (age, calving season, etc.) on the shape of the lactation curve. He demonstrated a number of properties of this function in subsequent studies (see Wood, 1969, 1970a, 1970b, 1972, 1974, 1976, 1977, 1980).

A peculiarity of this function is the fact that it can be solved by non-linear as well as linear methods. Solving the incomplete gamma function nonlinearly requires a larger volume of mathematical calculations. By logarithmic transformation of the initial function, a linear model is obtained that can be solved by multiple linear regression. The results obtained for the parameters of the lactation curve (a, b and c) are then very easily obtained by dilogarithm transformation. The linear function corresponding to the incomplete gamma function has the form:

$$ln(y_n) = ln(a) + b\ ln(n) - cn$$

where:

- ln natural logarithm;
- y_n is the milk yield at moment n from calving;
- a is the initial milk yield;
- n days in milk;
- b is the increasing rate of milk yield up to peak;
- c is the decreasing rate of milk yield after the peak.

The advantage of using the linear form of the incomplete gamma function is the possibility of statistical processing of the obtained parameters.

Lactation curves were drawn, in relation to the parity, for the following traits: milk yield, fat percentage, protein percentage and lactose percentage.

RESULTS AND DISCUSSION

Influence of parity on milk yield and milk chemical components on normal lactation

One of the important factors influencing individual milk production is parity, which is generally correlated with cow age, with lifetime milk production varying from one lactation to another (Stanciu, 1999). The smallest milk yield is obtained in the first lactation, the production increases until the 4th lactation, after which it begins to decrease. The causes that determine the variation in the milk production from one lactation to another are multiple, the most important being the productive potential of the cow, the intensity of metabolism, the volume and structure of the udder, the capacity of the digestive system, the state of health, the body condition of the cow at calving, the regime of feeding before and after calving.

Maximum milk production is generally achieved in different lactations, in relation to the precocity of the breed and its degree of genetic improvement. From an economic point of view, it is important for cows to reach the maximum productive level at a young age and to maintain this productive level for as long as possible, and in first lactation to achieve a milk production as close as possible to the maximum production.

Table 1 shows the averages and indices of dispersion for the milk yield per normal lactation in Montbeliarde cows in relation to the parity.

The milk yield during the normal lactation increased with the parity, being on average 4974.49 kg in the first lactation, 5231.43 kg in the second lactation and 5788.73 kg in the third lactation.

The cows in lactation 1 had the lowest milk yield, the minimum value being 3325.1°kg of milk and the maximum value of 6608.9 kg of milk. Cows in lactation 2 had the minimum milk yield of 3288.6 kg, while the maximum yield was 7604 kg of milk. Also, cows in lactation 3+ achieved a minimum production of 2749.1 kg of milk and a maximum production of 12,239.1 kg of milk. The coefficient of variability for the amount of milk in cows in lactation 3+ had the highest value (24.43%) and the lowest coefficient of variability (13.84%) was obtained in cows in lactation 1.

Table 1. Averages and dispersion indices for milk yield (kg) on normal lactation according to parity in Montbeliarde cows / Mediile și indicii dispersiei pentru cantitatea de lapte (kg) pe lactația normală în raport cu ordinea lactației la vacile de rasă Montbeliarde

Parity	n	Average ± SEM	SD	V%	Min	Max
First lactation	76	4974.49±78.972	688.458	13.84	3325.1	6608.9
Second lactation	73	5231.43±93.095	795.403	15.20	3288.6	7604.0
Third+ lactation	218	5788.73±95.773	1414.065	24.43	2749.1	12239.1

The averages and indices of dispersion for fat yield per normal lactation in relation to parity in Montbeliarde cows are shown in Table 2. As the cows get older, the fat yield per normal lactation increases from 193.94 kg in primiparous cows to 204.87 kg in second-lactation cows and up to 229.4 kg in third-lactation cows.

From Table 2, it can be seen that the cows in lactation 1 have the minimum fat yield 116.1 kg, reaching the maximum fat yield of 282.4 kg. Also, cows in lactation 2 achieved the lowest fat yield at 110.4 kg and the maximum at 350.7 kg. Cows in lactation 3 and above had the lowest fat yield at 92.5 kg and the highest at 496.5 kg of fat. The coefficient of variability for the fat yield in cows was the highest in lactation 3 (28.26%) and the lowest was obtained in cows in lactation 2 (18.28%).

Table 2 .Averages and dispersion indices for milk fat yield (kg) on normal lactation according to parity in Montbeliarde cows / Mediile și indicii dispersiei pentru cantitatea de grăsime (kg) pe lactația normală în raport cu ordinea lactației la vacile de rasă Montbeliarde

Parity	n	Average ± SEM	SD	V%	Min	Max
First lactation	76	193.94±4.217	36.761	18.95	116.1	282.4
Second lactation	73	204.87±4.384	37.459	18.28	110.4	350.7
Third+ lactation	218	229.40±4.391	64.825	28.26	92.5	496.5

Table 3 shows the averages and indices of dispersion for the protein yield per normal lactation in relation to the parity in Montbeliarde cows. The protein yield produced during normal lactation increases with the age of the cows, the lowest value was recorded in secundiparous cows (176.17 kg), followed by primiparous cows (180.4 kg), and the highest value was obtained in cows in the third lactation (201.12 kg).

Lactation 1 cows had the lowest protein yield at 119.5 kg and the highest at 260.9 kg protein. Lactation 2 cows had the minimum yield of 102.9 kg protein, while the maximum yield was 288.1 kg protein. Also, lactation 3 cows achieved a minimum yield of 85.5 kg of protein and a maximum yield of 454.6 kg of protein. The coefficient of variability for the protein yield in cows in lactation 3+ had the highest value (27.51%) and the lowest coefficient of variability for the protein yield was obtained in cows in lactation 1 (15.74%).

Table 3. Averages and dispersion indices for milk protein yield (kg) on normal lactation according to parity in Montbeliarde cows / Mediile și indicii dispersiei pentru cantitatea de proteină (kg) pe lactația normală în raport cu ordinea lactației la vacile de rasă Montbeliarde

Parity	n	Average ± SEM	SD	V%	Min	Max
First lactation	76	180.40±3.258	28.401	15.74	119.5	260.9
Second lactation	73	176.17±3.655	31.225	17.72	102.9	288.1
Third+ lactation	218	201.12±3.748	55.335	27.51	85.5	454.6

Table 4 shows the averages and indices of dispersion for the lactose yield per normal lactation in relation to the parity in the **Montbeliarde** cows. Primiparous cows achieved the lowest lactose yield per lactation (246.87 kg), followed by secundiparous cows (260.12 kg), while the highest lactose yield was achieved by cows in the third+ lactation (287.47 kg).

Table 4. Averages and dispersion indices for milk lactose yield (kg) on normal lactation according to parity in Montbeliarde cows / Mediile și indicii dispersiei pentru cantitatea de lactoză (kg) pe lactația normală în raport cu ordinea lactației la vacile de rasă Montbeliarde

Parity	n	Average ± SEM	SD	V%	Min	Max
First lactation	76	246.87±4.961	43.25	17.51	150.5	356.1
Second lactation	73	260.12±4.815	41.14	15.81	171.8	432.7
Third+ lactation	218	287.47±5.037	74.36	25.86	132.2	631.3

Primiparous cows had the lowest lactose yield of 150.5 kg and the highest of 356.1 kg. Secundiparous cows had the lowest lactose yield of 171.8 kg, while the maximum yield was 432.7 kg. Also, cows in third+ lactation had the minimum yield at 132.2 kg lactose and the maximum yield at 631.3 kg lactose. The coefficient of variability for the lactose yield in cows at the third + lactation had the highest value (25.86%) while the lowest coefficient of variability (15.81%) for the lactose yield was obtained in cows in second lactation.

Table 5 shows the differences and statistical significance for the milk, fat, protein and lactose yields in relation to the parity in **Montbeliarde** cows. For the milk yield per normal lactation, very significant differences ($p<0.001$) were obtained between cows in the first lactation and those in the second lactation (-814.24 kg milk), as well as between cows in the second lactation and those in the third+ lactation (-557.3 kg milk).

Table 5. Differences and statistical significance for milk production indices according to parity in Montbeliarde cows / Diferențele și semnificația statistică pentru indicii producției de lapte în raport cu ordinea lactației la vacile din rasa Montbeliarde

Parity	Milk yield (kg)		Fat yield (kg)		Protein yield (kg)		Lactose yield (kg)	
	3+	2	3+	2	3+	2	3+	2
Lactation 1	-814.24***	-256.94^{ns}	-35.46***	-10.93^{ns}	-20.72***	4.23^{ns}	-40.6***	-13.25^{ns}
Lactation 2	-557.30***	-	-24.53***	-	-24.95***		-27.35***	-

ns – $p>0.05$; * - $p<0.05$; ** - $p<0.01$; *** - $p<0.001$

For milk fat yield, there were very significant differences ($p<0.001$) between lactation 1 and lactation 3+ (-35.46 kg fat), as well as between lactation 2 and lactation 3+ (-24.53 kg fat).

Between lactation 1 and lactation 3+ there were very significant differences (-20.72 kg, $p<0.001$) for the milk protein yield. Also, a highly significant difference ($p<0.001$) was observed between lactation 2 and lactation 3+ (-24.95 kg protein).

For the milk lactose yield, very significant differences ($p<0.001$) were obtained between lactation 1 and lactation 3+ (-40.6 kg), as well as between lactation 2 and lactation 3+ (-27.35 kg).

There were no statistically significant differences ($p>0.05$) between milk production indices during normal lactation between primiparous and secundiparous cows.

Evolution of the milk yield and milk chemical components during lactation according to parity

Age is generally correlated with parity, and milk production varies from one lactation to another. The lowest milk production occurs in the first lactation, then increases until the maximum level is

reached, after which it begins to decrease. The increase in milk production occurs until cows reach adult age, during which the cow's body development and the capacity of the digestive tract increase. The udder is not fully functional after the first calving, increasing its weight, capacity and improving its histological structure during the first 1-2 lactations. The greatest increase in volume and the most significant changes in structure occur between the first and the second lactations. The decrease in milk production in the last part of life is the result of the aging phenomenon, due to which the intensity of vital functions is gradually reduced, the capacity to consume and digest feed decreases and, above all, the capacity to restore the alveolar system during the breast rest period (Stanciu, 1999).

The analysis of variance demonstrated the existence of a very significant influence ($p<0.001$) of the parity on all the properties studied (Table 5).

The shape of the lactation curve for daily milk yield is shown in Figure 1. The parity had a highly significant influence ($p<0.001$) on the shape of the lactation curve for milk yield. Thus, primiparous cows (lactation 1) showed a flatter lactation curve, which started from an average level (14.6 kg) increased to 16.1 kg milk/day, after which the daily production decreased slowly until the end of lactation. Peak milk yield was reached, on average, 175 days after calving for cows in lactation 1. Tekerli et al., 2000, showed that in the first lactation, peak milk yield, as well as, over the entire lactation was lower, but lactation persistence was better. On the other side, the multiparous cows (3 and more than three lactations) presented a much sharper lactation curve. They started from a production of 14.43 kg of milk per day, reaching a maximum level of 20.1 kg of milk per day at only 82 days of lactation. Secundiparous cows showed a lactation curve similar to that of cows in lactation 3 and above, thus the minimum milk yield was 9.89 kg, reaching a maximum level of 17.9 kg at 115 days after calving.

The parity also influenced the milk fat percentage, which decreases from the first lactation to the fourth lactation by about 0.2-0.3%. After the fourth lactation, changes in fat percentage are non-significant (Figure 2). Significant differences ($p<0.05$) were found between the shapes of the lactation curves depending on the age of the cows. For cows in lactation 1 and lactation 3 and above, milk fat percentage was high at the beginning of lactation, after which it decreased to a nadir, only to increase again towards the end of lactation. Different is the shape of the lactation curve for cows in lactation 2 where the evolution of the percentage of fat started from 3.68% and gradually increased, almost linearly, until the end of lactation.

Similar to the lactation curve for milk yield, milk fat percentage in primiparous cows showed a flatter curve, starting from an initial fat percentage of 3.04%, reaching a minimum of 2.92% at 36 days after calving, and towards the end of lactation it rose to a threshold of approximately 3.17%.

Multiparous cows (3+ lactations) started from a higher initial level of 3.36% fat, reached a nadir of 3.26% in the first 32 days after calving, after which, towards the end of lactation, a fat percentage of 3.53% was obtained.

In secundiparous cows, the fat percentage in the milk towards the end of lactation increases abruptly, up to the value of 3.49%, with accelerate increase in the ascending phase until weaning.

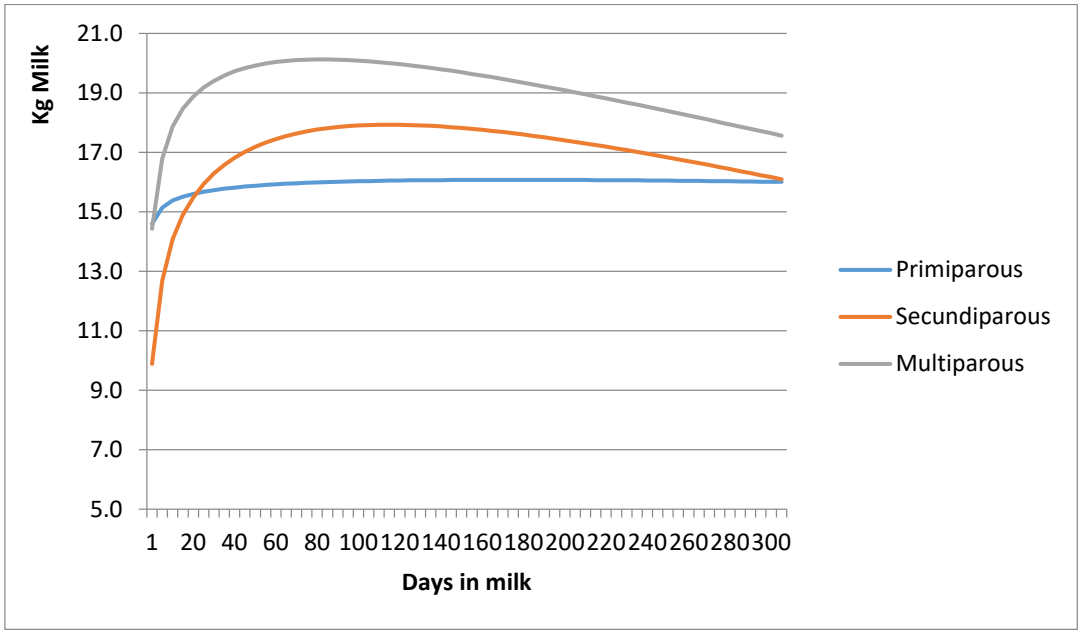


Figure 1. Evolution of milk yield during normal lactation according to parity in Montbeliarde breed / Evoluția cantității de lapte pe lactația normală în funcție de ordinea lactației la rasa Montbeliarde

The percentage of milk protein varied from one lactation to another, varying at the beginning of lactation between 3.01% in primiparous cows and 4.12% in cows in lactation 2 (Figure 3).

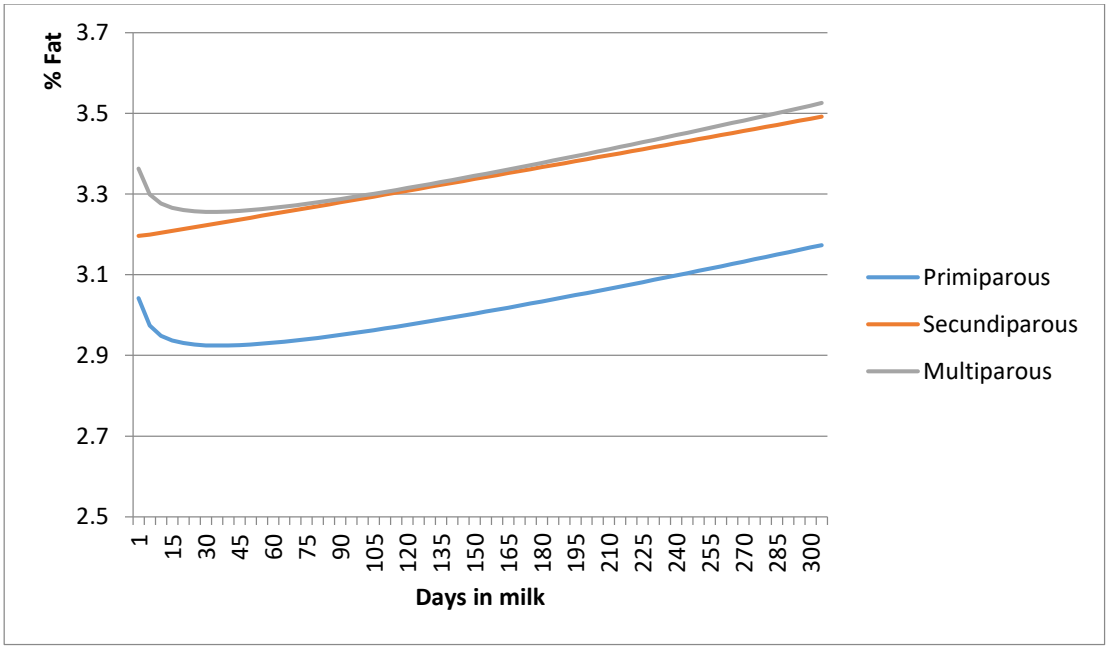


Figure 2. Evolution of milk fat percentage during normal lactation according to parity in Montbeliarde breed / Evoluția procentului de grăsime din lapte pe lactația normală în funcție de ordinea lactației la rasa Montbeliarde

As shape, primiparous cows had a flatter lactation curve, starting at an initial level of 3.01%, then reaching a nadir of 2.95% at 71 days postpartum, after the protein percentage increased slow until the end of lactation (2.98%). Secundiparous cows reached their minimum protein percentage of 2.76% at 224 days after calving, after which it increased very slowly, reaching 2.77% at the end of lactation.

Multiparous cows, with more than three lactations, showed a strong decrease in milk protein percentage, from the initial value of 3.92% reaching a nadir of 2.87% at 150 days after calving. After that, the milk protein percentage showed a more pronounced increase compared to secundiparous cows until weaning (2.94%).

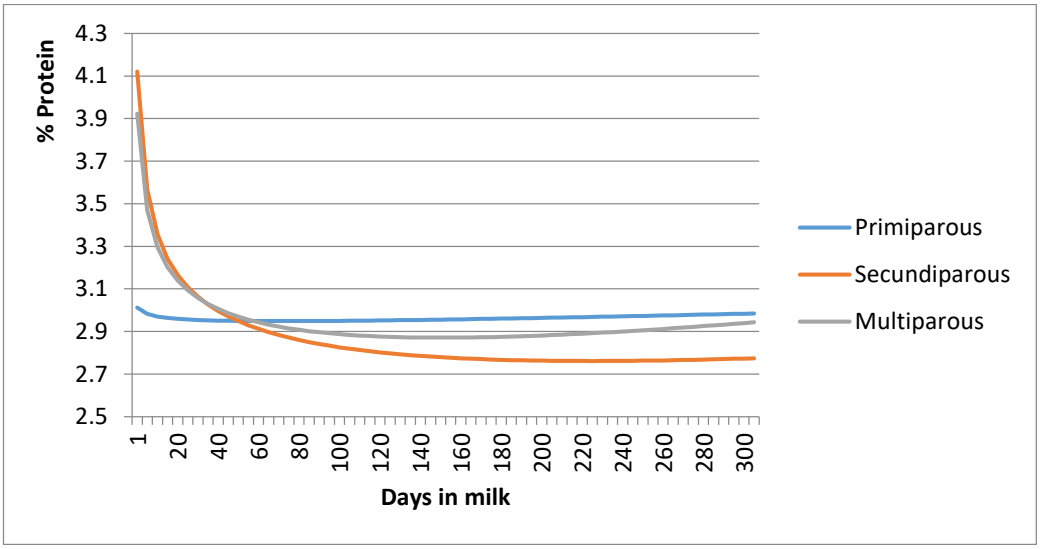


Figure 3. Evolution of milk protein percentage during normal lactation according to parity in Montbeliarde breed / Evoluția procentului de proteină din lapte pe lactația normală în funcție de ordinea lactației la rasa Montbeliarde

Parity had a significant effect on the shape of the lactation curve for milk lactose percentage ($p<0.001$, Figure 4).

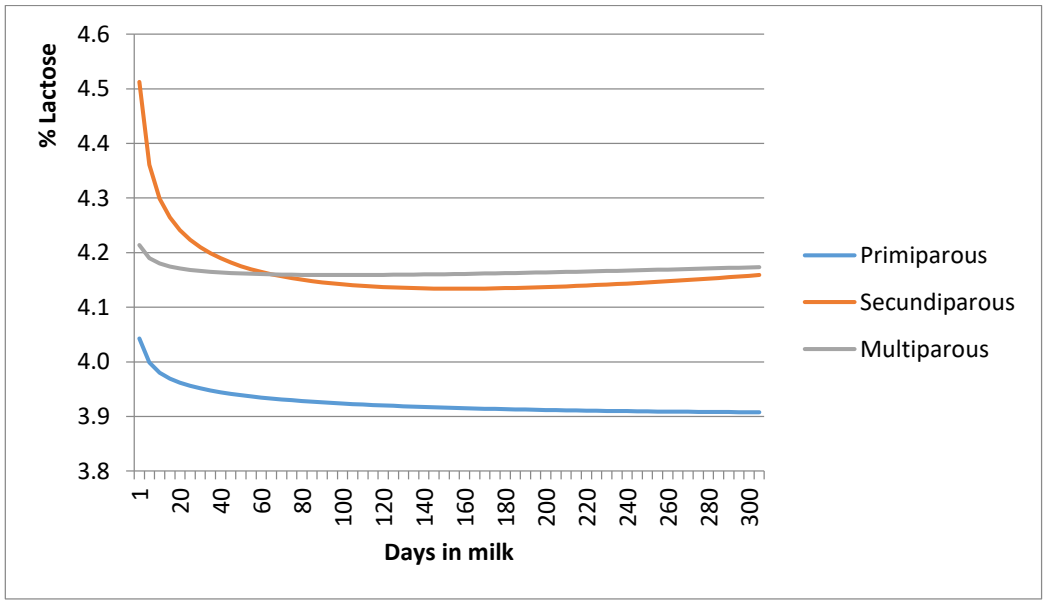


Figure 4. Evolution of milk lactose percentage during normal lactation according to parity in Montbeliarde breed / Evoluția procentului de lactoză din lapte pe lactația normală în funcție de ordinea lactației la rasa Montbeliarde

The lowest percentage of lactose in milk during lactation was recorded by cows in first lactation. In these cows, the milk lactose content decreased slightly from 4.04% at the beginning of lactation to 3.91% at weaning.

In the case of the other two groups of cows (secundiparous and multiparous) the lactation curve for the milk lactose percentage had an atypical shape, i.e., in both groups the lactose percentage decreased immediately after calving, reaching the nadir, after which growing slightly to weaning. Multiparous cows (3+ lactations) had a more flattened curve, where the percentage of lactose in milk started from a value of 4.21%, showing a slight decrease at the beginning of lactation, reaching a minimum of 4.16% at 99 days after calving, then remained close to this value until the end of lactation (4.17%).

Second lactation cows started at 4.51% lactose percentage, reached a nadir of 4.13% at 157 days after calving, and at weaning milk lactose percentage was 4.17%.

CONCLUSIONS

Parity significantly influenced ($p<0.05$) both milk production in normal lactation and the shape of the lactation curve for milk yield as well as fat, protein and lactose percentage.

Multiparous cows, which completed three or more lactations produced, during a normal lactation, 16 to 18% more milk compared to primiparous cows and 10 to 14% more milk compared to secundiparous cows ($p<0.01$). Between primiparous and secundiparous cows there were no significant differences ($p>0.05$) for milk, fat, protein and lactose yields per normal lactation.

Parity had a highly significant influence ($p<0.001$) on the shape of the lactation curve for milk yield. Thus, primiparous cows (lactation 1) showed a flatter lactation curve. On the contrary, the multiparous cows (3 and more than three lactations) presented a much sharper lactation curve.

Significant differences ($p<0.05$) for milk fat percentage were recorded between cows in lactation 1 and cows in lactation 3 and above. The percentage of milk fat was high at the beginning of lactation, then decreased to reach a nadir, to increase again towards the end of lactation.

The milk protein percentage showed variations in relation to the age of the cows, being at the beginning of lactation between 3.01% in primiparous and 4.12% in secundiparous cows. Multiparous cows (3+ lactations) had the lowest content of milk protein, of 2.87% on the 150th day of lactation.

Parity had a highly significant effect ($p<0.001$) on the shape of the lactation curve for milk lactose percentage. The lowest level of lactose in milk during lactation was recorded by cows in lactation 1.

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THE PRETABILITY OF THE TRANSYLVANIAN PINZGAU BREED FOR MEAT PRODUCTION

PRETABILITATEA RASEI PINZGAU DE TRANSILVANIA PENTRU PRODUCȚIA DE CARNE

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Abstract

*The **Pinzgau** breed is a local breed formed in the environmental conditions of the mountainous area of Apuseni and Bucovina. A late breed, resistant to specific cattle diseases, it makes good use of natural meadows. Unfortunately due to excessive cross-breeding it is in danger of disappearing. The breed has a modest milk production, at the same time it has major udder defects, which is why it is not suitable for mechanical milking. Under these conditions, we studied the readiness of the breed for meat production using the "meat cow" technology, where the heifers are kept with the calves next to them, from calving to weaning, the period when the calves suck all the milk of the mother. Our studies showed that the young male achieves, up to the age of slaughter (181), an average body weight of 410 kg, a slaughter yield of 54-56% and an average daily gain of 700 g. From the point of view of the quality of the carcasses, assessed by the EUROP method, they have a superior quality, falling into the R, O+ category, with a level of good meat dressing of the body regions and a reduced meat dressing in fat* (2). *After analyzing the results obtained regarding the growth rate of the youth, the yield at slaughter and the quality of the carcasses, we can conclude that the **Pinzgau de Transilvania** breed lends itself to meat production using the "meat cow" technology with real chances of relaunching the breed in the years following.*

Keywords: pretability, "meat cow", slaughter yield, growth rate, mechanical milking

Rezumat

*Rasa **Pinzgau** este rasă locală formată în condițiile de mediu ale zonei montane din Apuseni și Bucovina, o rasă tardivă, rezistentă la bolile specifice bovinelor, valorifică foarte bine pajiștile naturale, din păcate datorită metisărilor excesive este în pericol de dispariție. Rasa are o producție modestă de lapte, totodată are defecte ale ugerului, motiv pentru care nu se pretează la mulsul mecanic. În aceste condiții, am studiat pretabilitatea rasei pentru producția de carne folosind tehnologia "vaca de carne", unde vacile fătate se întrețin cu viței lângă ele, de la fătare până la întărcare, perioadă în care viței sug toată cantitatea de lapte a mamei. Din studiile noastre a rezultat că tineretul mascul realizează până la vârsta de sacrificare (18 l), o greutate corporală medie de 410 kg, un randament la sacrificare de 54-56% și un spor mediu zilnic de 700 g. Din punct de vedere a calității carcaselor, apreciate prin metoda EUROP, acestea au o calitate superioară, încadrându-se în categoria R, O+, cu un nivel de îmbrăcare bună în carne a regiunilor corporale și o îmbracare redusa a cărnii în grăsime (2). După analiza rezultatelor obținute în privința ritmului de creștere a tineretului, a randamentului la sacrificare și a calității carcaselor, putem concluziona că rasa **Pinzgau de Transilvania** se pretează pentru producția de carne folosind tehnologia "vaca de carne", cu șanse reale de relansare a rasei în anii următori.*

Cuvinte cheie: pretabilitate, "vaca de carne", randament la sacrificare, ritm de creștere, muls mecanic

INTRODUCTION

Transylvanian Pinzgau cattles have a conformation characteristic of mixed breeds with milk-meat, meat-milk production skills. Phenotypically they have a color specific to the breeding breed: dark red-chestnut, with a white stripe that starts from the withers towards the rear extremities of the trunk,

goes down on the inguinal region, up to the head of the chest, forming a ring of the same color on the calves and arms (Florian P., 2007).

The breed is well adapted to the current growing areas, body development is variable depending on the area of spread, respectively the specific climatic conditions.

The breed is resistant to environmental conditions, later and with lower production qualities in comparison with the domestic improved breeds, **Baltata Romaneasca** and **Bruna de Maramures**.

The interest in the meat from the **Transylvanian Pinzgau** breed is justified by the very good quality and by the fact that the increase in growth is consistent with the effectiveness of the breed in exploiting the meadows of the mountain areas (Ujica V., 2005).

From a qualitative point of view, the meat of this breed is among the best in the world. The fine fiber, the juicy red meat, with a light covering of fat, is remarkable (Bures D., 2006).

The breed is late compared to the other improved mixed breeds in the country, namely **Baltata Romaneasca** and **Bruna de Maramures**. At the same time it has major udder defects with asymmetrical quarters, long and thick nipples and modest milk production, 6-7 l per day.

Under these conditions, the **Pinzgau** breed does not lend itself to mechanical milking, and due to the small production, the expenses of manual milking are not justified.

The research aimed to study the readiness of the breed for meat production, using the 'meat cow' technology. In parallel we studied the quality of the meat after slaughter. With the help of the EUROP system we assessed the conformation of the carcasses and the degree of their coating with fat.

Starting from these premises, we set out to bring, through our studies, a substantial contribution to the knowledge of meat quality parameters in order to make better use of it.

Knowledge, the application of improved exploitation technologies, maintenance and feeding are the main factors in obtaining good results in the production process. Deviations from these desired or compromise solutions lead to a decrease in economic efficiency (Vaida Ghe., 2010).



Picture 1. Young Transylvanian Pinzgau bull / Tăuraș rasa Pinzgau de Transilvania

MATERIAL AND METHODS

Considering the modest milk production of the breed, we have left the suckling calves with their mothers until their instant weaning. On average the decrease in milk production occured at 7 months of lactation, when the calves were transferred to collective stalls for youth. Cattle were fed with the varieties available on the farm, respectively: hay, corn silage, concentrates and protein supplements.

We prepared appropriate feed rations and recorded the evolution of body weight up to the age of slaughter. Control slaughters were performed and we collected meat samples for physico-chemical analysis. The organoleptic properties of the meat were assessed and finally the carcasses were assessed using the EUROP assessment system.

The obtained data were systematized, processed and interpreted by specific statistical methods specific for such studies.

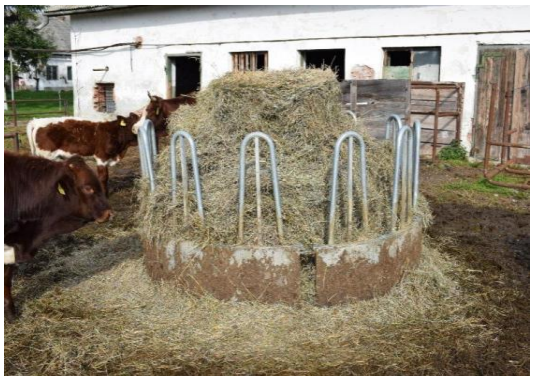
In order to carry out this research project, we studied the male youth of the **Transylvainian Pinzgau**, which form the core of the genetic conservation of the breed which, due to excessive cross-breeding, is threatened by extinction.

The number of animals that make up the population of **Pinzgau** was established in 2015-2016 by the purchase of adult cows, pregnant heifers, and female youth, totalling 25 individuals.

This herd of animals was identified and purchased from 10 different locations in the Bucovina area. Currently the total herd consists of 40 individuals, of which 22 are adult cows in different physiological states.

We mention that the population of **Pinzgau** from the station`s farm is very heterogeneous, especially in terms of body development, milk production and even the coat color. In this **Pinzgau** herd we also have several individuals of the **'Dorna Cow'**, whose basic color is black with the same white line on the upper-lower part of the body (Draganescu C., 2006).

Mother cows and their calves, which were the object of the study were kept together during the calving-weaning period in a shelter with free housing.



Picture 2. Feeding installment for hay / Tipul de hrănitoare pentru fân

RESULTS AND DISCUSSIONS

The **Transylvanian Pinzgau** breed has a rustic conformation. It is well adapted to the environmental conditions in which it was bred, namely the mountainous area. The breed is very resistant to specific cattle diseases. These cattle make good use of the meadows in the submontane and mountainous areas, with a very diverse and valuable floristic composition.

The milk production of the breed is very modest: on average, one cow produces 6-7 l per day. The duration of lactation - compared to other mixed breeds in the country - is of 210 days, compared to 305 days.

Unfortunately, major udder defects, such as asymmetrical quarters and thick and long nipples are characteristic for the breed. For these reasons, lactating cows are not suited for mechanical milking.

In these conditions, we resorted to the 'meat cow' exploitation technology, in which pregnant cows, including after calving, are kept together, in free stables, in simple shelters with a paddock for movement. Calves stay with their mothers and suckle the entire amount of milk produced. Calves stay with their mothers until weaning, which occurs on average at 210 days (7 l) when milk production deplets completely. Then the calves are weaned and transferred to collective stalls for youth.

The basic feed of lactating cows consists of good quality hay (15 kg/day) during the stable period, whereas in the summer they can be taken out to pasture. In addition to this diet, vitamin and mineral

supplements are also provided. It should be noted that the shelters comprise stalls for calving cows that stay in them for 5-6 days, after which they are allowed access to the exercise paddocks.

Body development of youth

According to the *'meat cow'* technology, newborn calves stay with their mothers until the average age of 7 months, when the mother's milk production deplets completely. At this point the calves are weaned, after which they will be fed with the existing assortments in the farm or household.

In order to study the growth rate of the youth, a series of measurements were taken for body weight, waist, rump height, chest circumference, chest depth and oblique chest length. These measurements were performed at regular time intervals: at birth, at 6 months, 12 months, respectively at 18 months.

After obtaining the data from the measurements, these were processed and statistically interpreted by calculating y, sy and V% (Czister L., 2008).

Table 1. Physical development of youth / Dezvoltarea corporală a tineretului

Specification	Birth	6 months	12 months	18 months
Body weight (kg)	40.00	186.42	325.00	410.00
Waist (cm)	78.08	101.28	115.37	125.60
Height at the rump (cm)	82.83	107.71	122.50	131.80
Chest circumference (cm)	76.75	128.71	159.25	171.80
Chest depth (cm)	28.08	44.28	56.25	62.60
Length oblica trunk (cm)	65.33	96.71	121.87	135.00

According to the data presented in Table no. 1, calves are born with an average body weight of 40.10 kg and reach 410 kg at the age of 18 months.

At birth, the calf's waist measures 78.08 cm. Waist size reaches 125.6 cm at the age of 18 months. Height at the rump increases from 82.83 cm at birth to 131.80 cm at 18 months.

Chest circumference at birth is 76.75 cm, reaching 171.00 cm at the age of 18 months. The depth of the chest is on average 28.08 cm at birth and measures 62.60 cm at the age of 18 months.

The oblique length of the trunk measures 65.33 cm at birth, reaching 135 cm at the age of 18 months.

The data in Table no. 2 summarizes the intensity of growth of body weight and dimensions in the period birth - 18 months of age. At the age of 6 months the measurements represent 46.25-81.70%, whereas at 12 months 80.64-93.12% of the parameters achieved at 18 months.

Table 2. Growth coefficient of body sizes (%) / Coeficientul de creștere a dimensiunilor corporale (%)

Age in months	Body regions					
	Weight %	Height %	Rump height %	Chest circumference %	Chest depth %	Oblique length of the trunk %
Birth	11.26	62.16	62.80	44.88	44.35	48.39
6	46.25	80.64	81.70	75.25	70.70	71.63
12	80.64	91.85	92.50	93.12	89.85	90.27
18	100.00	100.00	100.00	100.00	100.00	100.00

The chemical composition of meat

Immediately after the animals' slaughter and during storage, a number of biochemical transformations and a series of other processes occur in the meat, which, if carried out properly, positively affect the nutritional value and the organoleptic characteristics.

On the other hand, incorrect storage conditions lead to its degradation, worsening the quality of the meat.

In the meat industry, all the compounds of this raw material constitute, by its chemical composition, a favorable environment for the development of microorganisms.

Along with the evaluation of the carcasses of the slaughtered youth, we collected samples to determine the meat's chemical composition. The results are presented in table no. 3.

Table 3. Chemical composition of meat / Compoziția chimică a cărnii

Specification	Value %
Dry matter at 105°C	31.58
Fat content	6.02
Protein content	20.28
Water retention capacity	5.60
Bound water	21.01
Ash content	1.29
Thickness of muscle fibre	52
Meat color	0.38
pH	5.50

From the analysis of the data presented in the table, the values are in accordance to those quoted in literature: the dry substance content is of 31.50% (25-30%), the fat content of 6.02% (4.6%), the protein content of 20.2% (18-22%), while the water retention capacity has values of 5.0-5.6%, and the binding water of 21.0%.

Regarding the thickness of the muscle fiber of the meat, we found that it is 52 u, and the color of the meat expressed in Lange units has a value of 0.380, pink to red, specific to young age.

The pH of beef varies depending on its freshness (V. Ujica, 2005), with values ranging between 5-7. As the pH value indicates the time since slaughter and the storage conditions, the pH of 5.5 shows that the collected meat samples were fresh.

The organoleptic properties of meat

This group of properties belongs to the character that confers the palatability, flavor and attractiveness of the meat. The most important qualities in this group are: tenderness, juiciness, aroma and taste, marbling and color of the meat.

The organoleptic examination of the meat is done in bright spaces, without foreign smells, with a temperature range of 16-20 °C.

Tenderness is the basic criterion in the appreciation of meat (Temisan, 1990), it is defined as the '*easiness*' with which the meat is fragmented into small pieces by mastication.

Juiciness is given by the amount of juice in the meat and the water-protein ratio. It is reflected in two effects: the impression of moisture that appears during the first part of mastication, following the release of juice under the pressure applied by the teeth, and the permanent preservation of the sensation of juiciness, produced of muscle fat components that stimulate saliva secretion.

Following the consumption (chewing) of meat from the young male **Transylvanian Pinzgau**, we can conclude that this is tender and juicy.

The taste and aroma of the meat is another important characteristic that favors the secretion of saliva and gastric juice, indirectly participating in the process of digesting the substances from the meat.

The taste and aroma are specific to each species and depend on the sulfur and ammonia content of the meat. If these two substances are found in higher concentrations, the meat has a less pleasant taste and smell (Ujica - 2007).

After tasting, the prepared meat has a pleasant taste and aroma, highly appreciated by consumers.

The second group of properties that attract consumers are marbling, meat and fat color.

Marbling: this characteristic means the visible proportion of fat in the muscle tissue. In EU countries, meat with an average degree of marbling is preferred, i.e. an intramuscular fat content of 2.5-4.0%, a situation in which the meat is tender and juicy. In our study, the fat content was average (2-3%).

The color of the meat is one of the basic characteristics that attracts meat consumers. There is a correlation between color and quality.

For bulls, the pale pink color is preferred for veal, intense red for young bulls and dark red in adult animals. In our case the meat of young bulls had a pink to red color.

Fat color: the fat of bulls is generally of a yellowish-white color. The dark color of the fat is associated with the age or lack of freshness of the meat and with the conditions of meat storage.

In our experiment, the fat had a white - slightly yellowish color characteristic to young males.

Quantitative assessment of meat production

At the end of the research project, we performed control slaughters on a number of 5 bulls at the age of 18 months and an average weight of 410 kg. The slaughter was carried out at an authorized slaughterhouse, specialized for the slaughter of bulls.

The results obtained after the slaughter are presented in Table no. 4.

Table 4. Results after slaughter / Rezultatele în urma sacrificării

Specification		MU	Value
Live weight		kg	410
Hot case weight		kg	213
Cold case weight		kg	208
Slaugthering yield		%	54 - 56
Age at slaughter		days	540
Moss eye sourface	**Costal interval 8-9**	cm²	53
	Costal interval 11-12	cm²	79

Analyzing the data in the table, it shows that at the age of slaughter (18 l) the young males had an average live weight of 410 kg, the weight of the hot carcass being of 213 kg and after cooling 208 kg. The slaughtering yield was of 54 - 56%.

Various studies (C. Velea, 1999) have reported a salughtering yield varying between 44-58%, depending on the breed, sex, category and state of maintenance of the animals.

The slaughtering yield and the surface of the muscle eye in the slaughtered youth reveals the readiness of the breed for meat production. Even if breed that reaches maturity later than the **Baltata Romaneasca** or **Bruna de Maramures** breeds, we must mention that these animals were not fed in a fattening regime, but only to maintain their breeding condition.

In order to evaluate the carcasses after slaughter, they were cut. The data collected are presented in the table below.

Table 5. The structure of the casings / Structura carcaselor

Specification	MU	Value	%
Meat	**kg**	**155**	**74.7**
Fat	**kg**	**6.2**	**4.0**
Renal sebum	**kg**	**3.2**	**2.1**
Bones	**kg**	**27.0**	**17.8**
Flesh to bone ratio		**155:27 = 5.7:1**	
Case category		**A**	
Conformation		**R; O**	
Fat		**2-3**	

We note that the literature cites the following values of the carcass structure (C. Velea, 1999): meat 70-78%, fat 4.9%, bones 16-18%. Thus our results fall within the quoted limits.

The use of the EUROP method in assessing the quality of carcasses

For the assessment of meat production from a qualitative point of view, we resorted to the method imposed by the EC for the assessment of carcasses and their classification by authorized classifiers. (Movileanu G., 2009).

To assess the meat production of the youth, we slaughtered 5 young individuals, with an average age of 18 months.

The classification of cattle carcasses according to the EUROP method refers to 5 categories of animals. The slaughtered youth fall into category A- carcasses of young, uncastrated males up to 2 years old. Carcass classification is based on 2 criteria: conformation and the degree of fat coverage:

- for conformation, 5 classes have been provided, expressed by the letters E, U, R, O, P, ranging from superior to medium

- for the degree of fat coverage, 5 classes have been established , expressed numerically by 1,2,3,4,5, from skinny to maximum fat coverage. Each class refers to the conformation and the degree of fat coverage, which can be divided into 3 subclasses (-, =, +).

In the case of the youth from the "*meat cow*" experiment, the carcasses fall into conformation classes R and O+, with a good conformation and muscle development, well-rounded calf, thick back, fairly well-developed scapula.

The degree of fat coverage is situated between 2 and 3. Category 2 means a light coverage with fat, with the meat being visible everywhere. Category 3 means that the meat - with the exception of the thigh and back - is almost completely covered with fat, small deposits of fat in the chest cavity.

The figures below show aspects regarding: case, semicasing and youth meat in section.



Picture 3. Youth meat in the section / Carne de tineret în secțiune

CONCLUSIONS

Following the completion of the research project on the suitability of the **Transylvanian Pinzgau** breed for meat production we drew the following important conclusions:

The breed is well adapted to the environmental conditions of the mountainous area where it was formed. It resists to specific cattle diseases and, importantly, it makes good use of the meadows, either in the form of green grass or hay.

Compared to the group of mixed breeds in the country, it reaches maturity later: the calves first come into heat at the age of 22-23 months (considered the optimal age), with a body weight of 350 kg. The age of the first calving of the female youth is 31-35 months.

Body development is small compared to the **Baltata Romaneasca** breed. The weight of **Pinzgau** cows is on average 400-450 kg, with a waist of 127 cm, compared to the 550-600 kg weight and waist of 132 cm on average of the **Baltata Romaneasca** breed.

In addition to its mixed, rustic breed qualities of milk-meat-traction production, **Pinzgau** cows have major udder defects, asymmetrical quarters, long and thick teats, and for these reasons they are not suitable for mechanical milking.

The milk production of the cows is modest (1700 l/lactation) when compared to the **Baltata Romaneasca** cows (5500-6500 l/lactation) or to the **Bruna de Maramures** breed.

Considering the defects of the udder and the small production of milk per milked cow (of 6-7 l per day), we studied the suitability of the breed for meat production by applying the *'meat cow'* technology, which involves keeping cows together with their calves, which suckle the entire amount of milk until weaning (210 days), when the mammary gland stops secreting milk.

Calves left with their mothers during the calving-weaning period achieved a higher growth rate, with 80-100 g/day, compared to the calves whose mothers were kept in the classic system, whereby 5-6 days after calving the calves are fed with milk substitutes.

By using the *'meat cow'* technology, labor costs are eliminated for the manual or drum milking or for the purchase of the milking installation.

The maintenance of **Pinzgau** cows in the *'meat cow'* system, compared to the conventional system can bring a benefit to the farmers through the better values of the reproduction indices: calving interval reduced by 8 days, average number of inseminations/gestation 1.64 compared to 2.05, service period shorter by 5 days and the incidence of abortions lower, 0.17% compared to 1.44%.

The costs of setting up shelters for the 'meat cow' technology are greatly reduced, the consumption of milk substitutes is completely eliminated and the need for medicinal products is greatly reduced.

The ease of calving, the vigor of the calves at birth, the low percentage of ante- and post-partum diseases, and the sudden natural weaning of the calves do not affect at all the health status of the mammary gland in cows kept in the *'meat cow'* system.

After tasting and eating the meat from **Pinzgau** bulls, it was found to be tender, juicy, with a pleasant taste and aroma that were highly appreciated by consumers.

The yield at slaughter of 54 - 56%, the surface of the muscle eye of 53 cm^2, respectively of 79 cm^2 and the average body weight at slaughter (500 days) of 410 kg, reveals the suitability of the breed for meat production. This is true even if this breed reaches maturity later than the **Baltata Romaneasca** and **Bruna de Maramures** breeds, especially when considering that these animals were not fed in a fattening regime, but just to keep their breeding condition.

To assess the quality of the carcasses, we used the EUROP method, which showed that, after the slaughtering of the youth under study, the carcasses fell into the R and O+ classes, and presented a good conformation, as well as muscle development, well-rounded calf, thick back, and a well developed scapula.

The fat covering of the carcasses fell between grades 2-3, which means a light covering of the meat with fat, a commercial aspect much appreciated by buyers. The result of the evaluation of carcasses using the EUROP method is another argument that reflects the suitability of the Pinzgau breed for meat production using the *'meat cow*' breeding technology.

In conclusion, we can state that the keeping, breeding and exploitation of Pinzgau breed animals in the *'meat cow'* system is a viable option for cattle breeders in Romania, which can lead to the relaunch of meat production and the preservation of the genetic background of this breed, threatened by extinction.

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NUTRITIVE VALUE, USE AND IMPORTANCE OF MULBERRY LEAF FOR THE SILKWORM *BOMBYX MORI L.*

VALOAREA NUTRITIVĂ, UTILIZAREA ȘI IMPORTANȚA FRUNZEI DE DUD PENTRU VIERMII DE MĂTASE *Bombyx mori L.*

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Abstract

If the silkworm is the main interest point of sericulture, the mulberry is the beginning point since it produces leaves, the only feed supply for silkworm species Bombyx mori L. Due to the production of silk fibre, on the one hand, and the secondary products produced by the sector (pupae with specific by-products, respectively cakes, oils etc.), on the other hand, the breeding of silkworms is of great economic importance. The mulberry, specifically the quality of the mulberry leaves, is one of the most important factors in determining silkworm growth, production, and health status. The mulberry (Morus sp) is widely distributed in various climate zones, from tropical to temperate areas. The mulberry leaves have an excellent nutritional composition (proteins and amino acids, lipids and fatty acids, carbohydrates, vitamins and minerals, flavonoids etc.). Some volatile compounds contribute to the mulberry leaf's attractiveness, and one of the silkworm antennae receptors reacts significantly to cis-jasmone, which is thought to be the most alluring volatile component, even though it is naturally found in only trace amounts in the mulberry leaf. The digestive system and enzymatic equipment of the larva associated with the fermentative substances contained by mulberry leaves enable digestion and absorption of nutritive substances from the leaves, which are then converted into nutrients required for growth within the proper ranges and the production for obtaining a high-quality of silk fibres and valuable nutritional by- products. Given the crucial importance of the mulberries, new measures are necessary to grow and promote Bombyx mori silkworms, especially in rural regions.

Keywords: **mulberry, leaves, nutrients, silkworms, metabolism, applications**

Rezumat

Dacă viermele de mătase reprezintă punctul central de interes în sericicultură, dudul, prin producția de frunze, reprezintă punctul de plecare, ca unica sursă de hrană pentru viermii de mătase din specia Bombyx mori L. Creșterea viermilor de mătase are o importanță economică deosebită dată, pe de o parte, de producția de fibră de mătase, iar pe de altă parte de produsele secundare generate de sector (pupe cu subproduse specifice, respectiv turte, uleiuri etc). Creșterea, producția și starea de sănătate a viermilor de mătase, sunt aspecte care depind de o serie de factori, dintre care primordial este dudul, și anume calitatea frunzei de dud. Dudul (Morus sp.) este dispersat extensiv în diverse suprafețe climatice, de la zone tropicale la temperate. Frunza are o compoziție excelentă în substanțe nutritive (proteine și aminoacizi, lipide și acizi grași, carbohidrați, vitamine și minerale, flavonoizi etc.). Atractivitatea spre frunza de dud este dată de o serie de compuși volatili, iar unul din receptorii din antene, răspunde puternic la cis-jasmone, considerat compusul volatil cel mai puternic atractant, deși este prezent natural în cantități mici în frunza de dud. Sistemul digestiv al larvei și echipamentul enzimatic specific, asociat cu fermenții din frunza de dud, permit digestia și absorbția substanțelor nutritive din frunză, ce sunt convertite mai departe în nutrienți necesari pentru creșterea în parametrii corespunzători și obținerea unor fibre de mătase de calitate și a unor sub-produse valoroase nutrițional. Dată fiind importanța crucială a dudului pentru sector, se impun noi măsuri de extindere a suprafețelor cultivate cu dud și de promovare a creșterii viermilor de mătase, cu precădere în zone rurale.

Cuvinte cheie: dud, frunza, nutrienți, vierme de mătase, metabolism, aplicații

INTRODUCTION

Sericulture is a complex science that combines art and science to obtain silk fibre (as the main product) and secondary by-products (e.g., pupae with their waste cakes and oils), with multiple applications in various economic sectors (Jasmine and Mandal, 2014; Oduor et al., 2021). The economic value of sericulture depends on several ways of valorising products and by-products in the industry sector (Sharma et al., 2022).

The starting point of sericulture is the mulberry tree which belongs to the *Morus* genus and *Moraceae* family and has a wide geographic distribution in tropical and temperate regions. *Moraceae* is a family of plants from more than 64 species, subspecies, and a minimum of 100 varieties identified. The *Morus* word comes from Latinus *"mora*" and means, most probably, *delay* due to the slow development of its buds.

M. alba is the dominant species (Jan et al., 2021). The leaves are very important from the ecological point of view and as feed for silkworms being the single diet for *Bombyx mori (B. mori)*. The pupae are directed principally to obtain silk production.

Greeks and Romans have cultivated mulberry since ancient times. Since 220 D.H., Emperor Elagabal was wearing a silk coat. The clergy members in England have worn silk robes since the 1500s. Mulberry and silk industry play a major role in Virginia (Tang et al., 2016; www.wikipedia.com).

Mulberry (*Morus sp*.) is a fast-growing plant adapted to temperate, tropical, and subtropical climates. In Romania, sericulture first developed in Transylvania and Banat in the 14th century (1348). In the 18th century, the Turks introduced sericulture practices in Moldova and Muntenian. For supporting sericulture, 60000 mulberry saplings were distributed for free in Moldova in 1845, for being cultivated in the South of the region. In Banat, mulberry is regarded as a symbol. In the 18th century, the Habsburgs introduced mulberry to feed silkworms. Sericulture developed in this area, such as Banat becoming the first silk exporter (Doliș, 2008). Leaves were considered the single feed for silkworm *Bombyx mori* L. (Sanchez, 2000; Thaipitakwonga et al., 2018).

Due to the essential oils, flavonoids, terpenes, and flavonoids used by silkworms for digesting processes, along with the nutritional content of the leaves, there may be an unpleasant odour. Palatability issues can be due to this odour (Finke, 2002; www.feedpedia.com). The ability to obtain high-quality cocoons depends on how effectively the nutritional components of the mulberry leaf are used. Pupae represent approximately 60% of the dry cocoon (Tassoni et al., 2022).

1. NUTRITIONAL VALUE OF MULBERRY LEAVES

The studies show that mulberry leaves contain toxic substances and have higher palatability than other vegetable leaves (Srivastava et al., 2006). The composition depends on variety, age, harvest time, and environmental conditions. The primary nutrients from the mulberry tree are proteins, carbohydrates, vitamins, sterols, and minerals (Raghuvanshi et al., 2019; Alipanah et al., 2020). Such as Srivastava et al., (2006) mentioned, the powder of mulberry leaves has a content in water ranging between 5.11–7.24%, crude protein (CP) between 15.31–30.91%, ash 14.59–17.24%, neutral detergent fibre 27.60–36.66%, fat 2.09–4.93%, carbohydrates 9.70–29.64%, and energy between 113–224 kcal/100 g.

Generally, the CP composition is comparable to that of most legumes. Several authors (Sanchez, 2000; Raghuvanshi et al., 2019) determined a CP level ranging from 15–28%, while Sanchez-Salcedo et al. (2017) found a level of 13.4–19.4%, Iqbal et al. (2012) a level of 18.41–24.63%, and Adeduntan and Oyerinde (2010) reported a level of 21.24–21.66%.

Compared with other plants, the fibre level is lower, although it depends on many factors such as season, age, etc. Calcium concentration is about 0.14–0.24%, and phosphorus is 1.8–2.4% (Sanchez, 2000). In 2000, Liu et al. specified that the leaves have a higher nutritional value in spring than in autumn.

Furthermore, Srivastava et al. (2006) highlighted that mulberry leaves contain important quantities of beta-carotene (14.688 mg/100 g) *vs* other plant leaves consumed usually (spinach, amaranth, fenugreek). Furthermore, iron and zinc, acid ascorbic and antioxidants are contained in mulberry leaves.

Two new flavonoids were also identified in leaves (Wang et al., 2009). Phytochemical components isolated from leaves and fruit extract from mulberry *Morus rubra* were reported by Sharma (2010). Recently, the antioxidant potential of extracts from different parts of mulberry plants leaves, roots, and fruits have been investigated by many authors (Andallu and Varadacharyulu, 2002; Arfan, 2012).

As perennial plants, the mulberry tree is characterised by a deep root system, high biomass production and remarkable adaptability. The mulberry leaf has a strong absorption capacity that is believed to be helpful in controlling air pollution. It has demonstrated an encouraging result for stabilizing the adverse effects of heavy metals in various polluted soils (Ghosh et al., 2017).

The attractivity of silkworms for mulberry leaves intake can be explained by 20 volatile compounds. From a neutral fraction of essential oil of old mulberry leaves, Ɓ-Ɣ hexanol and α-Ɓ hexenal compounds attract the larvae. Twenty olfactive receptors are active in the antennae of silkworm larvae. From these receptors, one named *cis-jasmone* is considered the most strong and most attractive volatile, although it is in small quantities in leaves (0.3–300 ng). When the olfactive receptor is triggered to become active, insects move to the odour source. Citral also attracts silkworms when administrated within 30-300 ng, but not in small amounts. Linalol, hexenal, hexyl acetate and acetate 2-hexenyl presented an attractive activity at 30 ng, or 3 ng (Tanaka et al., 2009).

2. PROCESSES OF DIGESTION AND ABSORPTION IN SILKWORMS

The digestion and absorption of nutritional compounds from mulberry leaves present particularities due to the specificity of the digestive system described below.

It is essential to the fact that throughout the life cycle of the silkworm, only in the larval period does the silkworm consume feed based exclusively on mulberry leaves. In this phase, silkworms use nutritive compounds for growing and development and accumulate reserves for successive periods of growth.

2.1. The digestive tract of the silkworm

In the silkworm's body structure, such as seen by the larva's dissection, immediately under the skin at the median line of the dorsal surface of the larva, there is a dorsal vessel that surrounds many muscles and fat. Along the inside of the body is the digestive tube in the form of a straight tube with the trachea on both sides, and its dorsal side is the sericigenic gland. Beneath these organs are muscles.

Three main parts characterize the larva: the foregut (stomodeum), where feed is fragmented and stored; the middle intestine (mesenteric), where take place the digestion and absorption processes and the posterior intestine (proctodaeum), where waste is collected and decomposed substances are excreted or reabsorbed.

The foregut has the oral cavity, oral cavity, pharynx and oesophagus. At the end of the foregut, there is a cardiac valve that retain for a while, shredded leaves, to avoid regurgitation. The middle intestine (stomach) is the most significant part of the digestive tube. Goblet cells (intraepithelial, unicellular, scattered mucin secretors in simple epithelia) secrete the digestive fluid, and the cylindrical cell absorbs the digesta. The foregut and hindgut have a chitinous lining, and the peritrophic membrane in the midgut protects the epithelium from damage caused by feed particles. The posterior intestine has three components, the small intestine, cecum and rectum; a pyloric valve placed at the end of the small intestine protects and regulates the passage of feed particles from the middle to the posterior intestine. At the posterior segment of the digestive tube, the water is absorbed (a part re-enters the circuit), and the

undigested substances are eliminated. The rectal muscles exert mechanical pressure on the excreta (https://hbmahesh.weebly.com; Doliș, 2008).

2.2. Enzymatic equipment of silkworm

The enzyme involved in the digestion process is secreted in saliva and the middle intestine, the last having a vital role in digestion and absorption. The intestinal microorganisms can facilitate digestion. In other words, digestion consists of converting insoluble substances into soluble ones, while the impermeable substances become permeable. The most intense digestion process is in the middle intestine, where enzymes are secreted.

The digestive juice includes salivary and gastric juice. Saliva is a solution weakly alkaline and contains amylase. The gastric juice is strongly alkaline, pH of 9.2–10.3. The main enzymes contained in it is tyrosine (protease enzyme), lipase (the enzyme that acts on lipids), amylase, maltase and glycogenase (enzyme for decomposition of carbohydrates), oxidase, peroxidase, catalase, tyrosinase (enzymes oxidative). Amylase breaks down starch into destrins and maltose.

2.3. Digestion and absorption of silkworm

2.3.1. *Digestion*

The digestive system of the larva is well developed, while at pupae and moth are vestigial and non-functional.

Larva uses the digestive system to digest and absorb nutrients and other substances from consumed leaves. Macromolecules and other complex substances, such as proteins, polysaccharides, fats, nucleus acids etc., are taken over from mulberry leaves and subjected to a metabolic process. These substances must be beaked down by catabolic reactions in the small molecules, such as amino acids, fatty acids, and simple sugars, for passing into tissues and are used by own body cells for energy, growth, reproduction and, on the other hand, as substances required for body tissue synthesis. This process is named digestion (https://drive.google.com/file/d/1_H4Nps4-pkOX_K31Ni1QGgKy4BscnBiq/view).

The silkworms have considerable variation in the digestion, absorption and conversion capacity of the nutritive substance into body substances, depending on leaves quantity, climate variations, and feed (Rahmathulla and Suresh, 2012).

It is generally accepted that nutritive parameters are correlated with feed intake and silk production. The conversion of mulberry leaves into silk is highly correlated with the digestion capacity of the silkworm. A more accurate economic indicator for producing cocoons is the effectiveness of the conversion of mulberry leaves intake in silk or the conversion rate of active substances from leaves (Rahmathulla and Suresh, 2012). Only 620.70 kg of 2472.80 kg mulberry leaves produced/ha cultivated with mulberry plants are digested by silkworms and converted to 211.20 kg silk (Priyadharshini et al., 2017).

At *Bombyx mori* silkworm the haemolymph composition consists of 24–30 mg/mL protein and more than 1500 mg/mL lipids, while pupae have a weight that ranges between 705–885 mg (Mahmoud, 2017).

2.3.2. Absorption

The absorption of nutritional substances implies the passages from the digestive tube into the blood directly or indirectly through the lymph. Among the leaves' components, certain elements, such as glucose, soluble salts in the water etc., are absorbed directly by epithelial-specific cells in the middle intestine. Still, proteins and other macromolecules of large size can't penetrate through the cell wall. In this regard, by the decomposition into simple particles must be easily assimilable.

There are differences between the protein in mulberry leave and the protein from the silkworm body. The specific protein of mulberry leaves is broken down by an enzyme in amino acids and peptones

and then is absorbed in the middle epithelial intestine and transported to organs through haemolymph. Into organs, the amino acids and peptones are re-synthesised in animal protein, a process named assimilation. The fat is re-synthesised in fatty acids and glycerol. Behind this synthesis, the fat can be synthesized from carbohydrates. An important part of mulberry leaf carbohydrate is synthesised in fat into the larva body.

3. EFFECTIVENESS OF FEED UTILISATION

Appropriate feeding is a critical indicator of the economic value of silkworm development and a factor in determining the superiority of specific breeds or hybrids' productive characteristics (growth, development, and silk production (Rahmathulla et al., 2005). The efficacity of digestion implies a superior conversion of nutrients from silkworm into silk.

The conversion efficacity of ingesta (ECI) represents an important economic indicator of the assessment of cocoon production. The digestion and absorption process depend on hybrid/breed, the environmental factors, sex and quantity of feed.

Several methods to improve the rate of conversion in silk were highlighted by many authors (Benchamin et al., 1984; Chandrakala et al., 1999; Rahmathulla et al., 2005). The methods focus on respecting specific environmental conditions are the, growing the variety/ hybrids with superior characteristics, appropriate mulberry variety, diets according to qualitative and quantitative requirements, and respect for specific growing procedures. A high feed intake in larval phase 5 positively influences body weight and silk production. Females consume more feed than males, and monovoltine breeds ingest and digest a higher quantity of feed. The digestibility of leaves is higher in monovoltine species, although in polyvoltine breeds, diet digestibility is significantly higher (Tzenov et al., 1999).

A positive correlation is found between leaves intake and the protein level in silk fibre. The leaves' intake influences as well the reproduction activity and quantity of biomass accumulated. The newly created hybrids valorise the feed better compared to the traditional one.

In the larvae phase, digestibility remains more or less constant from the first day up to the middle of the growing phase; after that, a decline is registered up to the spinning process. The efficacity with which the feed is digested is converted to body mass decreases with age (Rahmathulla et al., 2005). The quantity of dry matter consumed and digested, ECI, the final weight of the larva and the weight of the cocoon increase significantly, being correlated with water quantity from leaves. A less water content extends larval phases.

The mean of parameters relies on ingesta and digesta of silkworm feed with three varieties of mulberry Kenmochi (*Morus bombycis Kodiz*), black mulberry (*Morus nigra* L.), and native mulberry (*Morus alba* L.) on the entire larval period, and they were presented by Alipanah et al., (2020), respectively 34% DM, leaves ingested 5.16 g, excreta 2.20 g, leaves digested 2.42 g, digestibility 44.2%.

Figure 1 shows the biological cycle of silkworm growing, production and health, and potential industry application.

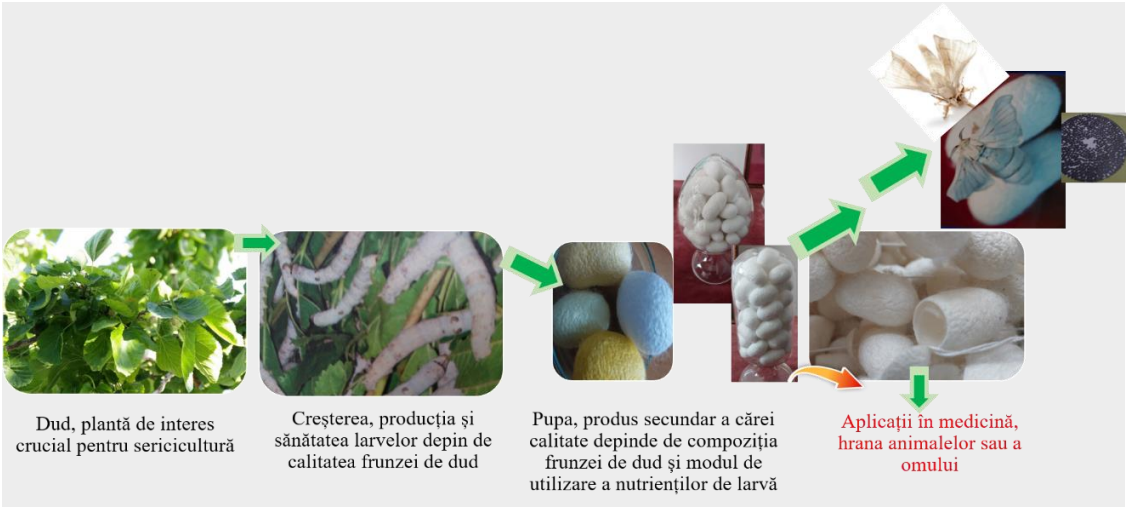


Figure 1. Biological cycle, nutritional quality and applications of the silkworm growth / Ciclul biologic, calitate nutriţională şi aplicaţii ale creşterii viermilor de mătase

4. IMPORTANCE OF MULBERRY CULTIVATION

All those described above highlight mulberry leaves' primary role in the silkworm diet. Of crucial importance is that leaves are stocked with all the nutrients the larva needs for body functions (growing, reproduction, silk production). In addition, the positive involved for health through the conversion of nutrients in compounds beneficial to the insect's health is also important. From an economic point of view, silkworms also have an important role. However, this species cannot survive without mulberry plants.

The valuable components from leaves and fruits make these plants a suitable functional food for human health besides the primary nutritional value (Kadam, 2019).

Practically, the protein contained in mulberry leaves is transformed into silk that includes 2 types of protein (fibroin and sericin). This means that the silk produced in this way can be used commercially for silk-coats. It is estimated that silk obtained from silkworms greatly contributes to the global production of silk (approx. 90%) and is a very attractive economic activity, especially for the rural population, significantly contributing to several social groups. Behind these aspects, many nutritional and medicinal benefits are demonstrated by the mulberry plant. Thus, mulberry leaves are used for numerous other things besides feeding silkworms. The leaves and fruits are protein and vitamins-rich and were exploited long ago as feed to animals in many countries. Due to its high nutritional value and savoury, the mulberry fruit can be considered a valuable food.

Mulberry bark and wood are also useful for making paper and sporting goods. The evaluation of several medicinally important pharmacological compounds present in the mulberry plant has recently opened new avenues of medical science research. Furthermore, studies demonstrate antioxidant effects, antivirals, anti-inflammation, hypolipidemic, anti-hyperglycaemic, neuroprotective activities (Pan and Lou, 2008), anti-HIV, anti-hypotensive and cytotoxic of different mulberry species (Du et al., 2003). Furthermore, fruits, roots and leaves extract can be used in cosmetics, dermatological skin care products, gels, and many others due to a greater capacity for capturing free radicals.

The mulberry is very important from the economic point of view and for the environment, taking into consideration many aspects of its role as feed for animals, phytomedicine, and cleaning up contaminated soils and air etc. (Ghosh et al., 2017). Large quantities of wastes from the sericulture industry (such as parts of mulberry plants and excrement of silkworms), nutritive-rich, are generated during the growth period of silkworms fed with mulberry leaves. It is estimated that 15 tonnes of waste

(including crop and agricultural waste) are generated annually from one hectare of the mulberry farm. Identifying ways to utilize these wastes properly can open up new opportunities for income generation for sericulture farmers. However, the surface cultivated with mulberry trees recorded a significant decline in Europe, including Romania, and immediate measures must be taken to revitalize and expand cultivated areas. In this way, the Government Rules (H.G. no. 695/2022) on the management and administration of the Research Station for Sericulture Baneasa-Bucharest for the further development and restoration of the value of the sericulture sector is very important.

CONCLUSION

Besides the aspects that rely on the economic importance of growing silkworms due to silk production and valuable by-products, mulberry leaves are a central point of interest. The mulberry (genus *Morus*, family *Moraceae*) is widely dispersed in various climatic areas, from tropical to temperate zones. Mulberry leaves have become very important for sericulture, especially since they are the only feed source for the species *B. mori* L. silkworms.

The nutritional value of mulberry leaves, the protein and amino acids, lipids and fatty acids, carbohydrates, vitamins and minerals, flavonoids etc., are adequate for growth and good health status. We can mention that mulberry has economic and environmental importance, considering many aspects of their role in various sectors, such as animal feed, phytomedicine, and cleaning up the soil of pollutant substances and the atmosphere. Large quantities of sericulture wastes, such as leftover plants and excreta of silkworms rich-nutritive are generated from this industry. Identifying correct ways of waste valorisation opens new opportunities for generating income for farmers practising sericulture. In particular, during the early stages of growth, the silkworm's digestive tract possesses properties that enable it to consume the leaf and absorb nutrients in a relatively high proportion. The silkworm's enzymatic equipment and the fermentation process in mulberry leaves considerably aid in the nutritional principles' ability to be digested.

New initiatives are needed to increase the mulberry cultivation areas and encourage the development of silkworms, particularly in rural areas.

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STUDY ON THE *APINOSEM FORTE* EFFECTIVENESS IN RABBITS COCCIDIOSIS

STUDIU PRIVIND EFICACITATEA PRODUSULUI *APINOSEM FORTE* ÎN COCCIDIOZA IEPURILOR

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Abstract

Coccidiosis is a parasitic disease frequently encountered in rabbit farms, affecting especially young animals. Influenced by the factors of non-specific aggression, this parasitosis can cause, depending on the location, digestive disorders, weight loss, and hepatomegaly. In large farms, chemoprophylaxis provides protection for rabbits. In households, once diagnosed, a specific treatment is instituted. The veterinarian must evaluate the degree of toxicity, the cost, and the method of administration, but also the risk of blood absorption (in the case of hepatic localization) when it must choose the therapy. Sulfaveridin, Toltrazuril, Diclazuril, Sulfachloropyrazine, Robenidin, and Salinomycin are just a few of the anticoccidials whose effectiveness has been demonstrated, although currently natural products, natural extracts from plants, fungi, and microorganisms, Aloe vera and the unconventional use of liquid paraffin are becoming remarkable in the treatment of coccidiosis in rabbits. The present therapeutic study aimed to test the efficacy of Apinosem forte, a product obtained from natural plant extracts, in treating an experimental group consisting of 80 rabbits naturally infested with Eimeria spp. Coprological, necropsy, and histopathological examinations were performed; the therapeutic protocol was applied and the results were interpreted from a statistical point of view. We recommend Apinosem forte, administered 3 ml/5 l water, in the prevention and combating of coccidiosis in rabbits. It is the first report on the therapeutic results of Apinosem forte used in the prevention and control of this parasitosis in rabbits.

Keywords: rabbits, Eimeria spp., Apinosem forte, therapeutic efficacy

Rezumat

Coccidiozele sunt afecțiuni parazitare întâlnite frecvent în crescătoriile de iepuri, afectând mai ales animalele tinere. Influențate de factorii de agresiune nespecifică, aceste parazitoze pot determina, în funcție de localizare, tulburări digestive, scăderea în greutate, hepatomegalie. În marile crescătorii, chimioprofilaxia asigură protecția iepurilor. În gospodăriile populației, odată diagnosticate, se instituie un tratament specific. În alegerea anticoccidicului, medicul veterinar trebuie să evalueze gradul de toxicitate, costul și modul de administrare, dar și riscul absorbției sangvine (în cazul localizării hepatice). Sulfaveridin, Toltrazuril, Diclazuril, Sulfacloropirazina, Robenidin, Salinomicin sunt doar câteva dintre anticoccidicele a căror eficacitate a fost demonstrată, deși, în prezent, produsele naturiste, extractele naturale din plante, ciuperci și microorganisme, Aloe vera și utilizarea neconvențională a parafinelor lichide devin candidate remarcabile în tratamentul coccidiozei iepurilor. Studiul terapeutic prezent a urmărit testarea eficacității Apinosem forte, un produs obținut din extracte naturale din plante, în tratamentul unui lot experimental alcătuit din 80 de iepuri infestați natural cu Eimeria spp. Au fost efectuate examene coproparazitologice, necropsice, histopatologice; s-a aplicat protocolul terapeutic și s-au interpretat statistic rezultatele obținute, care recomandă Apinosem forte, administrat 3 ml/5 l apă, în prevenirea și combaterea coccidiozei iepurilor. Este prima raportare privind rezultatele terapeutice ale Apinosem forte utilizat în prevenirea și combaterea acestei parazitoze la iepuri.

Cuvinte cheie: iepuri, Eimeria spp., Apinosem forte, eficacitate terapeutică

INTRODUCTION

The most notable disease of rabbits, coccidiosis, causes massive economic losses, high mortality, and morbidity and is caused by fifteen species of *Eimeria* of varying pathogenicity. Rabbit coccidiosis is a ubiquitous infection caused by obligate intracellular protozoa belonging to the genus *Eimeria* and is considered a major cause of significant morbidity and mortality [3, 8, 35]. Three forms of coccidiosis are known: intestinal coccidiosis, in which the protozoan invades the epithelial cells of various regions of the intestines, leading to moderate to severe damage, depending on the virulence of the species, hepatic coccidiosis, the site of predilection for the species *E. stiedae* and otorhinopharyngeal localization [3, 8].

The occurrence of coccidiosis in rabbits is aggravated by poor hygiene and high animal densities that allow the spread of the parasite. Oochists have a remarkable ability to survive in the environment, which makes parasitological control difficult [8, 35].

Currently, the attention of researchers is directed toward different natural strategies regarding the treatment and prevention of parasitic infestations. Studies show that proper hygiene combined with strict biosecurity and effective therapy plays a significant role in preventing and spreading these conditions [19].

Despite their success in the poultry industry, vaccination attempts with live or attenuated vaccines have had unsatisfactory results in rabbits. In the rabbit, coccidiosis control should address not to only prevention of disease and excess mortality, but also mild and subclinical infections, as even minor intestinal lesions can interfere with growth and feed conversion and thus profitability, state Bhat et al., 2010 [5]. Therapy of rabbit eimeria focuses on the products Amprolium and Toltrazuril [18]. The development of varying degrees of tolerance to coccidiostats drugs in feed and the side effects inherent to ionophore coccidiostats prompt producers to explore other control measures [27]. This is of particular relevance because, with the exception of some alternative rearing systems, most systems depend on pharmacological control of coccidiosis [12, 24].

According to the bibliographic studies, we note the therapeutic efficacy of various medicinal substances used to control this parasitosis. The therapeutic evaluation was based on the estimation of production data, body weight, body weight gain, feed consumption, feed conversion rate, performance index, and reduction of oocyst production indicating anticoccidial activity [11]. In the choice of the anticoccidial, when we talk about hepatic coccidiosis, the veterinarian must evaluate the degree of toxicity, the cost, and the way of administration, but, in particular, the risk of blood absorption [8].

Starting from the herbal food supplement (patent no. RO130489-B1) intended to prevent and combat microsporidiosis (*Nosema* spp.) which contributes to the massive loss of bee colonies in Europe and all over the world, we arrived at the product *Apinosem forte*, extract natural from plants, which proved to be effective in treating this parasitosis. Administration of the product to bees infested with *Nosema* spp. or in the prevention of the disease is not followed by the presence of residues in the products of the hive, thus fulfilling one of the most important European requirements, regarding human health [8].

Although synthetic anticoccidials remain the main agents of therapy, natural products, natural extracts from plants, fungi, and microorganisms, Aloe vera and the unconventional use of liquid paraffin are becoming outstanding candidates in the treatment of rabbit coccidiosis [1,10].

In this context, the aim of the study was to assess the therapeutic efficacy of the product *Apinosem forte*, a natural extract from plants, in the treatment of coccidiosis in rabbits.

MATERIALS AND METHODS

Animals taken in the study

The study was carried out over a period of one year, on a number of 80 rabbits naturally infested with *Eimeria* spp. During the study, the rabbits were hospitalized in the Clinical Veterinary Hospital of

the Faculty of Veterinary Medicine Timișoara. They were raised in metal cages under the same microclimate conditions. The rabbits were aged between 2-6 months. They were given water at will. No other antiparasitic treatment was administered to them.

Coproparasitological examination

Fecal samples were examined in the laboratory of the Parasitology and Parasitic Diseases discipline of the Timișoara Faculty of Veterinary Medicine. The coprological methods used for the identification and quantification of *Eimeria* spp. oocysts were the flotation method, respectively the Mc Master method [9]. Coprological methods were applied throughout the treatment to estimate EPGs.

Necropsy examination

It was performed in accordance with the necropsy technique [9].

Histopathological examination

In the succumbed individuals, we performed a histopathological examination from the liver sections parasitized with *Eimeria stidae*, respectively from the intestinal sections, using the usual work techniques [20, 22, 38].

The therapeutic protocol

In the present study, we administered the product *Apinosem Forte* – a product recommended for the prevention and combating of bee nosemosis and obtained through the collaboration between ULS Timișoara and the company Dulcofruct Cirast Focșani (Table 1; figure 1) [20].

Table 1: *APINOSEM FORTE* data sheet / Fișa tehnică *APINOSEM FORTE*

Product	**APINOSEM FORTE**			
Origin	Romania			
The description product	**Apinosem Forte – Stimulant adjuvant for preventing and combating nosemosis**			
Ingredients	Essential plant oils, plant maceration, and infusion, apple cider vinegar, garlic tincture			
Properties organoleptic	**appearance**	**Color**	**Smell**	**Taste**
	Liquid	Brown	plants	Specific
Administration	**Syrup**:-2 ml/1kg syrup. 200ml syrup with APINOSEM FORTE/hive **Cake**:4 ml/kg cake 500g with APINOSEM FORTE/hive **RECOMMENDATION**: In the case of prevention, two administrations in the spring and two administrations at the beginning of autumn are recommended (in total 4 administrations per year). The interval between administrations is 10 days. In the case of the visible presence of the disease, the preventive administration is not sufficient and it is recommended 4-5 administrations with a break of 4 days between administrations.			
Use	This product is used in the prophylactic treatment of *Nosema apis* and *Nosema ceranae* parasites			
PACKING	Bottles 50 ml, 200 ml			
Storage and validity	**STORAGE CONDITIONS**: at a temperature between -15ºC and + 35ºC **TERMS OF VALIDITY**: 12 months from the date of manufacture written on the packaging			
Author/Producer	**USAMVB Timisoara/SC CIRAST SRL-ROMANIA**			



Figure 1. ***Apinosem Forte / Apinosem Forte***

The rabbits were divided into three groups of 20 individuals each.
Batch I – treated with *Apinosem Forte* – 2 ml/5l water
Lot II – treated with *Apinosem Forte* – 3 ml/5l water
Lot III – treated with *Apinosem Forte* – 4 ml/5l water
Lot IV – infected and untreated
The product was administered daily, in drinking water, for 30 days.

Statistical interpretation

To determine the effectiveness of the treatment, the results were statistically interpreted by applying the Kruskal Wallis and Mann-Whitney tests, to determine the significant differences between the three infected and treated groups, respectively the infected and untreated group 4 (control group). Descriptive statistics established the differences between the characteristics followed throughout the experiment: degree of the infestation, color of feces, clinical signs, EPG value, and water consumption [44].

RESULTS AND DISCUSSION

All infested rabbits in groups 1, 2, 3, and the control group showed clinical signs: restlessness, depression, dry fur, loss of appetite, weight loss, bloody watery diarrhea, and dehydration.

Eimeria spp. oocysts were identified in the flotation examination (figure 2) and we determined in the Mc Master examination, a parasite load between 1500 – 58,000 EPG.

The results of the necropsy examination revealed the presence of intestinal lesions: thickening of the intestinal wall, small ulcerations, hemorrhages, desquamation of the intestinal mucosa, as well as liver lesions: hepatomegaly, the presence of numerous gray-whitish nodules.

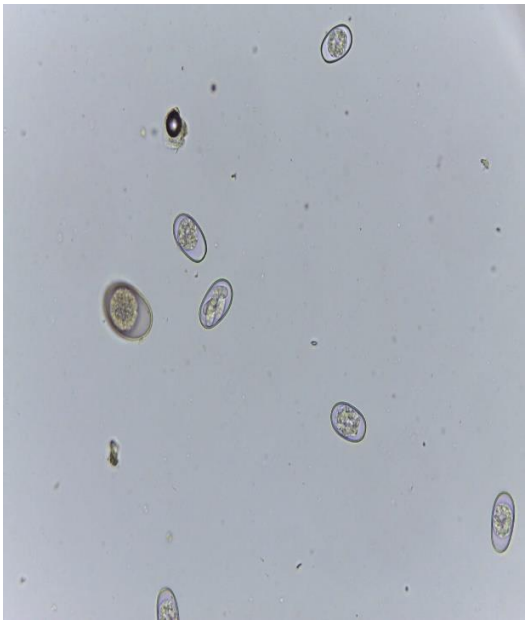


Figure 2. *Eimeria* spp. – oocysts / *Eimeria* spp. – oochiști

The histopathological examination revealed hepatic coccidiosis caused by *Eimeria stiedae*. The presence of extensive biliary hyperplasia with numerous intralesional coccygeal elements was found. The bile ducts were dilated and lined with hyperplastic columnar epithelial cells. Numerous developmental stages of the protozoa, ranging in size from 25-50 microns in diameter, were present within the epithelial cells. The bile ducts were surrounded by large amounts of fibrous connective tissue with lymphohistiocytic inflammatory infiltrates (figure 3).

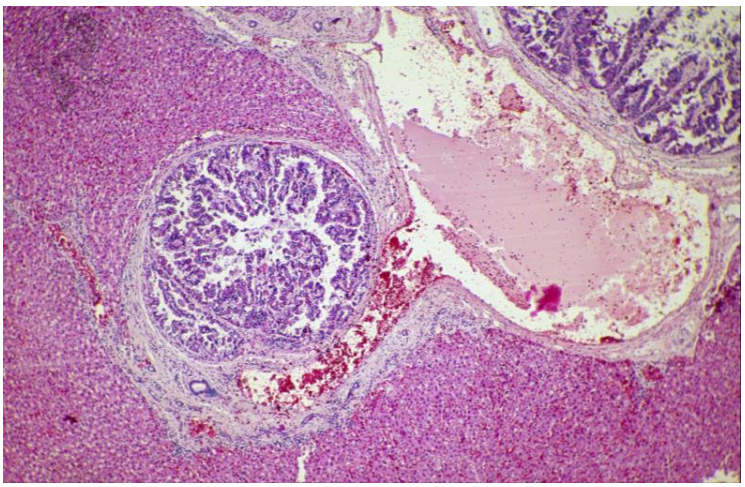


Figure 3. HP aspect on the liver - epithelial hyperplasia – *Eimeria stiedae* (H&E, 40x) / Secțiune HP în ficat - hiperplazie epitelială – *Eimeria stiedae* (H&E, 40x)

In the intestines infested with *Eimeria* spp., different stages of development of the protozoan involved in the etiology were highlighted. HP examination also revealed severe infiltration with inflammatory cells, mainly lymphocytes (figure 4, a-f).

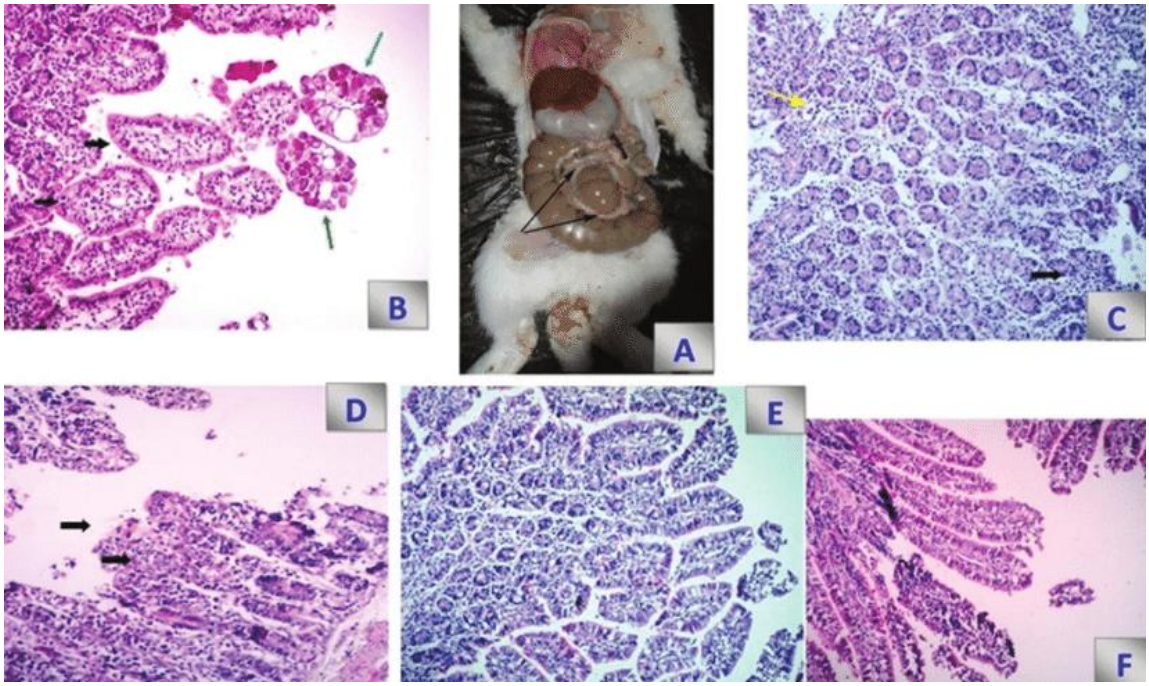


Figure 4. HP aspects on the *Eimeria* spp. infested intestines A. congestion B. cell necrosis, *Eimeria* spp. presence C, D, E, F. epithelial desquamation, lymphocytes infiltrated (H&E × 40) / Secțiune HP în intestin parazitat cu *Eimeria* spp. A. congestie B. necroza celulelor, prezente stadii de dezvoltare ale *Eimeria* spp. C, D, E, F. descuamare epitelială, infiltrate limfocitare (H&E × 40)

During the treatment, rabbits from the three infected and treated groups (L1, L2, L3) showed an improvement in health status, remission of clinical signs, and a significant ($P < 0.05$) reduction of EPG in feces in comparison with the infected and untreated group (L4). In the control group, the clinical signs did not remit, the EPG did not decrease, and 5 individuals succumbed 1 week after the start of experiment.

The results of the descriptive statistics are presented in table 2.

Table 2. Kruskal Wallis results / Rezultatele testului Kruskal Wallis

Statistics test [a,b]					
	Infestation	Fecal color	Clinical signs	OPG	Water consumption
Chi-Square	18,869	7,047	25,143	23,692	9,455
df	2	2	2	2	2
Asymp. Sig.	0,000	0.029	0,000	0,000	0.009
a. Kruskal Wallis Test					
b. Grouping Variable: Batches					

Analyzing the data obtained comparing the control group and the group that was administered ***Apinosem forte* 2ml/5l water**, we can notice significant differences in the case of infestation (U=30.00, p=0.000<0.05), fecal color (U=37.50.00, p= 0.000<0.05), clinical signs (U=22.5, p=0.000<0.05) and water consumption (U=72.00, p=0.000<0.05) (table 3).

Table 3. *Apinosem forte 2 ml* - Mann-Whitney test / Testul Mann-Whitney - *Apinosem forte 2ml*

	Infestation	Fecal color	Clinical signs	OPG	Water consumption
Mann-Whitney	30,000	102,000	37,500	22,500	72,000
	150,000	222,000	157,500	142,500	192,000
	-3,994	-.493	-3,808	-4,318	-2,282
	.000	.622	.000	.000	.022
	.000b	.683b	.001b	.000b	.098b

Between the control group and the group that was administered ***Apinosem forte* 3ml/5l water**, significant differences were evident in all the studied manifestations (table 4), the corresponding probabilities being all lower than 0.05.

Table 4. *Apinosem forte 3ml* - Mann-Whitney test / Testul Mann-Whitney - *Apinosem forte 3ml*

	Infestation	Fecal color	Clinical signs	OPG	Water consumption
Mann-Whitney	30,000	63,500	37,500	30,000	44,500
	150,000	183,500	157,500	150,000	164,500
	-3,994	-2,239	-3,808	-3,985	-3,348
	.000	.025	.000	.000	.001
	.000b	.041b	.001b	.000b	.004b

Analyzing the results after the administration of the treatment in the case of the ***Apinosem forte* 2ml** group vs the ***Apinosem forte* 4ml group**, significant differences were obtained only in the case of the fecal color variable (U=63.5, p=0.011<0.05) (table 5).

Table 5. *Apinosem forte 4ml* - Mann-Whitney test / Testul Mann-Whitney - *Apinosem forte* 4ml

	Infestation	Fecal color	Clinical signs	OPG	Water consumption
Mann-Whitney	107,000	63,500	112,500	105,000	109,500
	227,000	183,500	232,500	225,000	229,500
	-.243	-2,543	.000	-.482	-.134
	.808	.011	1,000	.630	.893
	.838b	.041b	1,000b	.775b	.902b

The results of the study reveal that the product *Apinosem forte*, administered 3 ml/5 l of water, showed the highest efficacy on *Eimeria* spp. oocysts.

From the perspective of clinical diagnosis, the results of the present study are consistent with those reported by other authors who identify in infected rabbits, typical clinical signs: watery diarrhea with streaks of blood, lack of appetite, dehydration, bloated abdomen, drowsiness, weight loss, hepatomegaly [2, 5, 16, 24, 25, 28].

Histopathological investigations reveal the diagnosis of chronic proliferative cholangitis marked with coccygeal intraepithelial elements and liver atrophy. Bibliographic histopathological studies confirm the appearance of the lesions identified in the present study: hypertrophic liver, dilated bile ducts, white nodules of variable size and creamy liquid content, on the liver, damage to the epithelium of the bile ducts with extensive hyperplasia of the epithelium, the presence of developmental stages of the protozoan in the epithelial cells of bile ducts, cytoplasmic vacuolations in hepatocytes [3, 4, 22, 40].

Therapeutic studies demonstrate the superior efficacy of the curative use in coccidiosis of rabbits of diclazuril and sulfachloropyrazine, substances established in the control of coccidiosis in birds, in favor of amprolium which proves to be ineffective [7, 13, 15, 17, 18, 23, 27, 30, 34, 36, 41, 42, 43].

A study by Peeters et al. [24, 29] demonstrated the efficacy of narasin in the treatment of mixed intestinal and hepatic coccidiosis. Robenidin and salinomycin have been widely used in Europe with varying efficacy against hepatic coccidiosis [26, 33]. Similarly, studies have reported varying efficacies following prophylactic and curative use of sulfonamides against coccidiosis [14, 37, 39]. The prophylactic and therapeutic use of toltrazuril has shown good results in the countries where it is used [11, 31, 37, 39].

In India, amprol, bifuran, and sulfur-based drugs have been widely used for the prevention of rabbit coccidiosis [5]. The prophylactic and curative use of diclazuril against rabbit coccidiosis has shown impressive results in Italy, France, and Spain [26, 41].

A study in Kenya looked at the curative (therapeutic) efficacy of sulfachloropyrazine, amprolium hydrochloride, and trimethoprim versus diclazuril which has never been used in Kenya, but has been shown to be effective [30, 41, 43].

Currently, there are no specific anticoccidials for rabbits in Kenya, and farmers use the anticoccidials used in the treatment of avian eimeriosis, not knowing the specific dose and efficacy for rabbits [6, 30, 32].

Studies on the effectiveness of the prevention and treatment of coccidiosis with natural extracts from garlic, pomegranate, and sweet wormwood plead for the introduction of these natural products in the control of protozoa [1, 20].

In the present study, we chose the product *Apinosem forte* – a natural extract from plants and garlic, used with great success in preventing and combating microsporidiosis produced by *Nosema* spp. [19, 21].

CONCLUSIONS AND RECOMMENDATIONS

The diagnosis of coccidiosis was confirmed following the coproparasitological, necropsy, and histopathological examinations.

Reduction to the disappearance of oocysts of *Eimeria* spp., the easy way of administration (in water), the remission of clinical signs, the return to the normal consistency of feces represent the advantages of the product *Apinosem forte*, administered 3 ml/5 l water, which we recommend in the prevention and combating of coccidiosis in rabbits.

It is the first report on the therapeutic efficacy of the product *Apinosem forte* used in the parasitological control of coccidiosis in rabbits.

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RESEARCH ON THE EFFICIENCY OF THE ARTIFICIAL INSEMINATION WITH FRESHLY DILUTED AND FROZEN SEMEN IN EWES DURING NATURAL ESTROUS

CERCETĂRI PRIVIND EFICIENȚA INSEMINĂRILOR ARTIFICIALE CU MATERIAL SEMINAL PROASPĂT DILUAT ȘI CONGELAT LA OI ÎN ESTRU NATURAL

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Abstract

In the context of the development and expansion of the sheep farming system, the main concern of sheep breeders has become the achivement of controlled reproduction and the practice of artificial insemination with semen from breeder rams. The study is carried out in a farm of 550 ***Merino de Palas*** *sheep in Constanța county, the owner aiming to improve the quality of the wool fiber. After about 3 weeks from the beginning of the mounting campaign, when the ewes in estrous were detected twice a day, in the morning and in the evening, the ewes came out to the mounting in a group due to the male effect. This was the moment when we started the double artificial insemination campaign, approximately 10 hours apart, on natural estrous, with semen from 2* ***Australian Merino*** *rams - UK import, aged 5 and 6 years. Thus, 308 ewes were artificially inseminated, respectively 226 ewes with freshly diluted semen and 82 ewes with frozen semen. After these 14 days, the two males were used for the natural mounting of 64 ewes in natural estrous, during the next 14 days, these being the control group. After the artificial insemination, a calving rate of 64.86 - 67.95% was obtained when inseminating with freshly diluted semen, 40.24% when inseminating with frozen semen and 72 - 76.92% after natural mounting of ewes from the control group.*

Key words: ewes, artificial insemination, semen

Rezumat

În contextul dezvoltării și extinderii sistemului de exploatare a ovinelor, principala preocupare a crescătorilor de oi a devenit realizarea reproducției dirijate și aplicarea inseminărilor artificiale cu material seminal de la berbeci amelioratori. Studiul este realizat într-o fermă de 550 oi ***Merinos de Palas*** *din județul Constanța, proprietarul urmărind ameliorarea calității fibrei de lână. După aproximativ 3 săptămâni de la debutul campaniei de montă, când s-a realizat depistarea oilor în estru de două ori pe zi, dimineața și seara, femelele au ieșit la montă în mod grupat datorită efectului mascul. Acesta a fost momentul în care am demarat campania de inseminare artificială dublă, la interval de aproximativ 10 ore, pe estru natural, cu material seminal de la 2 berbeci* ***Merinos Australian*** *– import Marea Britanie, în vârstă de 5 și 8 ani. Astfel au fost inseminate artificial 308 oi, respectiv 226 oi cu material seminal proaspăt diluat și 82 oi cu material seminal congelat. După aceste 14 zile, cei doi masculi au fost utilizați la monta naturală a 64 oi în estru natural, pe parcursul următoarelor 14 zile, acestea constituind lotul martor. În urma inseminării artificiale s-au obținut o rată a fătării de 64,86 - 67,95% la inseminarea cu material seminal proaspăt diluat, 40,24% la inseminarea cu material seminal congelat și 72 – 76,92% în urma montei naturale a oilor din lotul martor.*

Cuvinte cheie: oi, inseminare artificială, material seminal

INTRODUCTION

The present study was carried out as a result of the need to perform artificial insemination with semen from breeding rams. By applying the selection and improvement criteria within the Genealogical Registers, the ranking and sorting of the purebred rams that are at the top of the hierarchy for production in the desired selection direction was achieved. These rams from the top of the pyramid, respectively from the elite farms, are distributed to the selection and breeding farms but also to the production farms to improve the livestock productions. The number of males that reach the production farms is relatively small, the purchase being quite expensive. Thus, many sheep breeders have the tendency to excessively and irrationally use these rams in the natural mounting that leads to their physical and/or reproductive exhaustion. Another risk is the use for mounting without taking into account parentage, reaching a high degree of inbreeding. The solution to this situation is the exchange of rams between breeders or, easier to achieve, resorting to artificial insemination with semen from breeding rams from other farms or from elite farms. Under these conditions, it is necessary to check the quality of the semen from rams in terms of mobility and viability (Druart X. et al., 2009, Baril G. et al., 1993, Fatet A. et al., 2008) and establish its suitability for collecting, dilution and artificial insemination. Artificial insemination represents the most efficient reproductive biotechnology that has become a common practice for the sheep breeders in Europe. According to the website idele.fr, in 2017, in France, more than 800,000 inseminations were carried out in dairy sheep by means of nine artificial insemination centers, creating a herd that remains approximately constant for over 20 years. This trend has been maintained until now when there is a network of semen collection and preservation centers and frozen semen insemination centers, achieving a good diffusibility in time and space of the valuable genetic material.

In our country, sheep reproductive biotechnologies have been developed within the Research and Development Institute for Sheep and Goat Breeding Palas, Constanța and the Research and Development Stations for Sheep and Goat Breeding. Research in the field of reproduction has been oriented in the direction of increasing productivity and management of controled reproduction, through themes imposed by the needs of sheep breeders. All results have as their main purpose the dissemination of information, new or upgraded techniques and technologies in the production activity of the sheep sector.

MATERIAL AND METHOD

The present study was carried out on a farm of 550 **Merino de Palas** ewes, 17 **Merino de Palas** rams and 2 **Australian Merino** rams, exploited mainly for wool production. The farmer, engaged in wool sales contracts, is interested in the quality of the wool fiber, aiming to obtain specimens with a fineness below 20 microns. In this sense, he purchased 2 years ago, from Great Britain, the two **Australian Merino** rams, aged 5 years (hereinafter referred to as ram A) and 6 years old (hereinafter referred to as ram B), with a fiber fineness of wool of 17.5 microns and 13.91 microns respectively. After the first year of use at mounting and registration of calvings, we were asked to check the quality of the semen and advise him on how to carry out the mounting to maximize the use of the 2 imported males. Following the analysis of the semen and taking into account the age of the rams, we proceeded to collect and cryopreserve the ejaculates with a volume of over 1 ml and with a motility of 85-95% from the 6-year-old ram (ram B), making a stock of 150 medium sequins (0.5 ml).

In July 2022, the controlled breeding season began, by introducing test rams among the ewes, in the morning and in the evening, separating the females that show clinical signs of estrous and the natural mounting. After about 3 weeks from the beginning of the mounting season, when the ewes in estrous were detected twice a day, the ewes came out to mounting in a group due to the male effect, at which time we applied the artificial insemination protocol.

For 14 days, ewes in estrous were detected with rams provided with an apron, twice a day, in the morning (5.30 am) and in the evening (7 pm). Females detected in estrous were artificially inseminated 10-12 hours after detection, with the repetition of insemination at approximately 10 hours.

The collection regime of the 2 **Australian Merino** rams was 3 consecutive days followed by a rest day for recovery, collecting 2-3 ejaculates/day, depending on the number of females detected in estrous and the volume of the ejaculate at the first insemination. In the situation where the number of females was higher and the number of doses of freshly diluted semen was not sufficient, frozen semen from ram B was used for both inseminations (table 1).

Table 1. The situation of artificial insemination in Merino de Palas sheep, in natural estrous, during the 14 days period / Situația inseminărilor artificiale la oile Merinos de Palas, în estru natural, în perioada de 14 zile

Experiment day	No. Of ewes detected in estrous	Total number of ewes artificially inseminated with freshly diluted semen	Number of ewes artificially inseminated with freshly diluted semen ram A	Number of ewes artificially inseminated with freshly diluted semen ram B	Number of ewes artificially inseminated with frozen semen ram B
1	20	20	14	6	0
2	26	26	19	7	0
3	18	14	14	Ram recovery break	4
4	26	10	Ram recovery break	10	16
5	23	21	14	7	2
6	21	19	10	9	2
7	18	10	10	Ram recovery break	8
8	24	8	Ram recovery break	8	16
9	23	22	16	6	1
10	21	18	11	7	3
11	24	13	13	Ram recovery break	11
12	19	9	Ram recovery break	9	10
13	24	18	12	6	6
14	21	18	15	3	3
Total	**308**	**226**	**148**	**78**	**82**

The protocol for obtaining diluted raw semen.

Semen collection is done with the artificial vagina using a dummy ewe in estrous, contained in a special stand. To perform the collection, the artificial vagina must meet the three basic conditions to achieve ejaculation: the lubrication of the vaginal shirt with neutral ointment, the temperature of 38-39^0 C, and the pressure by blowing air at the level of the cannula of the artificial vagina.

Immediately after collecting the ejaculate, the volume is visually assessed by reading the graduations of the collecting cup, obtaining between 0.9 and 2.4 ml. The consistency and color of the semen can be evaluated through the transparency of the collecting cup, which varies from white-slightly yellowish (normal color) to white-brown (Zamfirescu et al., 2004; Zamfirescu et al., 2011).

To determine the degree of dilution, the Accucell analyzer was used - produced by IMV Technologies (photo 1a). After calibrating the device and entering the volume of the ejaculate and the concentration of spermatozoa/ml target for the insemination dose (200x10^6 spermatozoa/0.25ml), the sample of 10 microliters of semen is introduced and the photometer will indicate the volume of diluent to be added and the number of doses obtained. To achieve the dilution, we use the OviXcell diluent, produced by IMV Technologies (photo 1b). Until the moment of insemination, the glass with the diluted semen is kept on the water bath so that the temperature gradually decreases and to be valid until the moment of artificial insemination.

The cryopreservation protocol of frozen semen.

In order to cryopreserve the semen, we proceeded to the collection with an artificial vagina, the reading of the volume and the qualitative analysis directly on the graduated glass, respectively the motility and viability by the colorimetric method. The semen was subjected to three phases of dilution: the initial dilution in a ratio of 1:1 with TRIS diluent containing 20% egg yolk. The operation is done on

a water bath at 30^0 C, followed by gradual cooling to $+4^0$ C, the intermediate dilution with the same diluent, at room temperature, the amount being calculated according to the sperm concentration. The next stage is the equilibration of the diluted sperm at $+5^0$ C for 3-4 hours, followed by the final dilution with diluent II (saline diluent with 20% egg yolk and 8% glycerin); the amount used of diluent II is equal to that of diluent I and is added in one or two batches. This final dilution is done a few minutes before aspiration of the sperm into medium sequins with a capacity of 0.5 ml or fine sequins of 0.25 ml. The sequins are sealed with polyvinyl powder, which gels in contact with the aqueous medium. Freezing is done in two phases: pre-freezing in liquid nitrogen vapor at a temperature of -120^0 C for different times depending on the form of freezing (fine sequins are pre-frozen for 2 minutes at 8 cm and 8 minutes at 2 cm above the level of liquid nitrogen and medium sequins pre-freeze 4 cm above the level of liquid nitrogen for 8-10 minutes) and final freezing by immersing the sequins in liquid nitrogen (at -196^0 C). The control of the freezing is done 24 hours after freezing, by checking the viability of the spermatozoa from each ejaculate of several sequins, storing in the cryobank only the ejaculates which after freezingthawing have a viability of over 40%.



1a – ACCUCELL



1b - OviXcell

Figure 1. The equipment and the used preservation medium / Echipamentul și mediul de conservare utilizat

The artificial insemination of the ewes was carried out on natural estrous, following the detection of estrous with test rams with an apron. The insemination was carried out with 0.25 ml of diluted raw semen, with an average concentration of 200 x 10^6 spermatozoa/dose. For artificial insemination, the vaginal speculum with its own light was used, with the help of which the place of sperm deposition was visualized, respectively at the level of the involte flora, and when the opening of the cervix allowed, the insemination pipette penetrated along the path of the cervix, at 0.5-1 cm. The second insemination was made with semen from the same ram.

After the completion of the 14 days of insemination, the study continued for another 14 days in which the natural mounting of 64 more ewes with the two rams was carried out. The ewes detected in estrous were distributed to the 2 rams, these managed to achieve up to 4 mountings/day – Ram A and a maximum of 3 mountings/day – ram B, as can be seen in table 2. The difference of the ewes detected in estrous during the respective days were mounted with **Merinos de Palas** rams, a situation we did not include in the present study.

Table 2. Distribution of natural mounts in Palas Merino ewes with 2 Australian Merino rams / Distribuția montelor naturale la oile Merinos de Palas cu 2 berbeci Merinos australian

Specification	Number of naturally mounted ewes														Total mounted ewes
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	
Ram A	3	3	4	3	4	2	3	2	1	2	3	3	4	2	39
Ram B	3	3	1	2	1	2	1	2	2	1	2	2	2	1	25
Total mounted ewes	6	6	5	5	5	4	4	4	3	3	5	5	6	3	64

RESULTS AND DISCUSSION

The flock of artificially inseminated ewes with the two forms of semen presentation, but also the naturally mounted one were supervised and subjected to oestrous detection with test rams, at intervals of 15-19 days after insemination/mounting to determine the non-pregnant females, respectively the rate of no return. At an interval of 50 days after insemination/mounting, the ultrasound examination was performed with the mobile ultrasound machine WED 3000, with a multifrequency probe, set to 3.5Hz to confirm the gestational state. Females that showed estrous in the next estrous cycle or were not confirmed pregnant were subsequently mounted naturally with rams according to the pair matching chart, not being included in the calculation of the reproductive indices of the present study.

Table 3 shows the reproductive indices obtained during this study and graphic representation 1 illustrates the comparative analysis of each reproductive parameter in the five variants of the study: artificial insemination with raw diluted semen from the two rams and with frozen semen from ram B, as well as the natural mounting with the two rams.

In this study, we did not take into account the age of the ewes and the previous reproductive status that could, in addition to the individual factor, influence the results and the correlations between the monitored indicators.

Table 3. Recorded breeding rates of Palas Merino ewes artificially inseminated/naturally mounted to Australian Merino rams during natural estrous / Ratele de reproducție înregistrate de oile Palas Merino inseminare artificial/montate în mod natural cu berbeci Merinos australieni în timpul estului natural

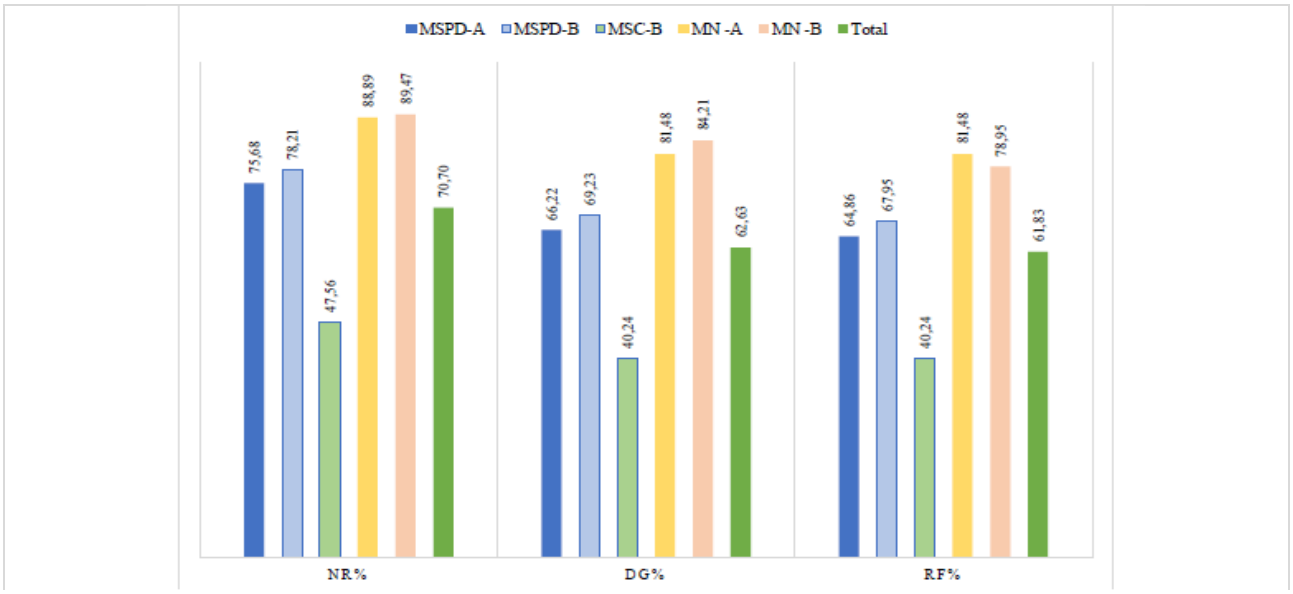
No. crt.	No of ewes	Specification IA / MN	No. Of ewes returned to estrous	NR%	No of ewes pregnant.	DG%	No. of calves	RF%	No of lambs	P%
1	148	MSPD-A	112	75.68	98	66.22	96	64.86	114	118.75
2	78	MSPD-B	61	78.21	54	69.23	53	67.95	64	120.75
3	82	MSC-B	39	47.56	33	40.24	33	40.24	40	121.21
4	39	MN -A	32	82.05	30	76.92	30	76.92	37	123.33
5	25	MN -B	19	76.00	18	72.00	18	72.00	21	116.67
Total	372		263	70,70	233	62,63	230	61,83	276	120,00

AI – artificial insemination; MN – natural mount; MSPD-A – freshly diluted semen - collected from ram A; MSPD-B – freshly diluted semen - collected from ram B; MSC-B – frozen semen - collected from ram B; MN -A – natural mount with ram A; MN -B – natural mount with ram B; NR %- – non-return rate; DG% – gestational diagnosis; RF% – calving rate; P – prolificacy

The difference between the rate of non-returns established at 15-19 days after artificial insemination/mounting and the ultrasound confirmation of the gestational state is generated by two situations: embryonic death without visible clinical signs due to the small gestational age or the females were not pregnant but did not manifest the specific signs of estrous (heats erased) or presented a short or longer estrous cycle not being captured in the monitored interval. In all five situations, the percentage of pregnant ewes is lower at the ultrasound diagnosis compared to the non-return rate. The best values are

observed in naturally mounted ewes (81.48-84.21%), followed by those artificially inseminated with fresh semen diluted (66.22-69.23%) and the lowest values are obtained following artificial insemination with frozen semen. The same situation occurs in the case of calvings, when the results of insemination with frozen semen register the lowest value (RF=40.24%). The results of this study are inferior to previous research when we performed artificial insemination on experimental lots, selected according to strict criteria, without differences between the animals in the batch, with previous normal cyclic reproduction and which were inseminated in the second estrous, therefore knowing the reproductive status at the time of insemination , the fecundity being 82% by using refrigerated semen (Nadolu et al., 2019). Superior results are recorded when artificial insemination is performed in ewes with synchronized and hormonally induced estrous, respectively 78% after artificial insemination with fresh diluted semen, but by using frozen semen the fertility was only 43% (Langford G.A et al., 1979).

Both artificial insemination and natural mounting being carried out during natural estrous, the prolificacy is within the specific limits of the breed (116.67 - 123.33%) not being influenced by hormones.

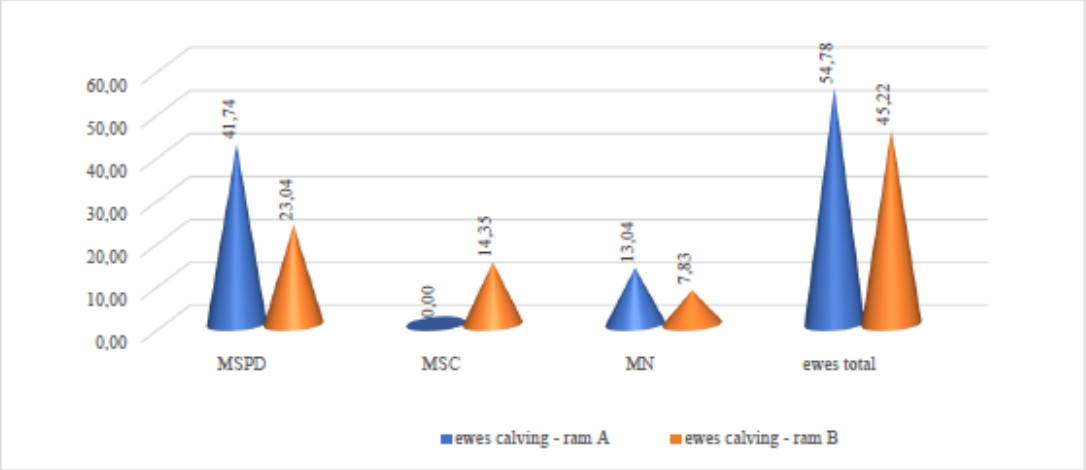


MSPD-A – freshly diluted semen - collected from ram A; MSPD-B – freshly diluted semen - collected from ram B; MSC-B – frozen semen - collected from ram B; MN -A – natural mount with ram A; MN -B – natural mount with ram B; NR %- – non-return rate; DG% – gestational diagnosis; RF% – calving rate

Figure 2. The reproductive indices variation in Palas Merino ewes artificially inseminated/naturally mounted with Australian Merino rams during natural estrous / Variația indicilor de reproducție la oile Merinos de Palas inseminate artificial / montate în mod natural cu berbeci Merinos australian în timpul estului natural

From the data analysis and graphical representation, the differences between the two males are observed. In natural mounting, ram B aged 6 years but with superior productive performance manages to mount fewer females (n=19) during the same time interval compared to ram A that mounts 32 ewes. Regarding artificial insemination, it is observed that the results obtained by using freshly diluted semen from ram B are better than from ram A (DG= 69.33% vs 66.22% and RF= 67.95% vs 64 .86%). These results can be explained based of the data observed at freezing. The semen of ram B, although it recorded smaller volumes, the suitability for freezing was good, and only the processed ejaculates that after freezing - thawing had a viability higher than 40% were kept in the cryobank.

During the 4 weeks study 187 ewes were inseminated/ mounted with ram A and 185 ewes with ram B, out of a total of 372 ewes.



MSPD – artificial insemination with freshly diluted semen; MSC – frozen semen; MN – natural mount

Figure 3. The efficiency of artificial insemination use in Merino de Palas ewes during natural oestrous / Eficiența utilizării inseminării artificiale la oile Merinos de Palas în timpul estrului natural

From figure 3 it can be seen that ram B, due to its age, could not be used for natural breeding or semen collection with the same intensity as ram A but the quality of the cryopreserved semen was good that the final percentage of pregnant ewes with this ram increased by 14.35%.

CONCLUSION

The use of reproductive biotechnologies, i.e. semen collection, semen processing and dilution, cryopreservation and artificial insemination, increases the main reproductive indices and thus the productivity of sheep farming.

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THE WEANING STRESS EFFECT ON CALF BEHAVIOUR AND PERFORMANCES IN ROMANIAN SPOTTED CALVES

EFECTELE STRESULUI DE ÎNȚĂRCARE ASUPRA COMPORTAMENTULUI ȘI PERFORMANȚELOR VIȚEILOR DE RASĂ BĂLȚATĂ ROMÂNEASCĂ

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Abstract

The aim of the study was to analyses the effects of weaning method on calf welfare based on body weight post weaning and behavior patterns. The study aimed to set the most appropriate weaning method in order to ensure the calves welfare. Study was carried out on 120 ***Romanian Spotted*** *calves weaned at 60 days of age (60 calves) either abruptly or with a 14-day pre-weaning period (60 calves). Comparisons between the weaning methods for body weight and feeding behavior were performed using one-way ANOVA assay with categorical factor "weaning method". A positive effect of the gradual weaning was observed on calves' body weight post calving. The abrupt weaning induced an 8.4 % loss in calves body weight within the first 10 days after weaning compared to 4.1 % recorded in gradual weaning (P≤0.001). Gradually weaned calves allocated 174.11±6.31 min. / day for feeding divided into 9.2±0.61 meals compared to 104.26±9.71 min. / day feeding and 5.22±0.34 meals (P≤0.001) recorded in abruptly weaned calves. Performing gradual weaning decreases loss of calves' body weight also increasing the feeding behavior patterns.*

Keywords: behavior, calves welfare, Romanian Spotted, weaning method

Rezumat

Studiul urmărește efectele metodei de înțărcare asupra condiției de bunăstare a vițeilor, evaluată în baza dinamicii greutății corporale post înțărcare și a manifestărilor comportamentale specifice categoriei de vârstă. Scopul acestei analize a fost stabilirea unei metode de înțărcare fiabile în încercările de a asigura vițeilor o bunăstare deplină. Observațiile s-au desfășurat pe un lot de 120 de viței de rasă ***Bălțată Românească****, înțărcați la vârsta de 60 de zile, fie prin înțărcare bruscă (60 viței) fie prin asigurarea unei perioade de preîncărcare de 14 zile (60 viței). Comparațiile în ceea ce privește efectele metodelor de înțărcare asupra greutății corporale și a comportamentului s-au efectuat în baza protocolului ANOVA în care a fost inclus factorul de influență "metoda de înțărcare". Un efect pozitiv semnificativ asupra dinamicii greutății corporale post înțărcare a fost asociat metodei graduale. Înțărcarea bruscă a vițeilor a indus o pierdere în greutate de 8,4% în primele 10 zile după sistarea dietei lactate comparativ cu un regres de doar 4,1% asociat înțărcării treptate. Vițeii care au beneficiat de perioada de pre înțărcare au alocat consumului de furaje un interval mediu zilnic de 174,11±6,31 min., interval împărțit în 9,2±0,61 tainuri zilnice, comparativ cu cei înțărcați brusc, care au prezenta o durată redusă de consum 104,26±9,71 min. asociat unui număr de doar 5,22±0,34 tainuri (P≤0.001). Rezultatele obținute pledează evident în favoarea implementării înțărcării treptate a vițeilor, pierderile în greutate fiind semnificativ reduse în paralel cu accentuarea manifestărilor specifice comportamentului de ingestie a furajelor.*

Cuvinte cheie*: **Bălțată Românească**, **bunăstare viței, comportament, metodă de înțărcare***

INTRODUCTION

The weaning of calves is necessary in order to improve the farm's efficiency, reproduction activity of the dams and not at least to increase the quantity of milk available to humans (Meyers et al., 1999). Due to the weaning method several issues may occur both in dams or calves. The weaning constitutes as a second major stress factor after birth. Intensity and duration of the calves' response depend on many influential factors such as weaning method, age at weaning, housing or feeding program. The calves body weight decreases and behavioral patterns suggest a negative emotional state being essential elements in assessment of animal welfare condition (Sweeney et al., 2010). Generally, the weaning represents the total independence of offspring from their dam and ability to survive on its own. Despite of its evidence, the endpoint of natural weaning is largely unknown and it is reported to vary surveys. Knowledge of weaning effects implies understanding of calves responses (body weight dynamics, behavior patterns and health status) according to "*honest signals of needs*" (Miller K., 2011). The negative effects of dams are intensely studied in beef breeds due to the natural nursing witch exert a negative impact on somatic development and subsequent performances (Borderas et al., 2009). In dairy or dual purpose breeds, the studies aim on the one hand the economic efficiency of farms according to the milk quantity and on the other hand, the efficiency in calves rearing assessment on daily gain and its costs. Economy is common practice to early weaning allowing use of vegetable forage in order to reduce costs. The intensive rearing systems require and imposes a technological efficiency in weaning, early and abrupt weaning being most often chosen. The effects of abrupt weaning are obvious in both calves' body weight dynamic and behavioral patterns. Moreover, undesirable behavioral patterns occur (frequent agonistic rounds, prolonged exploring behavior, reduced meals frequency). The institutional requirements as well as consumer concerns regarding animal welfare claim intensive and varied researches about the impact of applied technology on animal condition. The aim of the current research was to assess the effects of weaning method on calves' body weight dynamic and behavioral response.

MATERIAL AND METHOD

The study was carried out on livestock farm from the Research and Development Station for Bovine Arad, Romania (location: 46° 10' 36" N, 21° 18' 4" E, 107 m altitude, 582 mm annual average rainfall 21°C / -1°C average of temperature corresponding seasons summer / winter). In the current study, 63 Romanian Brown calves (41 females and 22 males) were randomly included. Calves were from eutocia, singles (n = 59) or twins calving's (n = 4 calves). Calves were separated from their dams within the first hour after birth and moved either individually (1.85 x 1.85 m) in straw-bedded pens, in the maternity until 14 days of age. Up to 90 days of age, the calves were housed outside the maternity barn in group pens (6 heads / box) up to weaning (except 3 calves housed individually due to several health issues).

The calves were fed with milk replacer (10 % of body weight) 6-8 kg / day (95 % DM, 22 % CP and 18 % Fat), alfalfa hay (84 % DM, 13 % CP) and concentrates (88 % DM, 17% CP, and 2.5 % Fat) ad libitum. The calves had constant visual and auditory contact with congeners from neighbouring pens, both in maternity and in external facility. The weaning was abruptly for 30 calves and gradual for another 33 calves, with 10 days pre-weaning period, 20 between day 90 and 100. After weaning, the calves were moved in the common paddock (n=63) where the feeding was allowed throughout 24 –h period with vegetal fodder and concentrate mixture. For all calves we recorded the body weight at weaning day and within the first 10 day after. Also, feeding behavior patterns were recorded. Behavior patterns were recorded for 24 hours in the weaning day (day 90 or day 100 for calves weaned gradually) using 4 cameras (Sony HDR-CX24OE). The recorded data were analyzed by The Observer XT specialized software. Data were expressed as means ± standard errors. Comparisons between calves were carried out

using the one-way ANOVA protocol, with categorical factor being considered "*weaning method*". All the statistical inferences were carried out using Statistica® software v. 13 (Hill and Lewicki, 2007). Decisions about the acceptance or rejection of statistical hypothesis have been made at the 0.05 level of significance. This research was mainly 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 were approved by the Scientific Council at the Research and Development Station for Bovine Arad (No. 50/29.10.2015).

RESULTS AND DISCUSSION

The weaning method exerts obvious effects on calves' body weight dynamics in the post weaning period. Pre-weaning feeding programs play an important role in both calves´ body weight and distress. Early feeding of forage allows a proper development of the rumen. Increasing the forage dietary can lead to a decrease level of stress by reducing the calves' milk requirements. Previous surveys have shown that calves that drink milk as much as they wish were healthier, recorded fewer visits to the vets, produced more milk as adults and shown an increased daily gain compared to calves nursed in conventional milk feeding program.

Although pre-weaning average daily gain is reduced, the differences fade post weaning, the calf's body weight being comparable (Gorgulu M., 2012). Higher milk amounts are desirable from an economic point of view in order to ensure an increased average daily gain (Diaz et al., 2001). However, the milk allowance and duration of the pre-weaning period influence the calves' body weight dynamics. The results obtained in the current research show decreased tendency of calves' body weight post weaning in both abrupt and gradual weaning methods, the intensity of its being different.

On the weaning day, no significant differences ($P>0.05$) were recorded between the studied calves (98.3±2.24 vs. 97.72±0.38 kg). The post weaning weights dynamics meets a different tendency, according to weaning method. The abrupt weaning calves recorded a decrease in body weight (98.3±2.24 vs. 92.16±0.59 kg, $P\leq0.004$) representing 6.6 % compared to gradual weaning calves recording a 3.73 % loss (97.72±0.38 vs. 94.2±0.26 kg, $P>0.074$). The length of the pre-weaning period exerts positive effects on calves´ body weight dynamics.

In this respect, the 10 days of pre-weaning period allocated in the current study proved to be sufficient in order to record a lower loss in body weight for gradual compared to abrupt weaning calves, similar results have been reported in previous research also by Sweeney et al., (2010). The calves' body weight dynamics proved to be strongly influenced by preweaning feeding strategy.

Intensified milk feeding programs providing higher milk amounts are able to determine the calves' growth dynamics in pre and post weaning time. Calves included in intensified milk feeding programs providing more milk amounts are able to increase their nutrient intake leading to a greater body weight compared to calves associated with conventional program characterized by restricted amounts of milk. (Appleby M., 2001; Jasper and Weary, 2002). This strategy allows an increased daily gain reach up to 1.2 kg / day. Comparatively, the conventional milk feeding program used in our study allows an average daily gain up to 0.65 Kg / day in pre-weaning follow by a loss of weight range between 3.6-6.2 % post weaning according to weaning method for both gradual and abrupt method respectively. Previous surveys regarding the influence of higher milk amounts in calves weight gain suggest a favorable impact on pre-weaning up to 100 g / day.

Also, the intensified milk feeding programs allow a growth tendency of calves' biometrics measurements in both, pre and post weaning. In dairy farms, intensified milk feeding programs involve increased costs. There are studies that proved these supplementary costs will be amortized during the first lactation (Gonyou and Stookey, 1987). The greater body weights as a result of pre-weaning intensified milk feeding programs could be maintained in post weaning period (Jasper and Weary, 2002).

According to conventional milk feeding program used in our study, the results recorded a significant body weight loss (P≤0.004) in abrupt compared to gradual weaning method. Slightly decrease of body weight was recorded for gradual weaning calves, according to time spent for it and meals frequency.

Table 1. Means (±standard error) for calves behaviour patterns according to weaning method / Mediile (±eroarea standard) manifestărilor comportamentale ale viţeilor în raport cu metoda de înţărcare

The weaning method	Parameters	Feeding behavior (min.)
Abrupt weaning (60 calves)	Meals frequency	5,7±0,37[a]
	Meal average duration	16,19±1,36[a]
	Daily meal duration	92,34±2,74[a]
Gradual weaning (60 calves)	Meals frequency	9,2±6,31[b]
	Meal average duration	18,92±1,4[b]
	Daily meal duration	174,11±6,31[b]

a,b Columns mean with different superscripts differ significantly at p≤0.05

Feed consumption is strongly influenced by technological factors. Particular importance is exercised by the calves' age of providing forage in ratio, forage quality and the amount of milk available in preweaning period. The confinement system exerts an important role, the common rearing being an incentive for calves feeding behavior. In our study, calves were reared in pairs up to 14 days of age, in common pens (6 head / pen) up to weaning and in the common paddock (30 heads / paddock) post weaning. The concentrate forage was administered since 4th day of age and water was freely available. A conventional milk feeding program with restricted milk allowance (10 % of body weight) was used. The vegetal diet structure was maintained in post weaning compared to the pre-weaning period in order to stimulate the consumption, the calves do not change their preferences. According to a conventional milk feeding program which provides restricted milk allowance, the current study recorded significant differences (P≤0.001) regarding meals frequency in gradually (8.1±0.31 meals / day) and abruptly weaned calves (6.3±0.17 meals / day).

Gradually weaned calves show an increase meals frequency (P≤0.001) in the post weaning period compared to abruptly weaned calves. Gradually weaned calves show an increased meals frequency comparable to meals frequency in calves feeding with higher milk amounts reported in previous surveys. Myers et al., 1999; Bach, 2012). Conventional milk feeding program in the pre-weaning period leads to an increased forage consumption and prolonged daily meal time. The effect was maintained also in the post weaning period. Nevertheless, prolonged meals do not indicate a high feed consumption. In intensified milk feeding programs and in abruptly weaned calves, previous studies found a negative correlation between meals time and forage intake. The current study found an average meal time of 14.9±0.3 min. / meal for abruptly weaned calves compared to a significant increased (P≤0.036) average meal time of 18.7±0.84 min. / meal in gradually weaned calves. These results were found by Miller et al., 2011) in a previous study (15.4 min. / meal). Conventional milk feeding program used in our study allowed a total daily meal range between 93.87±6.11 min. / day for abruptly and 151.47±4.28 min. / day for gradually weaned calves. Reducing of the milk amount administered further ceasing of it leading to an upward trend of forage feeding daily time in calves with range between 93.87±6.11 min. / day and 151.47±4.28 min. / day (P≤0.001) in abrupt and gradual weaning. These outcomes are similar to previous results reported by Overvest et al., 2016 (87-155 min. / day at weaning) with higher values in post weaning period (over 180 min. / day).

CONCLUSIONS

The weaning method has been shown to exert a significant influence in calves body weight dynamics, inducing a lose weight particularly in abruptly weaned calves. The current study recorded a weight lose up to 6.2 % in abruptly compared to 3.6 % in gradually weaned calves. The daily meals

frequency associated to abruptly weaned calves was lower (6.3 meals / day) compared to the higher frequency (8.1 meals / day) recorded for the gradually weaned calves. Also, total meals daily time have been reduced in abrupt weaning (93.87 min. /day) compared to 151.47 min. / day for spent by gradually weaned calves. The current findings still remain contradictory comparing to others previous studies, requiring further researches which include the specific parameters.

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A RISK-BASED „ONE HEALTH" PERSPECTIVE APPROACH - THE FUTURE OF DAIRY FARMS MANAGEMENT

VIITORUL MANAGEMENTULUI FERMELOR DE VACI PENTRU LAPTE: ABORDAREA CONCEPTULUI „ O SINGURĂ SĂNĂTATE" PRIN ANALIZA DE RISC

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Abstract

All food businesses, including food primary producers, are accountable on a legal level for ensuring the quality of their products. Primary production is the first crucial stage in the food chain, where food safety and traceability must be guaranteed. The maintenance of high-quality standards for milk and dairy products continues to be a challenge for farmers and manufacturers, who in turn ask the scientific community to provide them with appropriate tools for hazard identification and risk management. This overview describes the novelties of an integrated risk-based system that relies on the HACCP principle, corroborated by the incorporation of technological innovation, and the alerts from the multidisciplinary interactions of environment, animal health, and food safety SMART key indices of the "One Health" profile concept. This represents the future benchmark for primary dairy production. Here, farm characterization, agriculture practices (with particular attention to main crops, management of pesticides and fertilizers, and degree of groundwater pollution), animal nutrition (with particular attention to feeding quality, safety, and origin), animal health and welfare (anti-microbial and anti-parasitic drugs usage and management, udder health, etc.), milking techniques, milking parlour hygiene, and their consistent risk analyze are the main inputs used to develop a custom-focused grid of instruments for the top platforms in order to track a panel of analytical variables, unique to each dairy production farm. The overcoming benefit is that the above resumed integrated method of dairy production takes into account the effect that products entering and exiting the dairy farm have on the health condition of the larger network and ensures that food safety begins on the farm and does not negatively affect the environment or downstream consumers.

Keywords: one health, hazard analysis and critical control point, risk assessment, risk management;

Rezumat

Toți operatorii din sectorul producției agro-alimentare sunt responsabili din punct de vedere juridic de calitatea și inocuitatea produselor lor. Producția primară este prima verigă a lanțului alimentar unde este necesară garantarea siguranței alimentului și trasabilitatea produsului. Menținerea standardelor de calitate a laptelui și / sau produselor lactate reprezintă o provocare continuă pentru fermieri, dar și producători, care, la rândul lor solicită comunității științifice furnizarea de instrumente eficiente în identificarea pericolelor și gestionarea riscurilor asociate. Lucrarea de față descrie rezumativ noutățile unui sistem integrat, bazat pe evaluarea riscurilor directe dar și a celor asociate conform principiilor HACCP (Sistem de analiză a riscurilor și determinare a punctelor critice de control), coroborat cu integrarea inovării tehnologice exprimate prin alertele din interacțiunile multidisciplinare dintre mediu, sănătatea animală și siguranța alimentului, indicatori SMART ai conceptului „O Singură Sănătate". Acest concept reprezintă viitorul în definirea criteriilor de referință pentru producția primară de produse lactate. Aici, caracterizarea fermelor, practicile agricole, nutriția animalelor, sănătatea și bunăstarea lor, tehnologia mulsului, igiena sălii de muls și analiza consecventă a riscurilor potențiale la nivelul fiecărei etape din matricea proceselor sunt principalele elemente de intrare utilizate pentru a dezvolta un panel personalizat de instrumente pentru platformele de top în vederea exprimării printr-o serie de variabile analitice unice pentru fiecare fermă de vaci de lapte. Avantajul principal al acestei metode integrate la nivel industrial ia în

considerare efectul tuturor intrărilor dar și ieșirilor din ferma de vaci pentru lapte asupra stării de sănătate a rețelei de consumatori, fără efecte negative asupra mediului.

Cuvinte cheie: O Singură Sănătate, HACCP (Sistemul de analiză a riscurilor și determinare a punctelor critice de control), analiza de risc;

INTRODUCTION

The European White Book is a comprehensive guide that highlights the utmost importance of ensuring food safety in the food industry. It emphasizes that all food operators, from primary producers to manufacturers and distributors, have a significant ethical, scientific, and legal responsibility to ensure that their products are safe for consumption. This responsibility cannot be taken lightly, and it is essential that all parties involved take the necessary steps to guarantee that their products are of the highest quality and meet all safety standards. Any negligence or oversight in this regard can have severe consequences, not only for the consumers but also for the reputation of all operators from the food chain, including primary processors, like dairy farms [8].

Additionally, there is a growing awareness of issues relating to animal health and welfare. This is exemplified by past occurrences such as the BSE epidemic, the dioxin problems, and the *Salmonella* survey that affected various species in recent years. However, it is important to recognize that in recent years, there has been a growing emphasis on the interconnectedness of humans, animals, and the environment. This concept, known as *One Health*, has led to an increased understanding of the importance of prevention and safety measures in primary livestock production. It is important to understand that the issues we face today cannot be solved by a single solution. Instead, a comprehensive and all-encompassing approach is required to navigate these challenges successfully. This is where the One Health/One Medicine philosophy comes into play. This approach recognizes the interconnectedness between animal, human, and ecosystem health and seeks to improve them all simultaneously. By adopting a holistic mindset, we can create sustainable solutions that not only address the immediate problems but also ensure long-term health and well-being for all [14].

In order to ensure the well-being of dairy cattle and the safety of the products they provide, it is essential to prioritize access to clean water, safe food, and proper hygiene. These key components are integral to maintaining the health of dairy herds and preventing the spread of disease *(Fig. 1*).

Also, the One Health approach to dairy production medicine and management considers the interconnectedness of human, animal, and environmental health. By implementing effective farm management systems, such as *Dairy Dynamic Management*, and practicing the prudent use of antimicrobial agents, we can address these critical issues and promote the overall health and sustainability of the dairy industry. It is important to consider the entire system of dairy production, from animal welfare to environmental impact, in order to ensure a safe and responsible food supply. By prioritizing these key components and adopting a holistic approach, we can support the health and well-being of both dairy cattle and the communities they serve.

As we continue to learn more about the complex interactions between these various elements, it becomes evident that a comprehensive approach to food safety is essential, with prevention and safety measures at the level of primary production taking precedence, implying the following areas of approach: critical control points in the production of dairy products, prevalence of antibiotic-resistant mastitis pathogens, foodborne pathogens from farm to table, prudent use of antibiotics, residues of antibiotics in milk, dairy dynamic management and with big importance alternative treatments to antibiotics (*Fig. 1*).

To achieve this goal, food business operators [FBOs] often rely on a comprehensive and science-based approach to food safety known as the Hazard Analysis and Critical Control Point (HACCP) system or commonly named risk analysis. This system is designed to identify potential hazards and risks associated with food production and distribution and to establish specific control measures to mitigate

these risks, which implies that the farmer's agricultural practices align with animal welfare standards in addition to following the guidelines for food safety [2, 7].

The implementation of the Hazard Analysis and Critical Control Points (HACCP) methodology in the context of environmental changes and animal health management in dairy farms is a sensible and rational decision that encompasses seven guiding principles and twelve developmental steps. The 7 principles that have been established are primarily aimed at facilitating effective risk assessment, risk management, and the implementation of specific documentation and verification procedures. Additionally, they emphasize the importance of maintaining accurate and up-to-date documentation, as well as implementing rigorous verification procedures to ensure that all processes and procedures are functioning as intended [2, 7, 12].

The implementation of these management techniques in the dairy industry serves to reinforce the notion that worldwide dairy producers are committed to supplying milk that is not only safe and nutritious but also yields numerous health benefits. It is crucial that these practices are adopted in developing countries as well, as they have the potential to vastly improve the overall nutrition, health, and well-being of their populations. By prioritizing the implementation of these techniques, we can ensure that people across the globe have access to the same high-quality dairy products that we enjoy in the European Union.



Figure 1. The visual translation of the "One Health" concept by centralizing the active participatory factors within a milk cow farm / Translatarea vizuală a conceptului "O Singură Sănătate" prin centralizarea factorilor participativi activi în cadrul unei ferme de vaci pentru lapte [8, 12, 16]

Further, in this paper, we will explore „*an example*" how the implementation of HACCP principles can effectively control potential public health threats on dairy farms that are accessible to the general public, named „***multifunctional farms***". It will be described a clear example of a HACCP principles risk analysis for the biological hazard "***cuddling of the animals***" by the visitors. Multifunctional farms serve multiple purposes simultaneously. They may include commercial dairy farms that produce milk and sometimes cheese, but also offer the general public one or more additional services, such as camping or hosting groups of children. These farms provide educational opportunities, training, information, a venue for social gatherings, recreational amenities, animal "therapy," the opportunity to taste fresh animal products, and — for young children — "animal snuggling" [14].

Multipurpose farms actively encourage the equitable participation and access of children, adolescents, and adults through hands-on learning opportunities in a variety of social, recreational, educational, and professional endeavors. These initiatives are centered on agriculture, enabling

individuals to better their own lives and the environment. The necessity for such farms has grown over the past few decades, not the least of which is the widening knowledge gap between professional agriculture and urban residents. From the perspective of public health and safety, it is important to think about how HACCP may be applied to these kind of farms. It is important to mention that there is a specific "*hygiene code for city farms*" in various nations [13].

2. UTILIZING HACCP PRINCIPLES TO ENSURE PUBLIC HEALTH SAFETY ON OPEN DAIRY FARMS

2.1 Potential activities and services of city farms

City farms and commercial dairy farms that are accessible to the general public can offer a wide range of activities and services. Whatever activities and services are precisely offered depends primarily on the requirements and traditions of the host community. A type of list of these initiatives and services implies:

- food growing and food production in community gardens, with instructions;
- animal handling, animal husbandry and animal welfare-related activities;
- conservation and nature preservation-related activities (farm camping);
- young children's activities, including animal cuddling;
- young children city farm clubs (play schemes; excursions);
- senior citizen city farm clubs (including grandparents with grandchildren);
- visits and programs for particular groups (e.g. mentally disabled persons);
- venues for arts and crafts workshops (classes; demonstrations; practicals)
- visits of school classes; activities for instructing school children;
- summer holiday camp for children;
- a social meeting place in the format of a „farm café";
- venue for seasonal festivals, special events, or celebrations;
- evening recreation, conservation projects, and training programs;
- venue and support for local self-support groups;
- the basis for volunteering and for learning new skills;
- production of (raw) food products of animal origin.

In terms of public health, animal health, and animal welfare, farms with animals are required to protect both their visitors and the animals from potential risks. This includes bodily risks (trauma) brought on by an aggressive animal or unexpected behavior. In that regard, they are also required to abide with the General Food Law (EC regulation 178/2002). The hygiene directives (EU 852/853/854-2004) must also be followed by these farms when they offer visitors the ability to create food items on-site [14, 17].

2.2 Dairy farms open to the general public: context

The number of dairy farms has significantly decreased in many European countries and is likely to continue to decline in the upcoming years for the reasons outlined below. The choice to abandon farming is frequently influenced by urbanization and shrinking economic margins between farm income and production costs. In addition, many young people no longer have the desire to take over the farm. To boost herd productivity per man and per hectare, remaining farmers frequently expand their herds and adopt new technology. By using services or products targeted at residents as a method to create a „*better*" living in the future, hazards can occasionally be turned into opportunities [9].

Along with economic advantages, the growth of welcoming visitors to farms is frequently advantageous for a better public perception of the agricultural industry. Today's general populace knows very little about agricultural production. Only a small percentage of people have a history with farming, connections to farmers, or a sense of affinity with the place where the food they purchase originated. Strict hygiene regulations and the upscaling of farms are to blame for this. As a result, the public's

perspective is largely shaped by incidents involving animal health and food safety, as well as, the media's portrayal of those events [18].

The public perception of bioindustry and modern farm management, such as dairy farms with robotic milking or farms where cows are housed indoors all year round, might be harmed by negative press from animal welfare organizations. To sustain its right to exist, the agricultural sector, and especially more so in nations with dense populations, needs the compassion and understanding of the general public. By allowing people to learn more about the industry, farms that are accessible to the general public can help with this [4].

Direct animal interaction is one of the many created goods and services that include bed and breakfast, traditional or organic food production, camping, activities, and sports. The target audience for this service may include children, adults with physical or mental disabilities, or those looking to renter the workforce. In addition to the benefits of making farms accessible to the public, hazards may also exist [14].

This section uses the on-farm service of "***animal hugging***" as an illustration of how the HACCP principles be used to control public health hazards on dairy farms that are open to the public. By using HACCP, businesses can give customers more assurance regarding the calibre of their goods and services. Such an application's main goal is to protect users from potential dangers. Moreover, examples of and discussions about the potential (advisory) role of veterinarians in helping farmers apply HACCP will be provided [1].

2.2.1 The HACCP team (step 1 of the 12 developmental steps in HACCP)

The interdisciplinary HACCP team must be formed` before HACCP principles are used, as was described by *Codex Alimentarius*. The HACCP team will be tiny if there are just one or two individuals managing the farm, which is typical on most dairy farms. It is possible that in a small HACCP team, not all expertise is instantly available since distinct skills, particular knowledge, and experience suited to the product or process are needed for the formulation of a HACCP plan. It is advised in such circumstances to enlist external assistance for particular areas [5].

In areas like hygiene, public health (zoonoses), animal health and welfare, or animal handling practice, veterinarians with training in quality risk management and veterinary public health can be very helpful. So, the farmer, an employee, and a veterinarian can make up the HACCP team. As necessary, specialists can be added to the HACCP team to provide guidance in specific areas, such as caring for certain target visitor groups or building appropriate housing for animals used for cuddling [10].

2.2.2 Description of the provided service and the target group (step 2 and 3 of the 12 developmental steps in HACCP)

Once the HACCP team has been put together, it is necessary to provide a detailed description of the targeted service, as well as, the target group itself. The phrase "***animal snuggling***" which is used as an example in this paper, can be presented in a variety of ways. Either directly or through a fence, people can interact with animals. Also, the service can be targeted at various demographics and have a variety of goals. Contact with animals not only makes kids happy but also helps the sick, addicted to drugs or alcohol, overworked, and disabled individuals by instilling in them a sense of duty, self-worth, or positive sensations. The target group's mental and physical health may necessitate higher safety standards for the service.

Before choosing animals for animal cuddling, the service must be precisely defined. Some animal species or humans will be better suited for animal cuddling than others, depending on the service's objectives and its intended audience. Animal characteristics like age, body size, and natural behaviour must be considered, but unpredictable animal and possibly human behavior must also be considered when designing the selection and Quality Risk Management Program [17].

2.2.3 Development of flow diagrams of the production process (step 4 and 5 of the 12 developmental steps of HACCP)

Where appropriate, interactions between the various steps of a production process are also shown in a flow diagram together with their logical and chronological order. Several production procedures must be completed before the intended product, "*raw milk*," is shipped to the industry. These phases can be shown in the general flow diagram used to convey an overview description of the process steps. Detailed diagrams or charts can be used to work out secondary, detailed procedures like feeding various animal species, utilizing land, and treating animals while being fed. In addition to the previously mentioned basic flow diagrams for the raw milk production process, flow diagrams for services targeted at visitors must be produced in order to apply HACCP principles on dairy farms that are accessible to the public. *Fig. 2.* shows an illustration of a particular flow diagram of the animal cuddling procedure. When dairy farming and pet-holding procedures clash, complexity might result. To visualize interactions and points of contact between humans and animals, a (full) specification of the flow diagram must be produced depending on the farming areas where the service of "*animal cuddling*" is offered. For instance, animal hugging can be provided during calving, cow feeding, snuggling dry cows, feeding calves, and calves' grouping [16, 19].

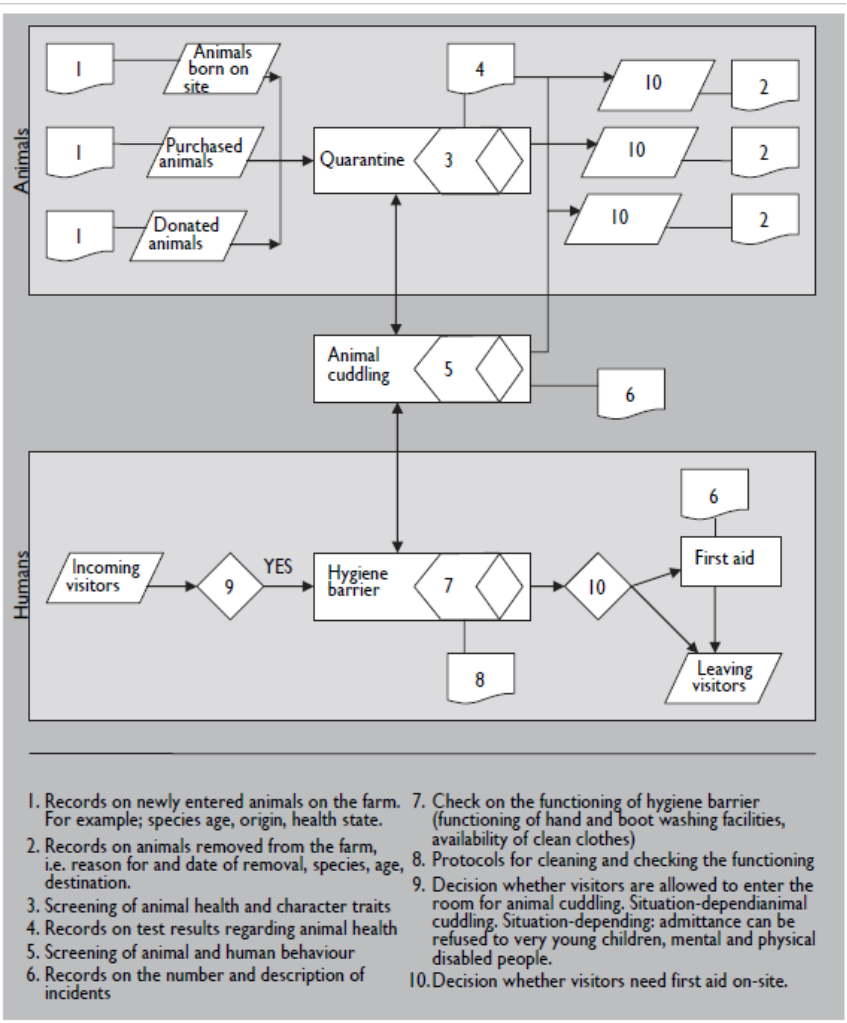


Figure 2. General flow diagram showing the phases of the interactive process between mean with animals by the "animal cuddling"/ Diagrama generală de flux a etapelor procesului de interacțiune a omului cu animalele prin "îmbrățișarea lor" [18, 19]

Before the HACCP team can audit the procedure to confirm that each step in the diagram is an accurate reflection of the actual scenario, the general flow diagrams discussed above must be modified to the unique circumstances of a single farm. The flow diagrams will raise awareness among team members and other farm visitors and will enable additional discussion about hazards and risks within the HACCP team.

Physical risks have a close connection to both animal and human behaviour. Individuals may sustain injuries as a result of animals biting, kicking, or scratching them, as well as, from poorly maintained buildings, fences, or machinery [21].

2.2.4 Hazard analysis (step 6 – principle 1 in the 12 developmental steps of HACCP)

Microbiological, chemical, and physical hazards and risks are the three most common categories of hazards. On dairy farms, unique managerial risks and hazards are identified. Keeping in mind the "*animal cuddling*" service, the risks can be separated into two categories: risks that affect the quality and quantity standards of the targeted product of animal origin and risks that affect the safety of the public's activities (zoonoses and injuries). In addition to having an impact on the amount and quality of raw milk, for instance, risks to animal welfare and the health of the production system might affect consumers that will receive products and services. Different risks and dangers must be evaluated depending on the product or service [1].

Also, microbiological dangers such as zoonotic bacteria, viruses, and parasites may be present on animal skin and in an area contaminated by animal faces (e.g., manure). According to the animals chosen for snuggling, their health status, and regional and national variations in the incidence of zoonotic pathogens, the precise results of the (microbial) hazard analysis will vary on each farm [6].

In case of Europe, pathogenic *Enterobacteriaceae*, such as *Salmonella spp., Campylobacter jejuni*, *E. coli O157*, and *Yersinia enterocolitica*, are zoonotic bacteria that can be common without symptoms. In addition, zoonoses such as dermatophytosis, zoonotic scabies, infectious ecthyma, giardiasis, and cryptosporidiosis should be considered, especially for elderly and young people, as well as, those with impaired immune systems. We must not ignore the possibility that brucellosis or tuberculosis still provide a risk to humans in some nations where they have not yet been completely eradicated.

People may come into touch with chemical risks on some public farms, such as cleaning supplies, pesticides, herbicides, and veterinary medications, when these supplies are, for example, left lying around the farm. Also, when animals are treated, such as with pour-on treatments of veterinary antiparasitic medications, chemicals can be discovered on their hair coats.

2.2.5 Risk Assessment (step 6 of the 12 developmental steps of HACCP)

The HACCP team will talk about the risks and determine which risk factors are prevalent on the farm throughout the risk assessment process. The team must decide the importance and priority of each hazard to be handled during the procedure. Risk assessment can be done using locally available veterinary epidemiological data or using an approach called adaptive conjoint analysis which gathers, validates, and ranks expert opinions on a given topic (such as the applicability of specific risk factors for disease). The third way to assign a weight to hazards is to qualitatively evaluate both their likelihood of happening and their potential effects should they do so. To the best of their knowledge and experience, the members of the HACCP team undertake this weighing. Focusing on the risks associated with "*animal hugging*," which are outlined in Table 1, other risk factors can be identified [20].

Visitors who have direct contact with animals and those who are present in an environment that is contaminated with animal waste (such as manure) have significant risk factors in connection to the threat posed by "*animals harboring zoonotic diseases*". Also, many visitors to farms that are open to the public, such as young children and pregnant women, tend to be in a more weakened condition of health, making them more susceptible to skin lesions than other visitors (*Table 1*).

Table 1. Risk factors associated to biological hazard concerning the pathogenic zoonoses transmission / Factori de risc asociați hazardului biologic privind transmiterea zoonozelor patogene [19]

Direct contact of humans with animals
A stay in an environment which is contaminated with animal waste (manure; scabs)
Lesions of the human skin
Fragile state of health of visitors (young, old, pregnant, immuno-deficient people)

Besides zoonotic disease infections, other significant risks include head injuries from animal contact, animal bites, scratches, and animal kicking. Unpredictable animal behavior and visitor target group behavior can both be risk factors for these dangers (*Table 2*). Also, persons who have little expertise in handling animals and little knowledge of typical and abnormal animal behavior are more in danger than, say, well-trained animal keepers [20].

Table 2. Example of some specific risk factors for a certain hazard / Exemplu a unor factori de risc specifici unui anumit hazard [19]

Direct contact of humans with animals
Contact with animals through a fence
Unpredictable behaviour of animals (e.g. mother behaviour; male behaviour)
Unpredictable/undesirable behaviour of visitors due to e.g. ignorance, health or mental state
Limited knowledge of visitors about animal behaviour

To enable control, the targets for each POPA and each CCP standard or tolerance must be established. The circumstances and objectives of each dairy farm must be taken into consideration while setting targets. For the majority of CCPs and POPAs shown in *Tables 3* and *4*, stated objectives may be set with a zero-tolerance policy, such as prohibiting visitors from entering certain farm areas. Objectives for the POPA's "*Exposure of visitors to animal waste is as low as achievable*" standard can be established in reference to bacterial plate agar counts, which are common in the food business. For instance, allowing no more than a particular amount of colony forming units per cm^2 for aerobic growth and having zero tolerance for *Enterobacteriaceae* on the plate agar obtained from a dinner table. Furthermore, it suggests that a sanitation strategy needs to be implemented.

Table 3. CCP and POCA associated to microbiological hazards at the zoonotic carries animals / PCC și PA asociate hazardelor microbiologice la animalele purtătoare de patogeni zoonotici

No contact between visitors and animals is allowed	CCP
Animals are free from selected zoonotic pathogens	CCP or POPA[1]
Exposure of visitors to animal waste materials is as low as possible	POPA
Isolation of sick animals	CCP[2]

Besides from zoonotic disease infections, other significant risks include head injuries from animal contact, animal bites, scratches, and animal kicking. Unpredictable animal behavior and visitor target group behavior can both be risk factors for these dangers. Also, persons who have little expertise in handling animals and little knowledge of typical and abnormal animal behavior are more in danger than, say, well-trained animal keepers.

Table 4. CCP and POCA associated with physical hazards as a result of physical aggression against animals during contact with them (bitten, hit) / PCC și PA asociate hazardelor de natură fizică ca rezultat a agresării fizice a animalelor în timpul contactului cu acestea (mușcat, lovit) [19]

No contact between humans and animals is allowed	CCP
No direct contact of humans with 'dangerous' animals is allowed	CCP
Avoiding situations which provoke dangerous behaviour of animals	POPA
Sufficient information for visitors about natural and abnormal animal behaviour	POPA

2.2.6 Critical Control Points, CCP, and Points of Particular Attention, POPA (step 7– principle 2 – of the 12 developmental steps of HACCP

A critical control point (CCP) is a step, series of steps, or procedure in the production process that can be measured or observed, that is connected to the risk of concern, at which process control can be applied, where control is necessary to prevent or eliminate a safety risk, and where related corrective measures must ensure the complete restoration of control once it has been lost. A control point is referred to be a Point of Particular Attention, or POPA, when it is not a CCP but is nonetheless thought to be extremely relevant despite not meeting the CCP criteria.

2.2.6.1 CCP's and POPA's in relation to the hazard: 'animals carrying zoonotic pathogens'

To effectively avoid zoonotic pathogen infections and animal-caused illnesses, people should be prohibited from coming into direct contact with animals and from entering cowsheds or pastures (CCP). When the primary objective of the service known as "animal hugging" is described as having direct touch with animals, implementing this control point will not be desirable. Nonetheless, in circumstances where infections are known to be common, the establishment of such a control point for specific populations, such as very young children or immune-compromised individuals, may be advised. Furthermore, when animals are recognized as zoonotic infection carriers, this control point must be put into practice. This issue can be controlled with the use of adequate fencing and supervision. More control points (POPA) are required when direct human interaction with animals is permitted.

It is preferable for animals that have been exposed to people to have a priori negative tests for specific zoonotic infections. Only zoonotic infections that can be reliably and quickly tested, such as by visual examination of clinical indications or through on-site and laboratory tests, can have critical control points established. Sadly, there aren't many zoonotic infections for which there are fast, dependable, and inexpensive diagnostic. In addition to this issue, it is impossible to rule out the reintroduction of zoonotic infections on many farms where animals and tourists come and go. To minimize hazards to the public's health, it is crucial in these open systems to optimize the herd's health state. It will be beneficial to have a stringent policy (good farming practice guidelines) regarding the acquisition and addition of additional animals to the herd. Also, it's important to limit (as much as possible) visitors' exposure to animal feces to lessen the likelihood that they'll contract zoonotic diseases.

Several levels of hygiene can be the focus of this point of special attention. For instance, by cleaning the animal snuggling area of animal droppings at least once every two hours or by cleaning the hair coats of animals before exposing them to guests [19].

2.2.6.2 CCP's and POPA's in relation to the hazard: 'trauma as a result of being hit, kicked, bitten or scratched by an animal'

Some animal species and individual animals can be classified as being dangerous in nature depending on the exact farm scenario, target service, and target group.

Male animals that are typically utilized for animal snuggling, such as bulls and stallions must be excluded. Some creatures can also be categorized as harmful. For instance, due to their more forceful or unpredictable behavior, animals in estrus may act less desirably. Yet, a two or four-month-old free-ranging calf can also be classified as dangerous due to its relatively large size and forceful (inquisitive) behavior when it is around young children. CCP can be characterized as the prohibition of exposing

visitors to harmful animals. Even animals that are normally trustworthy can act dangerously under certain circumstances, such as when they feel threatened. In order to prevent situations that cause animals to behave dangerously, supervision and a proper environment (i.e. housing) are required (POPA). In addition, in order to avoid potentially dangerous circumstances, employees and volunteers must possess or acquire appropriate knowledge of natural animal behavior. Posters with instructions could be put up throughout the property to let guests know what is expected of them and avoid potentially harmful situations. When visitors must take an exam to gauge their level of understanding, this area of focus could turn into a crucial control point. Targets for adequate knowledge could be set in such customized control programs [19, 20].

2.2.7 Targets associated to CCP's and POPA's (step 8 – principle 3 – of the 12 developmental steps of HACCP)

To enable control, objectives must be established for each POPA and each CCP standard/tolerance. KPI's must be determined based on the conditions and objectives of each dairy farm. The majority of CCPs and POPAs shown in Tables 3 and 4 can have their stated targets set on a zero-tolerance basis, such as prohibiting guests from entering certain portions of the farm's grounds. Targets can be set in relation to bacterial plate agar counts, which are utilized in the food business, for the POPA's "*Exposure of visitors to animal waste is as low as practicable*" KPI. For example, a zero-tolerance for *Enterobacteriaceae* on the plate agar taken from a dining table and a restriction on the maximum number of colony-forming units per cm2 for aerobic growth. It also suggests that a sanitation strategy has to be implemented [21].

2.2.8 Establishment of a monitoring system for CCP's and POPA's (step 9 – principle 4 – of the 12 steps of HACCP)

It is necessary to design a particular monitoring system for all CCP or POPA combined.

Regular tests on, say the presence of zoonotic infections will be required in order to optimize and sustain the health state of a herd. Depending on the pathogen, this may be accomplished through a visual assessment of the clinical symptoms or with laboratory testing following a sample of blood, urine, or feces to look for pathogen carriers or shedders.

The majority of sanitary procedures to reduce visitor exposure to animal waste as much as possible (POPA) and to prevent harmful circumstances (POPA) can be observed visually. Visitors should be watched, for instance, to ensure that they are dressing appropriately and wearing appropriate footwear, and cleaning their hands.

Visual inspection is a practical way to assess the cleanliness of the environment.

Agar bacterial count or contact plates should ideally be used to regularly inspect cleaning methods. Monitoring lists can be created for the CCP and POPA examples shown in *Tables 3* and *4*. The following information will be included on these lists: the CCP or POPA of concern; their standard/tolerance or target values; the location in the production process where it must be monitored; the frequency of the monitoring; the method of monitoring (for example, visual; testing after sampling); the responsible person to do it; and the action to be taken once monitoring has revealed loss of control [17].

2.2.9 Establishment of intervention methods & corrective action plans (step 10 – principle 5 – of the 12 developmental steps of HACCP)

The critical limit or target of a process stage can be identified using monitoring data. To ensure that the CCP or POPA has been brought under control once more or at the very least that its influence has been lessened, corrective measures must be implemented [3].

2.2.10 Corrective actions in relation to the microbiological hazard: 'animals carrying zoonotic pathogens'

Visitors must not be exposed to any animals that are suspected of being carriers of zoonotic illnesses until the monitoring data from the daily visual health inspection or routine sample shows signs

of zoonotic diseases. Entrance must be temporarily denied when a visual assessment reveals an inadequate degree of hygiene. The level of hygiene can be raised by changing the cleaning procedure or frequency or by redesigning cowsheds and stables to be more sanitary.

Corrective measures must be performed when visitors do not follow the farm's prescribed regulations. These measures may include warnings, bringing attention to the restrictions, or even denying entry. Examples of prescription regulations include requiring visitors to wear appropriate clothing while around animals and washing their hands following contact with them. Further visitor behavior prescription criteria might be introduced based on the animal species present [12].

2.2.11 Corrective actions in relation to the physical hazard: 'trauma as a result of being hit, kicked, bitten or scratched by an animal'

As was indicated in the last example, when people act irresponsibly, such as when potentially deadly animal behavior is ignored, corrective actions must be performed. Animal-related injuries to humans require prompt first aid or specialized medical attention. To be prepared for this task, farm workers must receive frequent first aid instruction and training. If an animal consistently causes problems or displays undesirable behavior, it must be prevented from snuggling or even replaced by another animal, or, if necessary, another species of animal [11].

2.2.12 Establishment of verification procedures and record keeping (step 10 and 12 – principles 6 and 7 – of the 12 developmental steps of HACCP)

Verification processes must be created in order to ascertain whether the HACCP-like program is operating properly. Preferably, the person in charge of carrying out the monitoring and remedial actions should not execute the verification procedures. A local veterinarian with the necessary expertise can complete this task, other competent outside parties can.

The HACCP-like program and its records, deviations, and product dispositions must all be examined as part of the verification process, and it must be confirmed that CCPs and POPAs are maintained under control. Where practical, validation operations have to involve procedures that verify the effectiveness of each component of the HACCP-like program. It is probable that external verification through auditing will soon need to be adopted in addition to these internal validity checks. The latter might ultimately result in certification for these types of dairy farms.

When a HACCP-like program is used, the results of the hazard identification and risk evaluations, as well as the determination of CCPs, POPAs, and their critical limits or objectives, must be documented. Additionally, written procedures and documentation of CCP and POPA monitoring activities (frequency, methods, results, responsible person), as well as, the associated corrective measures for improvement (CCP or POPA, date, area, type of measures taken, effects), are crucial for helping the farmer confirm that the HACCP-like program is operating in accordance with the goals. Furthermore, short- and long-term evaluations can be carried out using the stored papers.

The Good Dairy Farming Codes of Practice and their precise operating instructions on the farm are another area of documentation in such Quality Risk Management programs [13].

CONCLUSIONS

Dairy farms that are open to the public must deal with specific hazards relating to the activities carried out by lay persons visiting the farm in addition to risks associated with the production of milk. A risk management program similar to HACCP can be used to control safety risks for visitors entering the farm. The HACCP concept focuses on hazard detection, risk analysis, and risk management. As a result, it might be very appropriate for such use.

This paper demonstrates that a HACCP-like program may be created and implemented for dairy farms that are open to the public, in order to manage the microbiological, chemical, and physical risks to the public's health. The HACCP-like program can be used to demonstrate to third parties that safety

hazards associated with farm products and on-farm services are kept as under control as possible in case of complaints in addition to catastrophe prevention. It can also be utilized as a marketing tool (including potential official accreditation).

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RESEARCH ON THE FATTENING APTITUDES OF CROSSBREDS OBTAINED BY CROSSING TSIGAI SHEEP WITH FRENCH MEAT BREED RAMS

CERCETĂRI PRIVIND APTITUDINILE PRODUCTIVE ALE METIȘILOR OBȚINUȚI PRIN ÎNCRUCIȘAREA OILOR ȚIGAIE CU BERBECI DIN RASE DE CARNE DIN FRANȚA

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Abstract

Crossing of local sheep breeds with specialized breeds for meat production in order to obtain crossbreeds with superior meat characters is a rapid method of increasing quantitative and qualitative meat production. The main aim of our research was to study the productive abilities for meat production of crossbreeds obtained by crossing ***Tsigai*** *breed from the rust variety with rams of French meat breeds, namely* ***Vendeen, Blanche du Massif Central*** *and* ***Berrichon du Cher****. The experiences took place within the biobase of the RDSSGB Secuieni Bacau research station in the period 2018-2020. The results obtained show that the heterosis has a higher manifestation on the productive performance in the F1 crossbred lambs (****Berrichon du Cher*** *x* ***Tsigai****) with very significant differences being found (P<0.001) in the average body weight of all ages compared to the maternal breed, followed of F1 crossbreds (****Vendeen*** *x* ***Tsigai****) with highly significant differences (P<0.001) in the average body weight at birth, 100 days and at 6 months and with significant differences (P<0.05) at the age of 60 days. As a result, the* ***Berrichon du Cher*** *and* ***Vendeen*** *breeds have a better degree of genetic combinability with the local* ***Tsigai*** *breed, the resulting F1 crossbreeds having, in the case of intensive fattening, clearly superior performances to the local breed, being able to be used by farmers at any age after weaning. The F1 crossbreds (****Blanche du Massif Central*** *x* ***Tsigai****) can be used by farmers only after the age of 100 days, the weight gain up to this age being much lower than the other two crossbred variants.*

Key words: meat production, sheep, crossbreeding, fattening, crossbreeds, meat breeds

Rezumat

Încrucișarea raselor locale de ovine cu rase specializate pentru producția de carne în vederea obținerii de metiși cu caractere superioare pentru carne este o metodă rapidă de creștere a producției cantitative și calitative de carne. Scopul principal al cercetărilor noastre a fost de a studia aptitudinile productive pentru producția de carne a metișilor obținuți prin încrucișarea oilor ***Țigaie din varietatea ruginie*** *cu berbeci din rase de carne din Franța, respectiv* ***Vendeen, Blanche du Massif Central*** *și* ***Berrichon du Cher****. Experiențele s-au derulat în cadrul biobazei stațiunii de cercetare SCDCOC Secuieni Bacău în perioada 2018-2020. Rezultatele obținute arată că heterozizul are o manifestare mai ridicată asupra performanței productive la mieii metiși F1 (****Berrichon du Cher*** *x* ***Țigaie****) constatându-se diferențe foarte semnificative, (P<0.001) la greutatea corporală medie de la toate vârstele față de rasa maternă, urmați de metișii F1 (****Vendeen*** *x* ***Țigaie****) cu diferențe foarte semnificative (P<0.001) la greutatea corporală medie de la naștere, 100 zile și la 6 luni și cu diferențe semnificative (P<0.05) la vârsta de 60 de zile. Ca urmare, rasele* ***Berrichon du Cher*** *și* ***Vendeen*** *dispun de un grad mai bun al combinabilității genetice cu rasa locală* ***Țigaie ruginie****, metișii F1 rezultați având în cazul îngrășării în sistem intensiv performanțe net superioare rasei locale, putând fi valorificați de către fermieri la orice vârstă după înțărcare. Metișii F1 (****Blanche du Massif Central*** *x* ***Țigaie****) pot fi valorificați de fermieri doar după vârsta de 100 zile, sporul de greutate până la această vârstă fiind mult inferior celorlalte două variante de metiși.*

Cuvinte cheie: producție de carne, ovine, încrucișare, îngrășare, metiși, rase de carne

INTRODUCTION

Currently, on an international level, research in the field of sheep breeding is directed towards the creation of mixed types with high precocity and prolificacy, with a strong capacity for adaptability and high combinability with an increased attention to the improvement of reproductive aspects (Pop et al., 1983; Tafta, 2010). Sheep breeding for meat production has a priority role worldwide (Taftă et al., 1997; Pascal, 2013). The interest in the quantitative and qualitative increase in meat production determined the creation of specialized breeds for this production, the development of methods for the production of lambs for meat and high-performance technologies for growing and fattening young sheep.

Breeding for meat production of local sheep breeds is an extremely important field of activity. The socio-economic changes combined with the requirements and regulations of the European Union regarding the exploitation of meat production and the changes in the genetic structure of the sheep herds highlight the need to carry out and develop some activities regarding the quantitative and qualitative improvement of meat production in local sheep breeds.

The improvement for meat production aims to improve some morphoproductive characteristics such as: body mass, growth speed, reduction of feed consumption, increase of prolificacy, improvement of slaughter yields, meat-bone ratio, tissue composition of carcasses (Mochnacs et al., 1978; Taftă, 1983; Maier and Domnicar, 1989; Pascal, 2015).

Crossing local breeds with breeds specialized for meat production in order to obtain crossbreeds with superior meat characters is a rapid method of increasing production and carcass quality using the advantages of the phenomenon of heterosis (Sandu, 1993). This method of increasing meat production and improving the carcasses intended for delivery has begun to make its way timidly among sheep breeders in Romania.

In our country, the sheep flock is predominantly made up of breeds with a high degree of rusticity and very heterogeneous, with mixed productions. The increased interest in sheepmeat and animals with a high growth rate to give a higher quality carcass has created an opportune environment for research into crossbreeding to increase meat production. Recently, increased attention has been paid to this branch of sheep breeding, the strategy of the national authorities being to encourage the establishment and development of fattening farms and the opening of new markets for sheep meat, Romania has the potential to deliver quality young sheep for all tastes, here referring both to the requirements of the market in Europe and to the requirements of the market in Arab countries that prefer carcasses from rustic sheep breeds.

The main aim of our research was to analyze the productive abilities for meat production of crossbreds obtained by crossing **Tsigai** ewes from rusty variety (Tsi) with rams of meat breeds from France, namely **Vendeen** (V), **Blanche du Massif Central** (BMC) and **Berrichon du Cher** (BC).

MATERIAL AND METHOD

The experiences took place within the biobase of the RDSSGB Secuieni Bacau in the period 2018-2020. In the research, the direct crossing method was used between breeding rams for meat production originating in France and autochthonous **Tsigai** sheep of the rust variety. The goal was to obtain F1 mestizos with superior qualities to the native breed in meat production.

In order to carry out the research in this direction, the products of the pure breed **Tsigai** and the F1 crossbreeds were monitored, being 4 batches of lambs, which were distributed after weaning for fattening in an intensive system. To evaluate the body development of the products obtained, weighings were carried out at birth, at 60 days, at 100 days and at 6 months.

Animal feeding and accommodation

All batches of lambs received the same treatment throughout the experiments. Thus, during the nursing period, the lambs benefited from mother's milk and permanent access to combined starter feed (18% crude protein), alfalfa hay of the best quality, water and mineral blocks for licking. After weaning, lambs were fed ad libitum fully granulated fattening lamb feed containing 16.8% crude protein, alfalfa hay and water. Mineral blocks for licking were also administered to each batch throughout the fattening period. The way of valorization of feed was followed by weighing administered feed and unconsumed leftovers, thus tracking the evolution of feed consumption during the growth and fattening periods and over the entire experimental period.

The intensive fattening system adopted consisted in the maintenance of the young sheep in collective boxes with access to the paddock, the watering being provided with constant level watering cans, with a watering front of 2 linear m, the feeding with concentrates was carried on in high feeders to eliminate wastage and facilitated ingestion, and hay was administered in gridded feeders with a feed front of 3 linear meters on each side, ensuring the required feed front per head of animal.

The health status of the animals that were the subject of the research was constantly monitored during the experiments, performing all the necessary diagnostic, treatment, deworming and vaccination actions.

Monitoring body growth and development

To evaluate the body development of the obtained products, body measurements were performed at birth, at the age of 60 days, at 100 days and at the end of the fattening period, namely at the age of 6 months. At the ages mentioned above, the body weight of the lambs was also determined using the animal scale (accurate to four decimal places) for the best possible accuracy.

In order to ascertain the gain through the effect of heterosis and the combinability of the breeds involved in the crossbreeding program, the performance of the meat production of the crossbreeds was compared with the performance of the pure breed batch **Tsigai**, rust variety, subjected to fattening under the same environmental conditions as the crossbreeds.

Body measurements of length and height were taken using a zoometer, widths with a compass, and perimeters with a tape measure. Later, the data obtained from body measurements were used to calculate the main body indices that give us information on body size, compactness and skeleton.

Assessing the state of fattening in live animal and in the slaughtered animal

Fattening status was assessed on the live animal by palpation of body regions with rich muscle mass and manipulative points, as well as by free assessment using the point method. For a correct and conclusive evaluation of the quality of the carcasses, 5 lambs from each batch were slaughtered. The evaluation of the slaughtered animal was done by determining the body mass of the live animal and the carcass resulting from the slaughter in order to be able to calculate the "*slaughter yield*" which is expressed in percentages and is calculated as the ratio between the mass of the carcass and that of the live animal multiplied by 100, and the "*commercial yield*" when added to the weight of the carcase and organs. The measurements performed on the slaughtered animal were used to calculate the main indices of the carcasses, for a better picture of their quality.

Statistical data analysis

The data recorded following the observations as well as the determinations made were processed using modern statistical processing methods. Microsoft Office Excel 2016 was used to calculate all statistical parameters (mean, standard deviation, coefficient of variation and standard error of the mean) and the t-test (Student) to determine the significance of the difference between the mean values of the characteristics analyzed. Differences were considered statistically significant at $P<0.05$ and indicated by specific signs of differentiation. The results obtained for all the characteristics analyzed were then presented in the form of tables and graphs, and their interpretation was made by comparing them with data from the specialized literature.

RESULTS AND DISCUSSION

As a result of the crosses, the first generation of hybrids was obtained between the **Tsgaie** females of the rust variety and the three imported breeds, which were evaluated regarding the performance related to meat production (body growth and development, feed consumption, carcass quality, etc.), in order to determine the effect of heterosis for meat production.

The obtained data indicate a tendency to manifest the positive type of heterosis for the performance related to meat production in the three mixed populations. This statement is supported by the fact that the three groups of mestizos had a better body development compared to the maternal race for all characteristics analyzed (Table 1).

Table 1. Heterosis effect for body weight determined at specific ages /
Efectul de heterozis pentru greutatea corporală determinată la anumite vârste

Batch	n	Age	UM	The weight corporal in F1 mestizos	The weight corporeal to Tsigai sheep rust variety	Differences (heterozis)	
						Real	$\frac{\overline{X}_{F_1} - \overline{X}_P}{\overline{X}_P}$ x 100
F1 (**Vendeen** x **Tsigai**)	39	birth	kg	5.05	3.97	1.08	27.20
	30	60 days	kg	16.30	14.44	1.86	12.88
	25	100 days	kg	28.75	23.99	4.76	19.84
	25	6 months	kg	44.60	31.84	12.76	40.08
F1 (**Blanche du Massif Central** x **Tsigai**)	30	birth	kg	4.26	3.97	0.29	7.30
	30	60 days	kg	15.61	14.44	1.17	8.10
	27	100 days	kg	25.87	23.99	1.88	7.84
	25	6 months	kg	42.51	31.84	10.67	33.51
F1 (**Berrichon du Cher** x **Tsigai**)	20	birth	kg	5.20	3.97	1.23	30.98
	20	60 days	kg	18.51	14.44	4.07	28.19
	16	100 days	kg	30.02	23.99	6.03	25.14
	15	6 months	kg	43.35	31.84	11.51	36.15

Following the analysis of the evolution of body mass, from birth to 6 months in the breeds studied, in the offspring resulting from the purebred **Tsigai** and in the hybrids **Blanc du Massif Central** x **Tsigai**, **Berrichon du Cher** x **Tsigai** and **Vendeen** x **Tsigai**, it appears that:

- the lowest weight at parturition was recorded in the purebred progeny (**Tsigai**) with an average of 3.97 kg, while in the hybrid progeny the first place was the progeny from **Berrichon du Cher** x **Tsigai** with an average of 5.20 kg at parturition, closely followed (5.05 kg) by the **Vendeen** x **Tsigai** crossbreds progeny;

- at 6 months the lowest weight was recorded in the pure offspring (**Tsigai**) with an average of 31.84 kg, while in the hybrid progeny the first place was the offspring from **Vendeen** x **Tsigai** with an average of 44.60 kg, followed by **Berrichon du Cher** x **Tsigai** crossbreeds with an average of 43.35 kg.

The data obtained for calving weight are close to those obtained by Borzan et al. (2017), respectively an average of 3.60 kg for the **Tsigai rust variety**, which falls within the breed standard, and in the hybrid progeny, the first place was the F1 crossbreeds (**Blanche du Massif Central** x **Tsigai**) with an average of 5, 19 followed by F1 crossbreds (**Berrichon du Cher** x **Tsigai**) with an average of 4.50 kg.

Regarding the average daily gain achieved from weaning (60 days) to the age of 100 days, the first place is the progeny from the **BC** x **Tsigai** breeds with 287 g, followed by the progeny obtained from the **Vendeen** x **Tsigai** crossbreeds with an average daily gain of 277 g, the **BMC** x **Tsigai** crossbreeds with an average daily gain of 239 g and respectively the pure offspring from the **Tsigai** breed with an average daily gain of 214 g/day (Table 2).

Table 2. The average daily gain recorded during periods of growth (kg) / Sporul mediu zinic înregistrat pe perioade de creștere (kg)

Batch	Nr. crt.	Specification	n	$\bar{X}$	$\pm s_{\bar{x}}$	s	V%	Min.	Max.
Tsi	1.	Average daily gain 0-30 days	30	0.190	0.011	0.062	30.339	0.093	0.350
	2.	Average daily gain 30-60 days	30	0.194	0.016	0.086	23.301	0.101	0.370
	3.	Average daily gain 60-100 days	30	0.214	0.009	0.049	22.840	0.105	0.321
V x **Tsi**	1.	Average daily gain 0-30 days	30	0.229	0.009	0.050	21.806	0.126	0.359
	2.	Average daily gain 30-60 days	30	0.253	0.020	0.104	41.162	0.149	0.601
	3.	Average daily gain 60-100 days	25	0.277	0.010	0.052	18.868	0.201	0.389
BMC x **Tsi**	1.	Average daily gain 0-30 days	30	0.198	0.008	0.046	23.031	0.116	0.285
	2.	Average daily gain 30-60 days	30	0.210	0.008	0.046	21.670	0.138	0.302
	3.	Average daily gain 60-100 days	25	0.239	0.012	0.061	25.442	0.157	0.410
BC x **Tsi**	1.	Average daily gain 0-30 days	20	0.214	0.008	0.040	20.205	0.128	0.361
	2.	Average daily gain 30-60 days	20	0.229	0.019	0.114	39.062	0.151	0.402
	3.	Average daily gain 60-100 days	16	0.287	0.009	0.048	16.866	0.201	0.419

The values obtained in the present study are superior to those obtained by Borzan et al. (2017), who found that for the average daily gain, the **BMC** x **Tsigai** crossbreeds are in first place, with an average daily gain of 240 g, while for the pure descent from the **Tsigai** breed, the average daily gain recorded was only 154 g /day. For the **Tsigai** breed, the SMZ value is higher compared to that reported in the specialized literature, namely 152 g/day (Pascal et al., 2013).

Regarding the effect due to heterosis, it is found that after testing the differences between the resulting crossbreeds and the local breed (Student test application), it has a higher manifestation on the productive performance of the F1 products resulting from the crossing of the females of the **Tsigai** breed with rams from the **Berrichon du Cher** breed (highly significant differences in mean body weight at all ages - Table 5), followed by F1 crossbreeds obtained from crosses with rams belonging to the Vendeen breed (highly significant differences in mean body weight at birth, 100 days and at 6 months and significant differences at 60 days -Table 3).

Table 3. Differences between average body weight in F1 crossbreds (Vendeen x Tsigai) and Tsigai breed rusty variety (kg) / Diferențe între greutatea corporală medie la metișii F1 (Vendeen x Țigaie) și rasa Țigaie varietatea ruginie (kg)

Specification	F1 (Vendeen x Tsigai)	Tsigai	Real difference	Differences (%)	The meaning of the differences
Birth weight	5.05	3.97	1.08	27.20	***
Weight at 60 days	16.30	14.44	1.86	12.88	*
Weight at 100 days	28.75	23.99	4.76	19.84	***
Weight at 6 months	44.60	31.84	12.76	40.08	***

NS – insignificant differences (P>0.05); * significant differences (P<0.05); ** distinctly significant differences (P<0.01); *** very significant differences (P<0.001).

Table 4. Differences between average body weight in F1 crossbreds (Blanche du Massif Central x Tsigai) and Tsigai breed rusty variety (kg) / Diferențe între greutatea corporală medie la metișii F1 (Blanche du Massif Central x Țigaie) și rasa Țigaie varietatea ruginie (kg)

Specification	F1 (Blanche du Massif Central x Tsgai)	Tsigai	Real difference	Differences (%)	The meaning of the differences
Birth weight	4.26	3.97	0.29	7.30	NS
Weight at 60 days	15.61	14.44	1.17	8.10	NS
Weight at 100 days	25.87	23.99	1.88	7.84	NS
Weight at 6 months	42.51	31.84	10.67	33.51	***

NS – insignificant differences (P>0.05); * significant differences (P<0.05); ** distinctly significant differences (P<0.01); *** very significant differences (P<0.001).

Table 5. Differences between average body weight in F1 crossbreds (Berrichon du Cher x Tsigai) and Tsigai breed rusty variety (kg) / Diferențe între greutatea corporală medie la metișii F1 (Berrichon du Cher x Țigaie) și rasa Țigaie varietatea ruginie (kg)

Specification	F1 (Berrichon du Cher x Tsigai)	Tsigai	Real difference	Differences (%)	The meaning of the differences
Birth weight	5.20	3.97	1.23	30.98	***
Weight at 60 days	18.51	14.44	4.07	28.19	***
Weight at 100 days	30.02	23.99	6.03	25.14	***
Weight at 6 months	43.35	31.84	11.51	36.15	***

NS – insignificant differences (P>0.05); * significant differences (P<0.05); ** distinctly significant differences (P<0.01); *** very significant differences (P<0.001).

Regarding the F1 crossbreds (**BMC** x **Tsgaie)**, it was found that the differences are insignificant in the average body weight achieved at birth, at 60 days and at 100 days, and the differences become very significant only at the age of 6 months, registering a better weight and implicitly a higher average daily increase in the period 3-6 months (Table 4).

In accordance with these results, it can be stated that the **Berrichon du Cher** and **Vendeen** breeds have a better degree of genetic combinability with the local **Tsigai** breed, rust variery, the resulting F1 crossbreds having, in the case of intensive fattening, clearly superior performances to the local breed, being able to be exploited by to farmers at any age after weaning. The F1 half-breeds obtained with the **Blanche du Massif Central** breed can only be used after the age of 100 days, the weight gains up to this age being much lower than the other two half-breed variants.

The results obtained confirm those reported by Mochnacs et al. (1978), which shows that in F1 crossbreds (**BC** x **Tsigai**) growth is improved by 4.97%, specific consumption is reduced by 9.08% and yield at slaughter increases by 4.73%, compared to the maternal breed. Maier and Domnicar (1989), in the crossbreeding of **Tsigai** sheep with **Romney-Marsh** rams, found that the F1 crossbreds achieved a genetic gain of 3.50 kg in terms of body weight compared to the **Tsigai** breed.

The calculated specific consumption was on average 6.3 UN/kg gain in the **Tsigai rust variety** group, 5.9 UN/kg gain in F1 crossbreds (**BC** x **Tsi**), 5.6 UN/kg gain in crossbreds (**BMC** x **Tsi**) and 5.5 UN/kg gain in crossbreds (**V** x **Tsi**). The lowest consumption was therefore recorded by the mixed-breeds (V x Tsi), which is 12.7% lower compared to the whole-registered **Tsigai** lambs. In the **Tsigai** breed, the consumption is similar to that reported in the specialized literature, namely 6.2 UN/kg weight gain (Ionescu et al., 1985).

Table 6. Specific consumption / Consumul specific

Specificare	Tsigai	Breed F1 (V x Tsi)	Breed F1 (BMC x Tsi)	Breed F1 (BC x Tsi)
Specific consumption (UN/kg gain)	6.3	5.5	5.6	5.9
Differences (%) compared to Tsigai	-	12.7	11.1	6.3

According to the findings, it can be said that, when it is desired to obtain larger amounts of meat, one of the methods can be the crossing with parents from the three breeds because in the expression of body development in the mestizo generation, the number of loci involved increases due to the differences between breeds in terms of relevant allele frequencies at these loci. The greater the difference in gene frequency, the greater the effect due to heterosis (Bonnes et al., 1991; Bodin et al., 1999).

Assessment of fattening status on the live animal consisted of palpation of regions with rich muscle masses and manipulative points, as well as free assessment using the point method. Following the evaluation of a number of 5 heads from each lot, it was found that in the animals from the **Tsigai** lot, there were 4 animals graded 2 (poor) and one animal graded 3 (good). Regarding the **V** crossbreds (Figure 2), four of them were rated four very good) and one was good. **BC** crossbreds were graded 4 and **BMC** crossbreds (Figure 1) received grades 4 and 3. Thus, it can be seen that the best fattening condition was the crossbreds (**BC** x **Tsi**).



Figure 1. BMC x Tsigai crossbred male at 6 months of age / Metis mascul BMC x Țigaie la vârsta de 6 luni



Figure 2. Vendeen x Tsigai crossbred male at 6 months of age / Mascul metis Vendeen x Țigaie la vârsta de 6 luni



a b c d

Figure 3. Carcases from the 4 lots / Carcase provenite de la cele 4 loturi
a -**Tsi**; b -**V** x **Tsi**; c - **BC** x **Tsi**; d - **BMC** x **Tsi**

Lambs slaughtered at 60 days of age (weaning) had live weights from 16 kg in **Tsigai** to 25 kg in **BMC** with a commercial yield of 52% in **Tsigai**, 54% in crossbreds (**V** x **Tsi**), 55% in crossbreds (**BC** x **Tsi**) and 57% in crossbreds (**BMC** x **Ti**). Slaughter yield without head, without organs and without gastrointestinal contents was 43% in **Tsigai**, 44% in **V** x **Tsigai** crossbreds, 44% in **BC** x **Tsigai** crossbreds and 47% in **BMC** crossbreds (Table 7, Figure 3, Figure 4).

From the analysis of these data, it can be seen that the best yield at slaughter was obtained by the half-breeds (**BMC** x **Tsigai**). For the **Tsigai** breed, the yield value at slaughter is similar to that reported in the specialized literature (Pascal et al., 2007).

Table 7. Slaughter yield and commercial yield / Randamentul la sacrificare și randamentul comercial

Specification	Tsigai	Breed F1 (V x Tsi)	Breed F1 (BMC x Tsi)	Breed F1 (BC x Tsi)
Slaughter yield (%)	43	44	47	44
Commercial yield (%)	52	54	57	55

Compared to the data found in the specialized literature, it is found that the values obtained for the slaughter yield are lower (Borzan et al., 2017), a fact that can be explained by the fact that the slaughter age in the case of our project was only 2 months, compared to the 4 months encountered in the cited experience. Also, Borzan et al. (2017), found that the best yield at slaughter was achieved by the mixed breeds (**BC** x **Tsigai**) being 50.6%, followed by (**BMC** x **Tsigai**) with 50% and the mixed breeds (**V** x **Tsigai**) with 48, 8%.

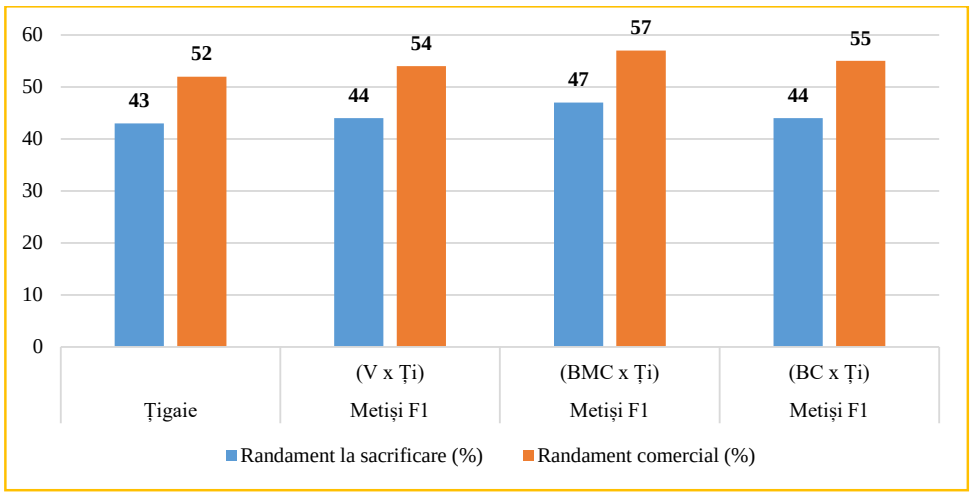


Figure 4. Slaughter yield and commercial yield / Randamentul la sacrificare și randamentul comercial

Following the average values of the carcase indices, it can be seen that the best carcase format has the half-breeds (**V** x **Tsi**) followed by the half-breeds (**BMC** x **Tsi**). The jigo format shows more obvious muscle masses in crossbreds (**BMC** x **Tsi**) followed by crossbreds (**BMC** x **Tsi**). The compactness of the carcass recorded the highest values in the **Tsigai** group, and the homogeneity of the carcass is the best in crossbreds with **BMC** being 100% as well as the best development of the jig represented by the index of jig development (Table 8).

Table 8. Average values of the main carcass indices calculated for the 4 batches / Valorile medii ale principalilor indici ai carcaselor calculați pentru cele 4 loturi

Nr. crt.	Lot	Carcass format index	Pulp format index	The compactness of the carcass index	The width of the carcass index	The development of the pulp index	Thorax depth index
1	**BMC** x **Tsi**	36.84	72.41	112.28	100.00	120.00	36.84
2	**V** x **Tsi**	37.25	70.37	119.60	110.52	112.5	39.21
3	**Tsi**	34.78	64.00	121.73	106.25	109.09	41.30
4	**BC** x **Tsi**	36.25	69.30	121.00	109.72	114.5	40.51

Following the heterosis effect for the main carcass indices calculated we observe a slightly negative heterosis effect for the carcass compactness index and the chest depth index. On the other hand, a positive heterosis can be observed for the size and development indices of the pulp, a portion of the carcass of increased importance in the capitalization of the carcass (Table 9).

Table 9. Calculation of the heterosis effect for the main carcass indices / Calcularea efectului de heterozis pentru principalii indici ai carcaselor

Lot	n	Indices	UM	Medium values half-breeds F1	Average values Tsigai rust variety	Difference (heterosis)	
						Real	$\frac{\overline{X}_{F_1} - \overline{X}_P}{\overline{X}_P}$ x 100
V x **Tsi**	10	carcass format	%	37.25	34.78	2.47	7.10
		pulp format	%	70.37	64.00	6.37	9.95
		compactness of the carcass	%	119.60	121.73	-2.13	-1.75
		the width of the carcass	%	110.52	106.25	4.27	4.02
		the development of the pulp	%	112.50	109.09	3.41	3.13
		thorax depth	%	39.21	41.30	-2.09	-5.06
BMC x **Tsi**	10	carcass format	%	36.84	34.78	2.06	5.92
		pulp format	%	72.41	64.00	8.41	13.14
		compactness of the carcass	%	112.28	121.73	-9.45	-7.76
		the width of the carcass	%	100.00	106.25	-6.25	-5.88
		the development of the pulp	%	120.00	109.09	10.91	10.00
		thorax depth	%	36.84	41.30	-4.46	-10.80
BC x **Tsi**	10	carcass format	%	36.25	34.78	1.47	4.23
		pulp format	%	69.30	64.00	5.3	8.28
		compactness of the carcass	%	121.00	121.73	-0.73	-0.60
		the width of the carcass	%	109.72	106.25	3.47	3.27
		the development of the pulp	%	114.50	109.09	5.41	4.96
		thorax depth	%	40.51	41.30	-0.79	-1.91

Based on these data, it can be said that the application of crosses represents a faster and more efficient solution for obtaining populations of a new type in which an improvement of characters can be found that require a long time for improvement through pure breed selection and the application of breeding programs breeding based on selection.

CONCLUSIONS

1. The **Berrichon du Cher and Vendeen** breeds have a better degree of genetic combinability with the local **Tsigai** from rusty variety, the resulting F1 crossbreds having clearly superior performance to the local breed in case of intensive fattening, being able to be used by farmers at any age after weaning.

2. The use of first-generation crosses of **Tsigai** ewes with **Berrichon du Cher** rams lead to an increase in meat production on the farm by 25.1 to 36.2% depending on the age at which the delivery is made and to obtaining clearly superior carcasses to obtained from the **Tsigai** breed.

3. The use of first-generation crosses of **Tsigai** sheep with **Vendeen** rams leads to an increase in meat production on the farm by 13 to 40% depending on the age at which delivery is made and to obtaining carcasses superior to those obtained from the **Tsigai** breed.

4. The F1 crossbreds obtained with the **Blanche du Massif Central** breed can be used by farmers only after the age of 100 days, the weight gains up to this age being much lower than the other two variants of hybrids.

5. Industrial crosses of first-generation remain an effective and fast solution for increasing meat production in farms that own herds of sheep from the **Tsigai** breed, the rust variety, using the benefits of

the heterosis effect, the farmer's additional investment being only the purchase of rams from the meat breeds.

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THE *IN VIVO* AND *IN VITRO* INFLUENCE OF SOME VEGETABLE EXTRACTS ON THE IMMUNOLOGICAL PROFILE IN CHICKENS

INFLUENŢA *IN VIVO* ŞI IN VITRO A UNOR EXTRACTE VEGETALE ASUPRA PROFILULUI IMUNOLOGIC LA PUII DE GĂINĂ

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Abstract

*Plant extracts are defined by their bioactive properties, thus providing the scientific basis for their use in various pathologies. The investigations were carried out on 28, 47-day-old broiler chickens (**Rock** x **Cornish**), divided into 4 equal groups treated orally for 7 days with: I –alcoholic extract of Calendula officinalis, 0.5 ml; II - Echinacea angustifolia, 0.5 ml; III - 70% alcohol, 0.5 ml; IV – untreated control. On days 0 and 7, the birds were antigenically stimulated with 0.5 ml of a 5% suspension of sheep red blood cells in saline, administered sc. Blood taken from all groups on days 0, 7 and 14 was processed by leukocyte counts (Natt-Herrick), evaluation of phagocytic activity (incorporation of carbon particles) and blast transformation test. Treatment with alcoholic extracts of C. officinalis and E. angustifolia did not significantly influence the total number of leukocytes during the experiment. The plant extracts used induced a stimulation of phagocytic activity for both extracts (Echinacea angustifolia: OD 0.157 ± 0.07 and Calendula officinalis: 0.152 ± 0.056 OD). Of the two plant extracts, only the Echinacea angustifolia extract (54.86% ± 7.76%) showed stimulating effects in the group treated in vivo with the same extract. Thus, plant taxonomy defined the immunological efficacy of the tested extract in chickens.*

Key words: plant extracts, Calendula officinalis, Echinacea angustifolia, chickens, immunity, phagocytosis, blast transformation

Rezumat

*Extractele vegetale sunt definite de proprietăţile lor bioactive, oferind astfel bazele ştiinţifice pentru utilizarea lor în diferite patologii. Investigaţiile au fost efectuate pe 28 de pui broiler de 47 de zile (**Rock** x **Cornish**), divizaţi în 4 loturi egale tratate per os timp de 7 zile cu: I- extract alcoolic de Calendula officinalis, 0,5 ml; II –extract alcoholic de Echinaceea angustifolia, 0,5 ml; III –alcool 70º, 0,5 ml; IV netratat. În zilele de 0 şi 7 păsările au fost stimulate antigenic cu 0,5 ml suspensie de hematii de oaie 5 % în ser fiziologic, administrată sc. Sângele prelevat de la toate loturile în zilele 0, 7 şi 14 a fost prelucrat prin numărarea leucocitelor (Natt-Herrick), evaluarea activităţii fagocitare (înglobarea particulelor de carbon) şi testul de transformare blastică. Tratamentul cu extracte alcoolice de C. officinalis şi E. angustifolia nu a influenţat semnificativ numărul total de leucocite pe parcursul experimentului. Extractele vegetale folosite au indus o stimulare a activităţii fagocitare pentru ambele extracte (Echinaceea angustifolia: DO 0,157 ± 0,07 şi Calendula officinalis: 0,152 ± 0,056 OD). Dintre cele două extracte vegetale doar cel de Echinaceea angustifolia (54,86 % ± 7,76 %) dovedeşte efecte stimulatoare la lotul tratat in vivo cu acelaşi extract. Astfel, taxonomia plantei defineşete activitatea imunologică a acesteia la puii de găină.*

Cuvinte cheie: extracte vegetale, Calendula officinalis, Echinacea angustifolia, pui de găină, imunitate, fagocitoză, transformare blastică

INTRODUCTION

In recent years, the study of plant extracts has gained new momentum due to the favorable results obtained in the therapy of some conditions in which even synthetic active substances have proven less effective (Roitt et al., 1989, Dhama et al., 2018, Qassadi et al. col., 2023). Phytochemicals show promise in supporting and maintaining health. There are numerous structural compounds in plant extracts such as polyphenols, terpenoids, phenolic acids, essential oils, lectins, polypeptides and alkaloids. Due to the presence of these substances, some of which are secondary metabolites, plant extracts are characterized with different biological properties, thus providing the scientific basis for their use in different pathologies (Qassadi et al., 2023). By associating plant extracts with the preparations used in classical therapeutic protocols, it is possible to intervene in increasing the body's non-specific defense capacity through the complex immune stimulation effect it exerts. The problem is all the more urgent in veterinary medicine where, by using such extracts, various disturbances of the immune status induced by stress states can be corrected. These conditions are increasingly common in the conditions of an increase - increasingly artificial exploitation of birds and animals, associated with a diet that is not always correct and balanced (Zarnea 1980, Vior et al., 1982, Nawaz et al., 2021). Numerous extracts from different plants are currently used to increase the natural resistance of organisms to various infections or in their effective treatment. Moreover, some extracts are used for the partial restoration of the functional capacity of the immune system in subjects with congenital immune depression. We should also not neglect the anti-inflammatory effects that some plant extracts can exert and due to which the extracts can acquire a wide use of interest in therapeutics (Arstila et al., 1994, Benencia et al., 1995, Bernd et al., 1995, Bussing et al., 1997, Fernandez-Puntero et al., 1997). A less researched but promising field of study on the use of extracts is the discovery of new adjuvants for vaccines or the improvement of existing ones, a phenomenon considered essential for the development of modern vaccines. Thus the use of plants and extracts obtained from them, although known for millennia, is far from being a closed subject. In this context, the purpose of the study was to evaluate the potential of some plant extracts on the immunological profile of chickens. The interest that today is medicine and biochemistry gives to this field is constantly growing and results in the introduction into practice of new preparations, which acquire an increasing importance among modern prophylactic and therapeutic means.

MATERIAL AND METHOD

The investigations were done on 28 broiler chickens (**Rock** x **Cornish**), males and females, aged 47 days. The chickens came from the private farm. During the period before the experiment, the birds were kept in bunk batteries, 6 individuals/battery. The feeding was carried out in the first two weeks with combined feed prepared according to the 21/1 recipe, and later with type 21/2 feed. The average weight of the chickens at the beginning of the experiment was 1.5 kg. Later, the chickens were introduced into a breeding system in batteries on 3 levels, two individuals/box. During the entire period of the experiment, the birds were fed ad libitum, with feed obtained according to the 21/2 recipe, and the watering was done drop by drop, in the waterers in the box. No immunoprophylactic actions were performed during the experiment. The chicks were distributed in 4 groupes of 7 birds each. The groups were treated with plant extracts as follows: in group I, alcoholic extract of *Calendula officinalis* was administered for 7 days, in the amount of 0.5 ml, per bone; in group II the alcoholic extract of *Echinacea angustifolia*, in the amount of 0.5 ml/bone; in group III (control group) alcohol 70º, in the same quantity and by the same route of administration. Lot IV represented the absolute untreated control (environmental control and handling stress). Day 0 was considered the start day of the experiment. Blood samples collections were performed on days 0, 7 and 14. On days 0 and 7 the chickens were inoculated subcutaneously with a suspension of sheep red blood cells (SH, freshly prepared in the laboratory) 5%

in saline, administered in an amount of 0.5 ml. The samples were evaluated by the leukocyte counting technique, the evaluation of the phagocytic activity and the blast transformation test. The Natt-Herrick Tic® test (BioAnalytic) was used to determine the total number of leukocytes. To determine non-specific cellular reactivity, blood samples were collected by jugular vein puncture. To the blood samples, 3 µl of China ink was added. From the samples thus prepared, 100 µl were taken and transferred to 2 ml physiological serum; the mixture represented the control for the optical density conferred by the presence of plasma proteins and non-phagocytosed mucus. After homogenization by repeated overturning, the samples were incubated at 37ºC and to establish the optimal reading time, 100 µl of the blood and swab mixture were taken at 15 and 30 minutes. Each of these quantities was placed in 2 ml physiological serum. Finally, the tubes with a mixture of physiological serum, blood and tissue were centrifuged for 5 minutes at 800 revolutions/minute. The optical density of the supernatants was read in Sumal PE 2 at a wavelength of 535 nm in 96-well plates. Readings for controls were made against saline.

The values of phagocytosis were considered the ones read, expressed in units of optical density with physiological serum as standard. In parallel, the effects of the administered extracts on the reactivity of phagocytosis *in vitro* were investigated. The working variants assumed the same operations and in addition the addition of 3 microliters of extract at the initial moment. The extracts used were those of *Echinacea angustifolia* and *Calendula officinalis*, and the control, 70° alcohol.

The blast transformation test was used to determine specific cellular reactivity. For this purpose, the blood taken was diluted with RPMI 1640 medium (Sigma-Aldrich) supplemented with 5% fetal calf serum (Gibco) and antibiotics (penicillin 1,000 IU/ml; streptomycin 1,000 µg/ml). The obtained suspension was distributed in sterile plates with 96 wells, 200 µl/well. The experimental variants were: control (untreated), PHA M1 1 µl/well, alcohol 1.5 µl/well and alcoholic extracts of *Calendula, Arnica, Symphytum, Echinacea angustifolia, Echinacea purpurea* each 1.5 µl/well. The response to red blood cell lysate was also tested, the birds being inoculated with sheep red blood cells. Optical density of samples was performed at 610 nm wavelength in 96-well plates.

Initially, an average supernatant sample was made for each variant, homogenizing 12.5 µl from the two wells in a single tube. 12.5 µl of this mixture was added to 0.5 ml ortho-toluidine reagent, and after homogenization, the test tubes were kept in a water bath at 100ºC for 8 minutes. Each series of samples was identically worked with the glucose standard (with a concentration of 100 mg %) and initial medium.

The concentration of glucose in the samples to be researched was found starting from their optical density and the optical density of the glucose standard according to the formula: Cp = Ep/Es x 100, in which: Cp = sample concentration in mg glucose %; Ep = sample extinction and Es = standard extinction. Considering the residual concentrations of glucose after stopping cell growth and the initial concentration of the medium, it is possible to calculate the blasting index expressed as a percentage for both control and stimulated cultures. The reference value is the integral medium. The blasting indices induced by PHA and the various extracts were calculated using the formula: IS = glucose from the medium - glucose sample / glucose from the medium x 100; where, IS is the spontaneous blasting index. All the results obtained through these tests were processed statistically to obtain the mean values, standard deviations and variance using the usual formulas of statistical calculation. The significance of the differences was assessed using the t-student test.

RESULTS AND DISCUSSION

Following the evolution of the total number of leukocytes in group I treated with *Calendula* extract, it is found that at the first collection the parameter varies between 11,500-19,500/mm3, with an average of 16,571,429±2,636,736 (standard deviation, SD). At the second collection, the number of leukocytes showed variable values between 17,500 - 39,000/mm^3, the average recorded was 29,785

±8,459.99 (SD). At the third collection, the leukocyte values were between 19,000 and 44,500/mm^3, the average being 26,785.71 ±8,557.92 (SD) (Fig. 1). In group III treated with *Echinacea* extract, the total number of leukocytes varied from 18,000 to 39,000/mm^3 with a mean of 27,333,333 ±7,167,054 (SD). Later, an increase in the variation is observed from 17,000 to 47,000/mm^3, the average having the value of 28,357,143 and the standard deviation of 10,391,139, while at the third collection the values obtained varied between 18,500 and 37,500/mm^3, the average being of 28,500, and the SD was ± 6,331.14 (Fig. 1).

In group III, treated with alcohol, at the first harvest the minimum value was 27,500, and the maximum was 51,000/mm^3, with an average of 37,428.57 and a SD of 8,974.169; at the second harvest, a decrease in variation is observed from 22,000 to 49,000/mm^3, with an average of 30,357.14 and a standard deviation of 9,432.7. The values of the third collection were between 24,000 and 45,000/mm^3, with a mean of 33,857.14 and SD of 6,878.192 (Fig. 1).

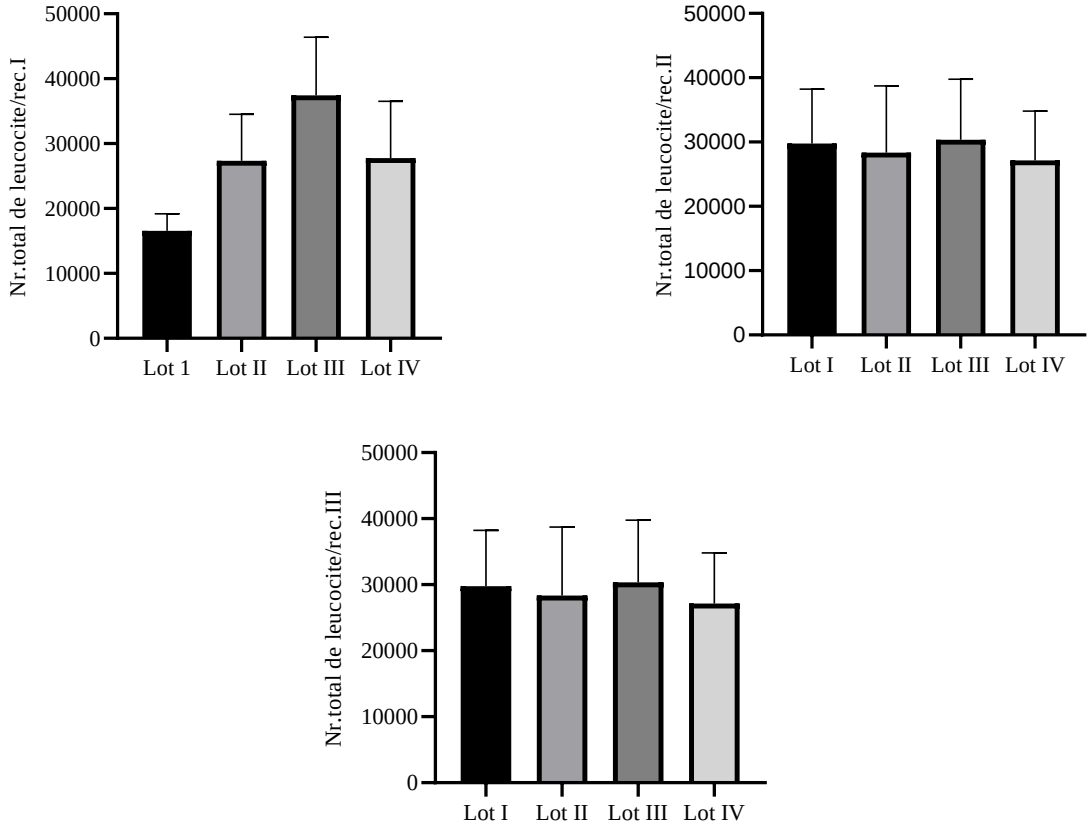


Figure 1. Total leukocyte count at the three sampling / Numărul total de leucocite la cele trei probe

In the control group, the total number of leukocytes at the first collection varied between 16,500 and 42,000/mm^3, with an average of 27,785.71 and a SD of 8,717.11. At the second harvest, an increase in individual values was observed, varying from 21,000 to 43,000/mm^3 with an average of 27,142.86 and a SD of 7,685.019. At the last harvest (III), the minimum value was 24,500, and the maximum value was 41,000/mm^3, with an average of 35,857.14 and a standard deviation of 5,406.125 (Fig. 1). Leukocytes represent some of the first effectors of defense (Czarnetzki et al., 1990). Their intervention can be destructive, with the aggressor microorganisms being incorporated, degraded and then eliminated, or constructive, when the aggressor microorganisms are incorporated, degraded and their constituents are

expressed on the cell surface, the antigenic information is transmitted to the immunocompetent cells and a response translated by the appearance of antibodies is elaborated (Rodenberg et al., 1994).

Establishing the total number of leukocytes can be a valuable indicator in estimating the efficiency of non-specific cellular protection. Phagocytosis represents one of the first intervention protection mechanisms, with a special significance especially in the prevention of bacteriosis and viruses. In this function, they are active, together with polymorphonuclear granulocytes and mononuclear cells of the macrophage type, whether they are in circulation or fixed in tissues. In the case of our study in the phagocytosis test of the control sample at harvest I, at T15 (minutes) a minimum value between 0.052 - 0.713 was found with a mean of 0.4247 ±0.172 (SD). The phagocytic activity of samples treated with alcohol at the same time have values that varied between 0.036 and 0.606, with a mean of 0.439 and a SD of 0.170. In the samples treated with *Echinacea* extract, at 15 minutes, the phagocytic activity values were between 0.032 and 0.796 with an average of 0.512 and a standard deviation of 0.187. Regarding the results of the second harvest, in the case of the control sample, at T15, the lowest value is 0.062 and the highest value is 0.197, the mean being 0.10 ± 0.04 (SD). In samples treated with alcohol, the lowest value was 0.14, and the highest was 0.291 with an average of 0.18 and a standard deviation of 0.07. Regarding the samples treated with Calendula extract, at T15, the minimum value is 0.067 and the maximum is 0.326 with an average of 0.17. In the case of samples treated with *Echinacea angustifolia,* the maximum value is 0.201, and the minimum is 0.067, with an average of 0.13 and a standard deviation of 0.045. At harvest II, it can be seen in the case of the control sample, at T15, that the lowest value is 0.21, and the highest is 0.378, with an average of 0.28.

In the case of samples treated with 70° alcohol, the values obtained at T 15 are 0.063 the minimum value and 0.366 the maximum value. Samples treated with *Calendula* show a fluctuation of phagocytic activity between 0.25 and 0.566 with an average value of 0.24 and a standard deviation of 0.11. In the case of samples treated with *Echinacea angustifolia* extract, the phagocytic activity is variable, with values between 0.154 and 0.304, with an average of 0.22 and a standard deviation of 0.051.

At T 30, at the first harvest, in the case of the control sample, the minimum value is 0.049, and the maximum value is 0.745 with an average of 0.36 and a standard deviation of 0.16.

The phagocytic activity of the samples treated with alcohol (also at 30 minutes) shows values that vary between 0.036 - 0.606 with an average of 0.43±0.17 (SD). In the samples treated with *Echinacea* extract, at the same time, the phagocytic activity values were on average 0.46±0.18. At collection 2, in the case of the control sample, at T 30, the minimum value was 0.03, and the maximum was 0.158, with an average of 0.07±0.039 (SD). In samples treated with alcohol, at 30 minutes, the minimum value was 0.05, and the maximum was 0.187, with an average of 0.11 and a standard deviation of 0.12. Samples treated with *Calendula* the minimum value was 0.053, and the maximum was 0.341 with an average of 0.18 and a standard deviation of 0.08. In the case of samples treated with *Echinacea angustifolia* extract, the minimum value is 0.063 and the maximum is 0.201, with an average of 0.135 ±0.045 (SD).

At harvest III in the case of the control sample, at T 30, the lowest value was 0.097, and the highest was 0.362 with an average of 0.21 ±0.06 (SD).

In the case of samples treated with 70° alcohol, the values obtained at T 30 were 0.049 the minimum value and 0.373 the maximum value with an average of 0.23 ±0.08 (SD). Samples treated with *Calendula* show a fluctuation of phagocytic activity between 0.04 and 0.231 with a mean value of 0.15 ±0.05 (SD). In the case of samples treated with *Echinacea angustifolia*, the phagocytic activity is variable with values between 0.028 and 0.215, with an average value of 0.157 ±0.06 (SD).

Numerous methods have been devised for the evaluation of phagocytic activity. Thus, the phagocytic capacity of whole blood *in vitro* against different live or inactivated bacterial suspensions was tested. Other methods of investigating phagocytic activity assume the association of this function *in vivo*. Thus, the animal is injected intravenously with the supernatant of a suspension of carbon particles (Chinese ink), centrifuged at 6,000 revolutions/minute for 15 - 20 minutes, in an amount evaluated according to

the species. Before and at different intervals after inoculation, blood samples are collected, the optical density of the plasma is evaluated spectrophotometrically and a correlation is established between this and the phagocytic activity of the body. This method assesses the body's phagocytic activity, summing up the activity of both mobile and fixed phagocytes. The acute antimicrobial anti-inflammatory response is the result of complement activation, translated by polymorphonuclear leukocyte chemotaxis and increased vascular permeability (Giurgea, 1994, Ostrycharz et al., 2022). Phagocytosis, i.e. intracellular killing by hydrolytic enzymes, represents one of the most primitive protection mechanisms, which in higher animals are performed by specialized cells: microphages and macrophages (Giurgea, 1994).

Microphage activity can be monitored *in vitro* by the carbon particle incorporation assay. This test also gives the possibility of following the effects of some extracts or other compounds, directly, in the presence of ink particles.

The data obtained initially, in the untreated birds, suggest a pronounced phagocytic activity in the untreated variant and stationary phagocytic activity, more intense at the beginning of incubation and slower afterwards, in the case of the alcohol-treated variant. The treatment with the *Echinacea* extract, although it does not induce phagocytosis as intense as that of the control group, thus acting Following the one-week treatment with the two extracts and the inoculation, the intensification of the phagocytic activity of the untreated control variant is observed. Alcohol treatment reduces phagocytic activity compared to the control, while *Calendula* extracts ensure an increase in phagocytosis initially and then a stationary evolution. *Echinacea* induces a more intense stimulation than *Calendula*, but the effects compared to those of the alcohol control are inhibitory.

At the end of the experiment, although the antigenic stimulation was repeated, the phagocytosis values decrease both in the control group and in the variants treated with alcohol, except for the plant extracts. Both extracts determine in the final moment, the stimulation of phagocytosis, compared to the control and the group treated with alcohol.

Regarding the stimulation indices in the case of the control variant, a fluctuation of the stimulation indices was observed between 49.06% and 62.79% with an average of 55.71%±5.42(SD). Phytohemagglutinin acts as a more intense stimulant, thus the stimulation indices are higher than in the case of the control variant from 54.41% to 75.81% with an average of 60.32% ±7.13 (SD).

The alcoholic extracts used induced variable stimulation indices, ranging between 11.16% in the case of the application of the *Arnica* extract and 82.32% in the case of the application of the *Calendula* extract. The highest average stimulation index was recorded in the case of cultures treated with alcohol (56.57%) and the lowest in the case of treatment with *Arnica* (18.43%). Red blood cell lysate determined an intermediate response. In group II, in the case of the control variant, the stimulation index indicated values between 26.27%-61.39%, with an average of 45.98 ±11.97 (SD).

Phytohemagglutinin showed values of the stimulation index close to those of the control sample, with values between 28.13% and 61.62%, with an average of 50.13% ±10.74 (SD).

The alcoholic extracts used induced variable stimulation indices between 14.18% in the case of the application of the *Arnic*a extract and 68.37% in the case of the application of the *Echinacea angustifolia* extract. The lowest average stimulation index was recorded in the case of crops treated with *Arnica montana* (20.23%), and the highest in the case of the treatment with *Echinacea purpurea*. Red blood cell lysate determined an intermediate response (49.14%). When analyzing the results of the first harvest in groupes 3 and 4 in the case of the control variant, values between 38.13% and 66.97% were observed, with an average of 50.26% ±9.78% (SD).

In the case of phytohemagglutinin, the stimulation index values were between 49.06% and 66.51% with an average of 58.97% ±5.92% (SD).

The alcoholic extracts used induced variable stimulation indices, ranging between 13.72% in the case of *Arnica* extract application and 68.37% in the case of alcohol application (extract control). The lowest average stimulation index is registered in the case of cultures treated with *Arnica* extract (26.91

%), and the highest in the case of alcohol treatment (57.17 %). Red blood cell lysate determined an intermediate response (54.14%).

In group 4, in the case of the control variant, there was a fluctuation of the stimulation indices between 53.25% and 88.13%, with an average of 72.49% ±17.69% (SD).

Phytohemagglutinin acts as a more intense stimulant, thus the stimulation indices were slightly higher than in the case of the control variant from 51.86% to 91.86%, with an average of 74.55% ± 17.10%.as an inhibitor, allows sensing the phagocytic dynamics. In the case of alcoholic extracts, the minimum value of the stimulation index was 9.30% in the case of the use of the *Arnica* extract, and the maximum value of 91.86% in the case of the use of the *Echinacea purpureea* extract with an average of 46.24%. At harvest 2 in the case of the control variant, in group I, a fluctuation of the stimulation indices was observed between 36.02% and 54.03%, with an average of 41.87% ± 8.87 (SD).

The alcoholic extracts used induced variable stimulation indices between -4.02% in the case of the use of the *Arnica* extract (so a negative action) and 51.86% in the case of the use of the *Calendula* extract with the highest average value of 36.37% in the case of using *Echinacea angustifolia* extract and the lowest of 10.24% in the case of *Arnica* extract. Red blood cell lysate determined an intermediate response.

In group II, by analyzing the results in the case of the control variant, a fluctuation of the stimulation indices was observed between 30.12% and 49.37%, with an average of 40.81% ±8.14 (SD). Phytohemagglutinin acted as a more intense stimulant, so the stimulation indices were higher than in the case of the control variant (25.15% - 56.20%). The alcoholic extracts used showed a maximum value in the case of using the alcohol extract (50.30%), while the *Arnica* extract had a negative effect (-15.53%). The average values of the stimulation index were 7.44% in the case of the use of the *Arnica* extract and 33.36% in the case of the use of the alcohol extract, while the red blood cell lysate has a response close to that of the alcohol extract, respectively 33 .71%.

In the case of the control variant in group I at the second harvest, the stimulation index indicates values that vary from 23.59% to 43.47%, with an average of 36.59% ±7.58 (SD).

Phytohemagglutinin indicated a more intense stimulatory potential. In the case of alcoholic extracts, the stimulation indices showed values ranging from -10.56% in the case of the *Arnica* extract (so a negative action), to 59.31% in the case of the alcohol extract. The highest average stimulation index was recorded in the case of cultures treated with *Echinacea purpureea* extract (42.18%), and the lowest (16.98%) in the case of treatment with *Arnica montana*. Red blood cell lysate causes an intermediate response (42.05%). In group IV, in the case of the control sample, the stimulation index indicates values between 24.5% and 57.76%, with an average of 39.97% ±12.88% (SD). In the case of phytohemagglutinin, the values obtained were between 21.42% and 60.24%, with an average of 42.10% ±14.36% (SD).

Among the alcohol extracts used, the highest value of the stimulation index was obtained in the case of the use of the alcohol extract of 57.45%, while the extract of *Symphytum officinale* has a strong negative action (-13.04%). The highest stimulation index is recorded in the case of cultures treated with alcohol extract (36.77%), and the lowest in the case of treatment with *Arnica montana* (14.10%). Red blood cell lysate causes an intermediate response. Analyzing the results after the third harvest in the case of group I, the control sample showed a fluctuation of stimulation indices between 29.81% and 69.25% with an average value of 52.48% ±8.21(SD).

The alcoholic extracts used had values of the stimulation index between 10.86% in the case of the *Arnica* extract and 64.59% in the case of the use of the *Calendula* extract. The stimulation index had the highest value in the case of treatment with *Calendula* extract (56.65%) and the lowest in the case of treatment with *Arnica* (22.13%). Red blood cell lysate causes an intermediate response (54.65%). In group II, in the case of the control sample, the stimulation index had the maximum value of 65.83% and the minimum value of 30.74%, with an average of 50.13% ±13.76 (SD).

Phytohemagglutinin acted as a less intense stimulator, so the stimulation indices were lower than in the control variant, from 31.98% to 63.97%. In the case of the use of plant extracts, the stimulation index had the maximum value in the case of the use of the *Echinacea angustiolia* extract (67.39%), and the minimum value in the case of the use of the *Arnica* extract (22.04%).

In group III at the third harvest in the case of the control variant, a fluctuation of the stimulation indices was observed between 40.68% and 76.70% with an average of 58.11% ±12.13% (SD).

Phytohemagglutinin acts as a more intense stimulant, so the stimulation indices are higher than in the case of the control variant, from 51.55% to 78.88%, with an average of 63.66% ±10.80% (SD).

The highest value of the stimulation index was obtained in the case of the use of the alcohol extract (74.84 %) and the lowest value in the case of the use of the *Arnica* extract (24.04 %). In group IV, the control variant showed a fluctuation of stimulation indices between 51.24 and 80.12% with an average of 60.86% ±9.28 (SD).

Phytohemagglutinin induced stimulation index values between 55.89 and 74.84% with a mean of 63.26% ±6.44 (SD).

The alcoholic extracts used induced variable stimulation indices, ranging between 18.32% in the case of the use of the *Arnica montana* extract and 84.78% in the case of the use of the *Calendula* extract. The highest average stimulation index was recorded in the case of cultures treated with alcohol (62.73%), and the lowest in the case of treatment with *Arnica montana.*

The study of the immune system in birds is particularly based on the knowledge gained by investigating immunological effectors and mechanisms in chickens. The cell cultures made from bird blood mainly focus on the blasting of lymphocytes, separated on special media, by concentration gradient centrifugation. Thus, the roles of T lymphocytes can be highlighted, the most important of which is to recognize self and non-self determinants (Giurgea, 1997). The blasting activity of lymphocytes is influenced by environmental factors, including the compounds included in the culture medium. The test of blast transformation *in vitro* allows the evaluation of the efficiency and the comparison of the effectiveness of such compounds. Estimating the values obtained from the blast transformation test, it is found that at the first harvest, the spontaneous blasting indices are heterogeneous, even though the birds were not treated. This fact is due to the inhomogeneity of the birds in the groupes. This discrepancy can also be found in the case of PHA-induced stimulation.

In our study, among the extracts used *in vitro,* the *Echinacea purpurea* extract proved to be the most effective, followed by the *Echinacea angustifolia* extract and the *Symphytum* extract.

Despite all the antigenic stimulation, at the second harvest, the spontaneous blasting indices decrease, still being maximum in the groupes treated with extracts. The differences are not statistically significant. The best response to phytohemagglutinin is given by the group treated with alcohol. Among the plant extracts, *Echinacea angustifolia* extract has the best answer. The response to red blood cell lysate is the most intense in the same group.

At the last harvest, spontaneous blasting indices increase more in groupes not treated with extracts than in treated ones. The response to phytohemagglutinin is similar. They increase the responses to plant extracts in all groups, however, the maximum ones are found in the control group, whose cells were treated *in vitro* with the extract of *Echinacea purpurea*. Comparing these results with those of alcohol stimulation, the differences argue for inhibition.

Lymphocytes maintained in contact with other types of blood cells or isolated in the culture medium, have the ability to multiply under appropriate conditions, spontaneously or under the influence of various non-specific (PHA M, PHA P) or specific mitogenic substances (bovine enzootic leukosis virus) . The evaluation of the degree of blastization by different methods: the incorporation of titrated thymidine or ethidium bromide, the dosage of residual glucose from the culture medium, allows the calculation of the stimulation index, providing the image of the reactive capacity of the T or B subpopulations in the case of an antigenic stimulation in vivo.

The blastic transformation of the cells *in vitro* is due to the stimulation of the cellular metabolism as a result of the reaction between the antigen and the lymphocyte membrane structures.

Following the coupling of the antigen with the receptors on the surface of specifically sensitized lymphocytes, the cell membrane is activated triggering the transmission of a signal to the nucleus, which determines the initiation of DNA synthesis. As a result, there is an increase in the size of the cell, the nucleus becoming larger, with loose chromatin and basophilic cytoplasm.

CONCLUSIONS

The treatment with alcoholic extracts of *Calendula officinalis* and *Echinacea angustifolia* does not significantly influence the total number of leukocytes during the experiment, so that the final values (16,571.43 ± 2,636.74 respectively 27,333.33 ± 7,167.05) are close to the initial ones (26,785, 71 ± 8,557.93 and 28,500 ± 6,331.14, respectively). A slight decrease is observed in the case of the group treated with *Calendula officinalis* extract. Spontaneous phagocytic activity is diminished towards the end of the experiment, in the absence of antigenic stimulation (0.218 ± 0.060 OD). The alcohol-treated variant exhibits similar activity (0.230 ± 0.082 OD). The plant extracts used initially act inhibitory so that later, the *in vivo* treatment, combined with the *in vitro* one, induces stimulation in the case of both (*Echinacea angustifolia:* OD 0.157 ± 0.07 and *Calendula officinalis:* 0.152 ± 0.056 (OD). Spontaneous blasting indices are lower in the groups treated with extracts (group I: 52.48 ± 13.19 %, group II: 50.13 ± 13.177 %), compared to the one treated with alcohol (58.11 ± 12, 13 %) and untreated (60.87 % ± 9.29 %). Treatment with *Calendula* and *Echinacea* plant extracts reduces the mitogenic blast index (57.41 % ± 8.21 % and 53.15 % ± 11.16 %). Among the plant extracts, only that of *Echinacea angustifolia* (54.86% ± 7.76%) shows stimulating effects in the group treated *in vivo* with the same extract.

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THE ROLE OF LABORATORY DIAGNOSIS IN THE CONTROL OF THE SILKWORM DISEASES, THE CURRENT STATE, PROSPECTS AND THE IMPACT ON SERICICULTURE

ROLUL DIAGNOSTICULUI DE LABORATOR ÎN CONTROLUL BOLILOR CONTAGIOASE LA VIERMII DE MĂTASE, STAREA ACTUALĂ, PERSPECTIVE ȘI IMPACTUL ASUPRA SERICICULTURII

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Abstract

With a tradition of thousands of years in the world and several hundred years in our country, sericulture, a branch of animal husbandry whose object is the growth and multiplication of silkworms in order to obtain cocoons intended for processing, is almost becoming a rarity in many areas of the globe. It has a long history in Romania, starting from the 19th century, when it developed steadily until 1990, after which there was a dramatic decline in this field so that currently silk is only processed at a traditional level. The silkworm is an insect with complete metamorphosis (egg, larva, nymph, adult) and, at the same time, a biological model, and the best-known species of silkworms, whose cocoons can be used for silk production, are: ***Bombyx mori*** *breed (90% considered the most valuable, the only one held in the heritage, which feed on mulberry leaves),* ***Anthereae perny*** *breed (oak silkworm) and* ***Philosamia cynthia ricini*** *breed (Eri silkworm). After the dissolution of S.C. Sericarom SA, by H.G. no. 695/2022, the Baneasa Bucharest Sericulture Research Station was established, which currently operates under the Ministry of Agriculture and Rural Development (MADR) and under the scientific coordination of the "Gheorghe Ionescu-Şişeşti" Academy of Agricultural and Forestry Sciences (AAFS), having as its main object the research-development activity and the conservation of the sericulture genetic pool. The maintenance of the sericulture genetic pool (84* ***Bombyx mori*** *breeds, lines and hybrids) involves in addition to compliance with technical conditions and the performance of sanitary-veterinary examinations (direct microscopy and laboratory diagnostic methods for bacterial, internal parasitic, mycotic and viral diseases, according to the specific methodology. Thus, at the end of the active silkworm season (2022), morphological and microscopic investigations of the preserved silkworm genetic pool were carried out in the Laboratory of Silkworm Genetics, Breeding and Pathology of RSSBB in order to exclude the risk of the presence of pathogenic germs in the generations of silkworms from the next silkworm season (2023). The results of the laboratory examinations were negative for the samples taken in the analysis, a fact that allowed obtaining reproductive material free of diseases, of the best quality. The biological material free from infectious-contagious diseases can be utilized without restrictions by the main target beneficiaries (sericulture farmers, private silkworm breeders who obtain raw silk for economic purposes), who can be advised through specialist consultancy for the prevention and control of silkworm diseases, that can endanger their health and influence the quality production of silkworms cocoons.*

Keywords*:* ***diagnosis, silkworms, contagious diseases***

Rezumat

Cu o tradiție de mii de ani în lume și de câteva sute de ani la noi în țară, sericicultura, ramură a zootehniei care are ca obiect creșterea și înmulțirea viermilor de mătase, în vederea obținerii gogoșilor destinate prelucrării, aproape devine o raritate în multe zone ale globului. Ea are o istorie îndelungată în România, începând cu secolul al XIX-lea, când s-a dezvoltat constant până în 1990, după care s-a produs declinul dramatic al acestui domeniu, astfel că în prezent, mătasea se prelucrează doar la nivel tradițional. Viermele de mătase este o insectă cu metamorfoză completă (ou, larvă, nimfă, adult) și, totodată, un model biologic, iar cele mai cunoscute specii de viermi de mătase, sunt: ***Bombyx mori*** *(90% considerată cea mai valoroasă, singura deținută în patrimoniu, care se hrănește cu frunze de dud),* ***Anthereae perny***

*(viermele de mătase al stejarului) și **Philosamia cynthia ricini** (viermele de mătase al ricinului). După desființarea Sericarom SA, prin H.G. nr. 695/2022 a luat ființă Stațiunea de Cercetări Sericicole Băneasa București (SCSBB) care funcționează în subordinea Ministerului Agriculturii și Dezvoltării Rurale (MADR) și în coordonarea științifică a Academiei de Științe Agricole și Silvice „Gheorghe Ionescu-Șișești" (ASAS), având ca scop principal activitatea de cercetare-dezvoltare și conservarea fondului genetic sericicol. Menținerea fondului genetic sericicol (84 rase, linii și hibrizi de **Bombyx mori**) implică, pe lângă respectarea unor condiții tehnice și efectuarea de examene sanitar-veterinare (microscopie directă și metode de diagnostic de laborator pentru boli bacteriene, parazitare interne, micotice și virale), conform metodologiei specifice. Astfel, în cadrul Laboratorului de Genetică, Ameliorare și Patologia Viermilor de Mătase al SCSBB au fost efectuate, la sfârșitul sezonului activ sericicol (2022), investigații morfologice și microscopice ale fondului genetic sericicol aflat în conservare, pentru a se exclude riscul de prezență a germenilor patogeni la generațiile de viermi de mătase din sezonul sericicol următor (2023). Rezultatele examenelor de laborator au fost negative pentru probele luate în analiză, fapt care a permis obținerea de material de reproducție liber de boli, de cea mai bună calitate. Materialul biologic indemn de boli infecto-contagioase poate fi valorificat fără restricții către principalii beneficiari țintă (fermieri sericicoli, crescători particulari de viermi de mătase, care obțin mătase brută în scop economic), ce pot fi consiliați prin consultanță de specialitate pentru prevenirea și combaterea bolilor la viermii de mătase, boli care pot periclita sănătatea acestora și pot influența calitatea producției de gogoși de mătase.*

Cuvinte cheie**: **diagnostic, viermi de mătase, boli contagioase

INTRODUCTION

Sericulture is the science that deals with the study of the biology and technology of reproduction, growth and improvement of all sericulture species that produce cocoons in order to obtain natural silk. On the international level, the products obtained from silk represent one of the most expensive textile materials, also being used for non-textile purposes (silk protein used for medical use, cosmetics, and biomaterials). In many countries of the world, the sericulture sector is being developed (India, China, Japan, Thailand, France, Poland, Brazil, Cuba, Uganda, Kenya, Ethiopia, etc.), that tend to move to another performance (automation and digitization) (Yashaswini et al., 2020; Vankadara, 2021; Nalavadi et al., 2021).

Sericulture has a long history in Romania starting from the 19th century, when it developed steadily until 1990, after which the dramatic decline of this field occurred (Pau and Brailoiu-Tanase, 2006), so that currently silk is processed only at a traditional level. The Research Station for Sericulture Baneasa-Bucharest (SCSBB) currently operates under MADR and under the scientific coordination of ASAS (H.G. no. 695/25.05.2022), having as its main activity the research-development and conservation of the sericulture genetic fund (HG no. 695/2022; Law no. 319/2003; Law no. 72/2011; Law no. 45/2009).

Recently, in 2022, in Romania, the 26th edition of the Congress of the International Sericulture Commission (ISC) "*The New Concepts in Sericulture*" dedicated to sericulture specialists was organized at USAMV-Cluj, for the first time in Romania. The representatives of ISC member countries and prominent guests from the field of sericulture and the silk industry, from more than 15 countries, including India, China, Japan, Thailand, France, Romania, Poland, Brazil, Cuba, Uganda, Kenya or Ethiopia, participated.

Romania is a founding member of the International Sericulture Commission since 1959 and obtained from the ISC, in 2016, the Certificate of Recognition through which the Global Center of Excellence for Advanced Research in Sericulture and Promotion of Silk Production was created, based at USAMV Cluj-Napoca.

Sericulture is most developed in China, but also in Japan, India, Korea. In Europe, this field had the same trend as in Romania (*www.fzb.usamvcluj.ro*). There are also important points of work in Italy and Bulgaria.

The biological cycle of silkworms has 4 stages of metamorphosis: egg, larva, nymph (chrysalis) and adult (moth) (Bura et. al., 1995). Figure 1 presents the biological cycle of silkworms (www.wikipedia).

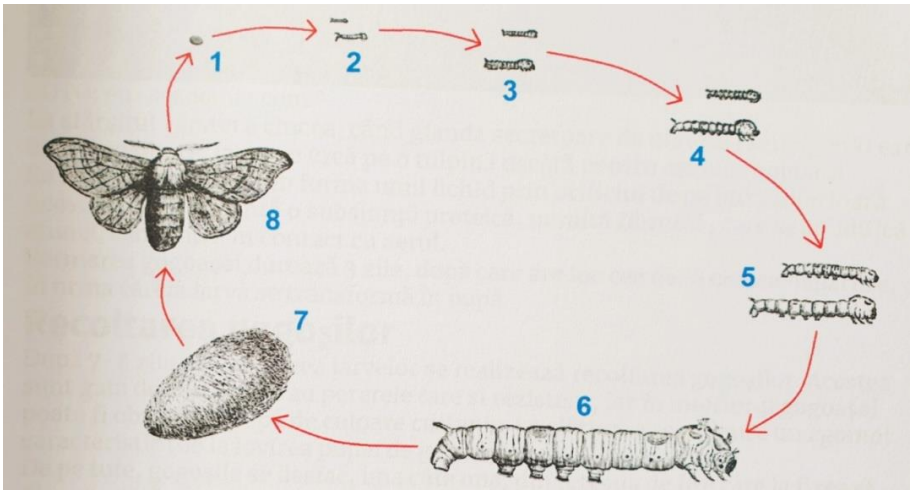


Figure 1. The biological cycle of silkworms: eggs, larve, nynph and adult / Ciclul biologic al viermilor de mătase: 1. Stadiul de ou; 2-6. Stadiile de larvă; 7. Stadiul de gogoașă; 8. Stadiul de fluture (www.wikipedia.com)

The silkworms (including the eggs and moth) are susceptible to numerous diseases (viral, parasitic, fungal and bacterial ones), with different routes of transovarian, oral, cutaneous transmission (Doliș, 2008), and currently there is no breed with totally resistance against them (Matei, 2002). Silkworm diseases are divided into two categories: infectious (viruses, bacteria, fungi, protozoa) and non-infectious (attacks by various arthropods, chemicals used in agriculture). The main factor in the production of the disease is the pathogen, without which no infectious disease can occur (Pau, 2000; Pau et al., 2006). Its development can be inhibited by good environmental conditions, and the physiological state of the host, (silkworm). All these elements have a special role in the occurrence of silkworm diseases; therefore, their prevention is of great practical importance. For most of the silkworm diseases, infection is achieved orally, along with the administration of feed (Shilpi and Praban, 2020). Therefore, avoiding contamination of the mulberry leaf, as a food source, represents an important step in the prevention of diseases in silkworms (Kumari et al., 2001; Matei, 2002).

The causes of diseases can be biological (pathogenic microorganisms, parasitic and non-parasitic insects), physical (mechanical injuries and physical irritations), chemical (chemicals used in agriculture), nutritional (food quality, over or under-nutrition), environmental (inadequate quality of food, temperature, humidity, ventilation, unfavorable lighting) or constitutional (differences due to genetic formation, developmental and sexual stage), which ultimately decrease vigor and reduce resistance.

The silkworm, a host for the development of various microbes, shows resistance thresholds in the prevention of diseases through the skin, digestive tract, intestinal juice, blood cells and the immune system (Pau et al., 2006).

Laboratory diagnosis plays a crucial role in ensuring the health and productivity of silkworms, and any disease can have a significant impact on silk production (Dolis, 2008). Laboratory diagnosis can help confirm disease and identify diseases early, allowing prompt treatment and prevention of further spread of diseases. Some of the common diseases that affect silkworms can cause symptoms such as loss of appetite, lethargy and abnormal behavior.

Laboratory diagnosis can help identify the specific pathogen causing the disease, guide the choice of appropriate treatment and management strategies (disinfection of necessity). In addition to diagnosing disease, laboratory analysis can help monitor the health and development of silkworms by assessing the

nutritional content of their diet, monitoring the quality of the mulberry leaves they feed on, and examining the genetic traits of different silkworm species.

In general, laboratory diagnosis is essential to ensure the health, productivity of silkworms, and by early identification and treatment of diseases, sericulture farmers can ensure a healthy and sustainable silk production process (impact on population productivity) (Kang and Guo, 2011).

In this context, the aim of the work was to evaluate the health status of the silkworm gene pool and highlight the importance of laboratory diagnosis in the control of contagious diseases in silkworms.

MATERIAL AND METHODS

Health monitoring through morphoclinical and laboratory examinations on moth of **Bombyx mori** was carried out in order to prevent and control diseases that may later develop in silkworms. In the Laboratory of Silkworm Genetics, Breeding and Pathology of RSSBB, at the end of the 2022 active silkworm season, investigations were carried out on the biological material of silkworms of the **B. mori** species, respectively on the 84 existing breeds, lines and hybrids that constitute the genetic background, maintained in conservation.

Monitoring sheets were drawn up that included the following: breed/line or hybrid, female characteristics, clutch morphology, results of direct microscopic examination and for pathogens, number of clutch cells removed and admitted for preservation.

As an example, we present below the methodology used to analyze an indigenous race, chosen randomly, with a total number of 140 samples, of which 70 female butterfly samples and 70 stem cells were analyzed (Figure 2 and Figure 3).



Figure 2. Femele moth of *B. mori* laying eggs / Femela de *B. mori* în timpul depunerii pontei (RSSBB)



Figure 3. Paper cell specially need for eggs laying / Celulă din hârtie pergament specială utilizată pentru depunerea pontei (RSSBB)

The morphoclinical examination sought the elimination of clutches with a reduced number of eggs, unfertilized eggs, unevenly arranged eggs or conglomerates.

The microscopic examination (LEICA microscope, 400x and 100x resolution) was performed based on the regulations of the International Office of Epizootics (OIE, 2016), adapted for samples of female *B. mori* moth (www.idsa.ro). To detect endoparasitises, the presence and number of spores of *Nosema* sp. was determined, to detect mycotic agents, the presence of fungal agents was determined, and to detect viral diseases, the presence of polyhedral crystals was determined.

Briefly, the working technique consists in triturating the female butterflies by placing them individually in special 18-compartment trays/mortars that are attached to the triturating machine, adding

distilled water and triturating until a thick liquid is obtained. With the help of a rod, a drop of the liquid in each mortar is deposited on a glass slide which is covered with a slide, and then subjected to microscopic examination. If pathogenic agents are observed on microscopic examination, the respective cell with the pontine is removed and destroyed by burning. If no pathogens are observed after examining about 10 microscopic fields for each sample, it follows that the female was healthy, so the eggs she laid in the cell are also healthy. After microscopic examination, the stem cells from healthy females were kept under appropriate conditions in the cold room (2.5-5ºC) for preservation.

RESULTS AND DISCUSSION

The analysis of the monitoring sheets regarding the morphoclinical examination of the moth showed the lack of even slight changes in the external appearance, finding they had developed properly, did not present physical integumentary injuries (intact integument, no changes in the integrity of the wings (wrinkling, malformations or shrinking of the wings) and legs (excrescences, anomalies), without skin color changes, lack of body fragility (dehydration), absence of smell (flacherie) and diarrheal excrement (nosemosis) (Figure 4 and 5). No typical symptoms of flacherie, grasserie or nosemosis were identified in *B. mori* moth.

The analysis of the monitoring sheets of the cells with the laying eggs related to each *B. mori* moth presented from a morphological point of view the concentric arrangement of the eggs, without identifying a scattered arrangement of them (nosemosis).



Figure 4. Clinically healthy *B. mori* butterflies/ Fluturi de *B. mori* clinic sănătoși (RSSBB)



Figure 5. Concentrically arranged B. mori eggs from clinically healthy females / Ouă de *B. mori* cu dispunere concentrică provenită de la femele clinic sănătoase (RSSBB)

Viral diseases of silkworms are produced by *Borrelina bombicys* with 2-6 microns polyhedra sizes, having triangular, hexagonal, tetragonal shape (Nuclear Polyhedra/NPV); or icosahedral, hexagonal, sometimes tetragonal or triangular polyhedra shape between 1 and 10 microns (Cytoplasmic Polyhedra/CPV) (Figure 6), as well as with globular viral particles of an yet undetermined structure (Flacherie infectious/FV) (Rahul et. al., 2019; Chopade et al., 2021).

Endoparasitic diseases (pebrina/nosemosis) are caused by the protozoan *Nosema bombycis* (Figure 7), with three stages of evolution, respectively spores, planont and meront (Chopade et. al., 2021).

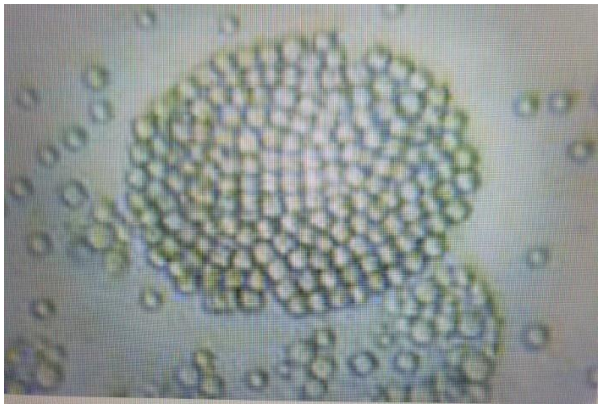


Figure 6. The morphological aspect of the cytoplasmic polyhedra virus / Aspectul morfologic al virusului poliedriei citoplasmatice (Liu, 2017)

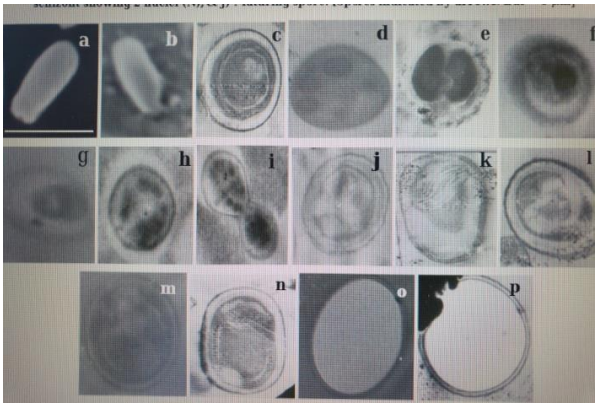


Figure 7. Mature spore of *Nosema sp.* and intermediate forms / Spor matur de *Nosema* sp. și forme intermediare (b-p) (Chakrabarty et al., 2012)

Mycotic diseases are caused by *Beauveria bassian*a (www.ansvsa.ro), a fungus that shows three stages in evolution: conidia, vegetative mycelium and aerial mycelium. The conidia have an oval, globular shape, visible as a white powder that penetrates the integument of the larva after germination (white muscardina) (Figure 8 and 9).

In aspergillosis, the pathogen is represented by fungi of the genus *Aspergillus sp*. (Chopade et al., 2021).



Figure 8. The morphological aspect of the colony of *Beauveria bassiana* / Aspectul morfologic al coloniei de *Beauveria bassiana* (Shanmugam and Seethapathy, 2017)

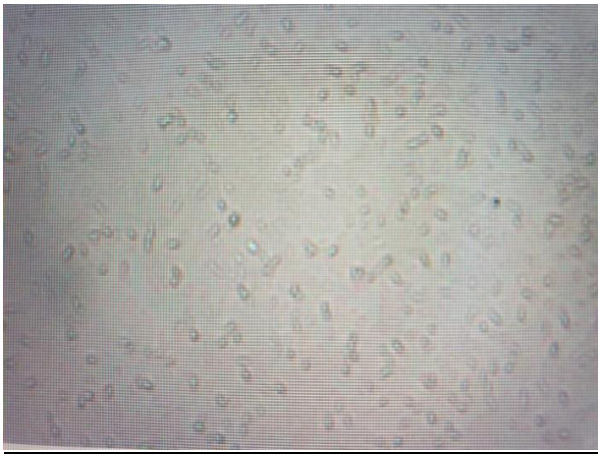


Figure 9. Conidia of *Beauveria bassiana* / Conidia de *Beauveria bassiana* (Shanmugam and Seethapathy, 2017)

Bacterial diseases are caused by bacteria of the genus *Bacillus*, with Gram positive bacilli measuring 1-1.5 x 3 microns, (Bacterial Septicemia) and bacteria of the genus *Streptococcus* sp. round diplococci or in the form of chains, Gram positive, 0.7-0.9 µ in size, (Infectious Anemia) (Chopade et al., 2021) (Figure 10 and 11).

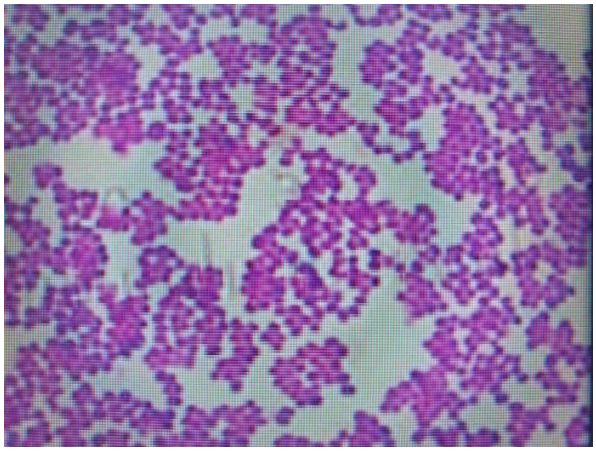


Figure 10. Morphological appearance of diplococci and streptococci / Aspectul morfologic al diplococilor și streptococilor (Rahul et al., 2019)



Figure 11. The morphological aspect of the bacteria *Bacillus sp.* / Aspectul morfologic al bacteriilor *Bacillus* sp. (Liu, 2017)

In the case of the example taken in the study, the microscopic examination of the analyzed samples (female moth of *B. mori*), no pathogens were identified: elementary particles of *Borrellina bombycis* in the form of polyhedral crystals (flacheria), chains of streptococci and staphylococci (infectious anemia), spores of internal parasitosis (nosemosis/pebrina), nor conidia, sterigma, conidiophores of mycoses (muscardina, aspergillosis).

For experimental purposes, during the inactiv season of March 2023, a eggs laying cell with 560 eggs (examined morphoclinically and microscopically) from a randomly selected breed was used in the Laboratory of Silkworm Genetics, Breeding and Pathology from RSSBB, preserved at 2.5ºC (diapause), with the aim of of verifying biological parameters of the laying eggs and hatching of the breed (% hatching and % viability). For the incubation of the eggs, the method of gradually raising the temperature and a relative humidity of 75-85% was used.

Table 1. Percent hatching and viability during the first 3 days of the experiment / Procentul de ecloziune și viabilitate în primele 3 zile de experiment

Hatching *B. mori*	No. hatched larvae / % hatching 1 day	No. hatched larvae / % hatching 2 days	No. hatched larvae / % hatching 3 days	No. unhatched eggs/ % hatching	Hatching percentage / Survival rate after 3 days
560 eggs	106 larve (18,93 %)	408 larve (72,86 %)	21 larve (3,75 %)	25 ouă / 4,46 %	95,54 % (100 %)

From the analysis of table 1, it can be seen that out of the total of 560 eggs that constituted the brood of a cell, the hatching percentage of the eggs in the breed in the experiment was 95.54%, distributed as follows: on the first day, 106 larvae hatched (18.93 %) (Figure 11 and 12), 2nd day 408 larvae (72.86%) and 3rd day 25 larvae (4.46%). During the experimental period (3 days after hatching), a 100% survival rate was observed for the larvae, which then entered sleep period 1 (moult 1) (Figure 13 and 14).

The high larval survival rate indicated that the preservation conditions of the sericicolic genetic material were adequate, and the eggs used in the experiment were able to withstand diapause stress and resume normal development.



Figure 11. Hatched eggs of *B. mori*, dormant season 2023/ Ouă eclozionate de *B. mori*, sezon inactiv 2023 (RSSBB)



Figure 12. *B. mori* larvae hatched on the first day, dormant season 2023 / Larve de *B. mori* eclozionate în prima zi, sezon inactiv 2023 (RSSBB)



Figure 13. *B. mori* larvae hatched for 2 days, inactive season 2023 / Larve de *B. mori* eclozionate de 2 zile, sezon inactiv 2023 (RSSBB)



Figure 14. B. mori larvae, age 4 days, during sleep 1 (moult 1) / Larve de *B. mori*, vârsta 4 zile, în timpul somnului 1 (năpârlirea 1) (RSSBB)

CONCLUSION AND RECOMMENDATION

The maintenance of the sericulture genetic pool involves sanitary-veterinary examinations (morphoclinical, direct microscopy), as well as laboratory diagnostic methods for silkworm diseases (bacterial, internal parasitic, fungal and viral), according to the specific methodology, adapted to the conditions of the Laboratory of Silkworm Genetics, Breeding and Pathology of RSSBB.

Health monitoring through morphoclinical and laboratory examinations on samples of *B. mori* moth, was carried out in order to prevent and control diseases that may later develop in silkworms.

Health monitoring through morphoclinical and laboratory examinations on samples of *B. mori* female mouth was carried out in order to prevent and control diseases that may later develop in silkworms, our study did not identify pathogens in the females taken in the study, and the eggs that deposited in the cell were healthy, resisting the stress of diapause, with the resumption of normal development.

The results of the laboratory examinations were negative for the samples taken in the analysis, a fact that allowed obtaining disease-free breeding material of the best quality.

Ensuring optimal microclimate conditions and prophylactic disinfection are mandatory conditions for ensuring the well-being and health of silkworms, in order to obtain and preserve disease-free silkworm biological material.

TRENDS AND PERSPECTIVES

- One of the main challenges in sericulture is the prevalence of diseases and infections affecting silkworms that can cause significant economic losses to sericulture farmers. To address these challenges, RSSBB's sericulture pathology researchers focus on developing new diagnostic tools and techniques (using DNA sequencing, advanced molecular biology techniques - PCR (Bebitha et al., 2020), identification of genes and markers associated with different diseases, understanding the mechanisms of pathogenesis (identification of key virulence factors and their interactions with silkworm host cells), investigating host-pathogen interactions (investigating the immune system, hemolymph and fat body), developing new strategies for disease management and investigating the role of environmental factors (temperature and humidity) on silkworm health.
- Another trend that could outline the future of pathology in sericulture is the development of new treatments and management strategies to control the spread of diseases (the use of probiotics and other biological agents to improve silkworm health).

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THE EXTENT OF SYSTEMIC IMMUNOLOGICAL CHANGES DEPEND ON HIPERSENSITIVITY LEVEL IN BOVINE TESTED FOR TUBERCULOSIS

AMPLITUDINEA MODIFICĂRILOR IMUNOLOGICE SISTEMICE DEPINDE DE NIVELUL DE HIPERSENSIBILITATE LA BOVINE TESTATE PENTRU TUBERCULOZĂ

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Abstract

Tuberculosis (TB), a multi-host zoonotic disease, still represents a great concern for One Health in many countries worldwide. The research, carried out on bovine from a TB positive farm (n=47, divided in three groups: positive, uncertain and negative by the skin test), where an incomplet stamping out programme was implemented, aimed at monitoring the in vitro changes of systemic immunity in relation to the skin test results. Blood was sampled twice, before and at reading of the skin test, and subjected to carbon particle inclusion and blast transformation tests to evaluate cell mediated responses to mitogens, vegetal extracts and bovine/avian PPD. Circulating immune complexes (CIC) and immune globulins (Ig) were quantified by PEG 4.2% and ZnSO424%o preciptation methods, respectively. The results indicated the lowest values for phagocyatosis in the positive animals, while the blast transformation was most stimulated towards avian and bovine PPD (93.51±24.26% and 91.21±18.04%, respectively). An increased humoral immune activity was also recorded in the positive group, which showed the highest CIC (0.281 ± 0.017ODU) and Ig (0.182 ± 0.004 ODU) values. A positive, statistically relevant (p<0.001) correlation between the phagocytic capacity, CIC, Ig and the cutaneous reactivity was poited out. All tests indicated extensive changes in innate and adaptive immunity, suggesting their use, although not golden standard could be beneficial in surveillance of TB immunity in positive herds.

Key words: tuberculosis, skin test, bovine, phagocytosis, blast transformation, immune complexes, total immune globulins

Rezumat

Tuberculoza (TB), o boală zoonotică, continuă să reprezintă un pericol major în cadrul One Health în numeroase țări din întreaga lume. Cercetarea, efectuată pe bovine dintr-o fermă TB pozitivă (n=47, grup: pozitiv, neconcludent și negativ la testul cutanat), în care s-a implementat un program de eradicare incomplet, a urmărit monitorizarea modificărilor in vitro ale imunității sistemice în relație cu rezultatele testului intradermic. Sângele a fost prelevat de două ori, înainte și la citirea testului cutanat și prelucrat prin teste de înglobare a particulelor de carbon și de transformare blastică (TTB) pentru a evalua răspunsurile mediate celular la mitogeni, extracte vegetale și tuberculină bovină/aviară (PPD). Complexele imune circulante (CIC) și imunoglobulinele (Ig) au fost cuantificate prin metodele de precipitare cu PEG 4,2% și respectiv ZnSO424%o. Cele mai scăzute valori pentru fagocitoză s-au observat la animalele pozitive, în timp ce TTB a fost stimulată către ambele tuberculine (93,51±24,26% și 91,21±18,04%). O activitate imună umorală crescută a fost observată la grupul pozitiv, cu cele mai mari valori CIC (0,281 ± 0,017ODU) și Ig (0,182 ± 0,004 ODU). S-a evidențiat o corelație pozitivă, relevantă statistic (p<0,001) între capacitatea fagocitară, CIC, Ig și reactivitatea cutanată. Toate testele au indicat modificări extinse ale imunității înnăscute și adaptive, sugerând că utilizarea lor ar putea fi benefică în supravegherea imunității la TB în efectivele pozitive.

Cuvinte cheie: tuberculoză, intradermoreacție, bovine, fagocitoză, transformare blastică, complexe imune, imunoglobuline totale

INTRODUCTION

Mycobacterium bovis, one of the much-studied members of the *M. tuberculosis* complex, causes bovine tuberculosis, a zoonotic disease affecting cattle worldwide, induced by contact with or consumption of contaminated animal products (Cousins, 2001). Although relatively drastic measures are first implemented on farms, the disease continues to cause production losses when insufficiently controlled (Ballarini et al., 1971, Dungworth, 1992). WOAH includes tuberculosis on the list of diseases common to several animal species, as a disease considered to be of socio-economic or public health importance in countries and of importance to international trade in animals and animal products (WOAH, 2022). Consequently, most developed countries have engaged in campaigns to eradicate *M. bovis* from the cattle population or at least to try to control the spread of the infection. The success of these eradication and control programs has sometimes questionable.

Mycobacterium bovis, having multiple hosts, is found in both domestic and wild animals, and this range of hosts can complicate attempts to control or eradicate the disease in cattle. Thus, bovids, leporids and felids are susceptible to *M. bovis* but relatively resistant to *M. tuberculosis*. Wild ungulates are generally susceptible to *M. bovis*, with few reports of *M. tuberculosis* being detected in them. The susceptibility of pigs (Thoen, 2006) and dogs to *M. bovis* and *M. tuberculosis* is approximately equal. In the case of human patients, currently *M. bovis* causes a relatively small number of clinically manifest cases among those reported (LoBue, 2006). However, *M. bovis* remains a pathogen of significant importance in wild and domestic animals around the globe, with some areas of the world map being white islands, where the availability of information on the disease is absent or vague (LoBue et al., 2010).

The genus *Mycobacterium* belongs to the category of microorganisms transmitted orally or by respiratory route, but which survive intracellularly, and which in the absence of specific serum prevents the attachment of lysosomes to phagosomes. Even in the presence of specific antibodies, when the enzyme content overflows into the phagosome, *Mycobacterium* shows resistance to this attack. It should be mentioned that the partial destruction of some antigens by macrophages allows the expression of some fragments which are then presented to the cells of the immune system inducing the specific response (Muscoplast et al., 1975, Neill and Pollock, 1994). The presence in the infected body of agents from the genus *Mycobacterium*, induces a particular state of reactivity, not transmissible through the serum of sick animals (Moraru, 1984, Olsen et al., 2010). There are studies that support the pathogenicity of the *M. tuberculosis* complex being a multifactorial phenomenon. However, in cases where the host response cannot destroy the bacillus due to immune-compromising conditions resulting in low CD4+ T-cell counts, such as immune suppression due to chemotherapy, stress or HIV, reactivation can occur, resulting in the release of bacilli and transmission of infection (Brosch et al., 2002).

Phagocytosis represents one of the first intervention protection mechanisms, with particular significance, especially in the prevention of bacterioses (Răducănescu and Bica-Popii, 1986). The role of neutrophils in tuberculosis infection remains less studied compared to that of CD4+ T lymphocytes and macrophages. Thus, changes in the transcriptional profile of *M. tuberculosis* following phagocytosis by neutrophils and how these changes differ from those caused by phagocytosis by macrophages remain unknown (Kondratieva et al., 2022, Queval et al., 2017).

In this function, polymorphonuclear granulocytes are active, together with mononuclear cells of the macrophage type (Maphasa et al., 2021), whether they are in circulation or fixed in tissues (Saragea, 1987). Numerous methods have been designed for the evaluation of phagocytic activity. Testing can be done using whole blood samples against different live or inactivated bacterial suspensions, *in vitro*, or by assessing the activity *in vivo* (Roitt et al., 1989, Thoen, 1994). The *in vitro* carbon particle inclusion test has wide applicability and is based on the principle that under appropriate temperature conditions, the phagocytic activity of heparinized blood continues (Kumar et al., 2019). The suspension of carbon particles added in a certain proportion is incorporated by micro- and macrophages over time. Following

the clarification of the supernatant the phagocytic activity can be evaluated by spectrophotometry (Roitt, 1991, Rothel et al., 1992).

The dominant immune response in tuberculosis is cellular (Roitt, 1991, Thorns and Morris, 1983, Wood et al., 1992). The *in vitro* blast transformation of circulating leukocytes is due to the stimulation of cellular metabolism as a result of the reaction between antigen and lymphocyte membrane structures (Saragea, 1987, Gindeh et al., 2020). The response of circulating T lymphocytes as well as that at the site of infection are considered important (Sable et al., 2005).

Lymphocytes maintained in contact with other types of blood cells or isolated in a culture medium, have the ability to multiply under appropriate conditions, spontaneously or under the influence of various antigens that infect the host organism. Following the coupling of the antigen with the receptors on the surface of specifically sensitized lymphocytes, the cell membrane is activated triggering the transmission of a signal to the nucleus, which determines the initiation of DNA synthesis (Roitt et al., 1989). As a result, there is an increase in the size of the cell, the nucleus becoming larger, with loose chromatin and basophilic cytoplasm (Moraru, 1984). More advanced methods, with a pronounced objectivity in expressing the results, are the labeling of cultures with tritiated thymidine (Muscoplast et al., 1975; Rothel et al., 1992) or the dosing of glucose consumption as a metabolic indicator, by automated reading of the results.

In tuberculosis infection, humoral effectors do not represent elements of the hypersensitivity and resistance mechanism, however, they are endowed with a special specificity for antigens (Muscoplast et al., 1975, Cole et al., 1998), even allowing antigen detection (Răducănescu and Bica- Popii, 1986). Serological diagnosis can be made difficult by the presence of non-specific autoantibodies, which can react with tissue antigens and cross-react with *M. tuberculosis* (Roitt et al., 1989, Saragea, 1987, Gindeh et al., 2020). In the case of the humoral immune response, the role of immunoglobulins is a major one. The activity of gamma-globulins depends on: their molecular structure, their concentration in the serum and the reactive capacity of the body (Moraru, 1984, Stites and Terr, 1991). The application of the zinc sulfate precipitation test for the dosage of total immuneglobulins provides a completion of the immunological picture in affected animals, even without identifying the classes of immunoglobulins involved. The test is based on the fact that at pH 7.4 the electric charge and the colloidal stability of gammaglobulins is lower than that of serum albumins, thus the test using Serb solution can be performed (Roitt, 1991).

Immune complexes are continuously formed in the case of coupling between Ag and Abs and they are efficiently removed by the reticulo-endothelial system. In some cases, the formation of immune complexes leads to hypersensitivity reactions. The formation of immune complexes occurs in different cases: long-term infections, autoimmunopathies, for example in the lungs, following repeated inhalation of antigens of a different nature; their presence indicates either an effective way of removing the antigen, or, especially in the case of their chronic presence in the body, the ineffectiveness to remove antigens (Rothel et al., 1992). The structure of the immune complexes is dependent on the nature of the antigen and the antibody involved in their formation, the antibodies involved usually being of the IgG type. The dosage of circulating immune complexes provides a valuable element in defining the immunological profile (Lyashchenko et al., 2017). Several methods have been developed for the assessment of these complexes. One of these methods, polyethylene glycol - precipitation (Thorns and Morris, 1983, Thoen et al., 2009), was also used in the present experiment.

The research sought to establish some correlations between skin allergic reactivity and the variation in the concentration of total gammaglobulins, circulating immune complexes, phagocytic function, *in vitro* leukocyte blastogenic capacity in cattle from a unit with notified tuberculosis that was subjected to erradication by extraction.

MATERIALS AND METHODS

The research was carried out on 47 animals divided into 3 groups (positive, uncertain, negative) based on the results obtained by the comparative skin test (TCS), performing two blood sampling on the technological flow: before intradermal injection of PPD (17 animals) and when reading the tuberculin test (30 animals). Blood samples have partially served the diagnosis of serological surveillance for enzootic bovine leukosis and brucellosis. Blood was collected on heparin (50IU/ml) for cellular tests and on procoagulant gel to obtain blood serum. The samples taken for cell tests were processed within a maximum of 4 hours after sampling and the sera were stored at -20ºC until processing. All assays were performed in duplicate.

a. Quantification of total immunoglobulins

In the wells of a 96-well plate, 3.3 µl serum were added to 196.7 µl Serb reagent. After shaking with the electric stirrer, the samples were incubated at room temperature for 30 minutes, then the optical densities were read on Sumal PE2 (Karl Zeiss, Jena) at a wavelength of 475 nm, against a blank sample represented by the reagent. The results were expressed in optical density units (ODU). Lipemic and hemolyzed sera were not tested.

b. Quantification of circulating immune complexes (CIC)

An amount of 193.4 µl of 4.2% polyethylene glycol solution in borate buffer was added to 6.6 µl of whole serum, using 96-well plates as a support for the reaction. After an incubation period of 60 minutes at room temperature, the optical densities of the mixtures were read spectrophotometrically on a Sumal PE2 apparatus (Karl Zeiss, Jena) at a wavelength of 450 nm, directly in the working plates, compared to the borate buffer +control research serum, tested under the same conditions. The levels of circulating immune complexes were calculated by performing the difference between the optical densities of the samples precipitated with PEG 4.2% and the corresponding samples treated with borate buffer. The obtained values were expressed in optical density units (ODU).

c. Carbon particle inclusion assay to assess phagocytic activity

To each sample 6 µl of China ink supernatant (centrifuged at 6,000 rpm for 20 minutes) were added. 150 µl of blood and india ink mixture were transferred into 2 ml of saline at 0, 30 and 75 minutes of incubation at 37ºC. Three variants were tested in parallel: control, with bovine tuberculin (12 µl) and avian tuberculin (12 µl). The tubes were centrifuged at the end of the incubation at 1500 rpm for 10 minutes. 200 µl were taken from the supernatant from each test tube and distributed in a 96-well plate. The reading was done with the Sumal PE2 spectrophotometer against saline, at a wavelength of 535 nm and the values were expressed in UDO.

d. The in vitro blast transformation test for the evaluation of mononuclear cell activity

The blood taken on heparin (50 IU/ml) was diluted with RPMI 1640 medium with the addition of 5% fetal calf serum (SFV) at a ratio of 1:5. The pH was adjusted to 7.2 – 7.4 with a 5% sodium bicarbonate solution and antibiotics (1000 I.U. penicillin and 1000 micrograms streptomycin/ml) were added to inhibit bacterial growth. The blood thus processed was distributed in sterile plates with 96 wells, each 200 µl/well. Several experimental variants were carried out: untreated control, treated with PHA (phytohemagglutinin, established mitogen), alcohol, alcoholic extracts of *Calendula officinalis, Arnica montana, Symphytum officinale, Echinacea angustifolia, Echinacea purpureea*, avian tuberculin and bovine tuberculin (each 1.5 µl), to compare the cellular response to different stimulating molecules. Each variant, including the untreated control, was tested in triplicate. The plates were incubated for 72 hours at 37°C in a CO_2 atmosphere. Initially, an average supernatant sample was obtained for each variant, homogenizing 12.5 µl from the three wells in a single tube. 12.5 µl of this mixture were passed on 0.5 ml ortho-toluidine reagent and after homogenization, the test tubes were kept in a water bath at 100°C for 8 minutes. A glucose standard (with a concentration of 100 mg%) and the initial medium were used

identically for each series of samples. Extinction was measured at a wavelength of 610 nm, in 96-well plates with the Sumal PE2 spectrophotometer.

The concentration of glucose in the samples to be tested is based on their extinction and the extinction of the glucose standard according to the formula:

$$C_P = \frac{E_P}{E_S} x100 \quad \text{where:}$$

C_P = sample concentration in mg glucose %
E_P = sample optical density
E_S = standard optical density

Considering the residual concentrations of glucose after the cessation of cell growth and the initial concentration of the medium, blastogenic indices expressed as a percentage can be calculated, both for control and stimulated cultures. The reference value in the case of the control culture is the complete medium and for the stimulated culture it is the untreated control variant.

The following formulas are used:

IS_M = (RPMI glucose – control glucose)/RPMI glucose x100,

IS_{PP} = (control glucose – sample glucose)/control glucose x100

where

IS_M is the spontaneous blastogenic index, and

IS_P is the blastogenic index induced by phytohemagglutinin or other variants.

The blastogenic indices induced by PHA and the various extracts were calculated according to the formula.

e. Statistical calculation

All the results obtained through these tests were processed statistically to obtain the average values, standard deviations and variation using the usual formulas in the Microsoft Excel program. The significance of the differences was assessed using the t-Student test. For the positive and uncertain groups, the correlation coefficients between allergic skin reactivity and phagocytic activity were calculated at all three moments.

RESULTS AND DISCUSSION

a, b. Correlations between skin reactivity and humoral immune response in tuberculin-positive cattle

The results of the evaluation of total Ig and CIC levels were presented in table 1. It can be seen that in both sampling the CIC value is the highest in the positive group (0.235; 0.281 ± 0.017 UDO), it decreases in the uncertain group (0.232 ± 0.067 UDO; 0.250 ± 0.043 UDO) and is reduced in the negative group (0.215 ± 0.079 UDO; 0.213 ± 0.044 UDO). For the positive and uncertain batches at the second sampling (performed when reading the tuberculin test), the values were increased, the difference being obvious in the case of positive animals. On the other hand, in the negative animals, an insignificant decrease is found.

In several diseases, CIC levels provide a measure of the body's reactivity, on the one hand, and the severity of the disease on the other. In microbial diseases, the excess formation of CIC can lead to their deposition in various organs, with the activation of the membrane attack complex (complement) and the consequent destruction of the noble tissue, the release of new antigens and the formation of this time antibodies against the modified self .

Table 1. Total Ig and CIC values in bovine based on their skin test results / Variația Ig totale și CIC la bovinele testate prin TCS, în fucție de categoria de reacție

Category	Sampling	CIC (UDO)	total Ig (UDO)
positive	I	0,235	0,185
	II	0,281 ± 0,017	0,182 ± 0,004
uncertain	I	0,232 ± 0,067	0,145 ± 0,026
	II	0,250 ± 0,043	0,150 ± 0,047
negative	I	0,215 ± 0,079	0,143 ± 0,052
	II	0,213 ± 0,044	0,142 ± 0,041

Due to the chronic character of the disease and the presence of the antigen in the body over a long period of time, it was assumed that CIC can occur in high concentration. Their dosage, correlated with the hypersensitivity tests or supporting it, could serve as a diagnostic test. Comparing the two sampling, in the case of the positive batch, the CIC level emphasizes the presence of an infectious process, compared to the negative batch, in which case, the two sampling reveal similar results. Since the differences between the groups are quite small, it is difficult to conclude that tuberculosis infection significantly influences the formation of CIC, at the time of testing. However, the highest values, consistently obtained in the positive batch, suggests this possibility.

Microbial aggression induces in the body an immune response translated by the secretion of gamma globulins with antibody activity and the appearance of sensitized cells, mediators of cellular immunity. In tuberculosis, the diagnostic role of antibodies is less important, the type IV hypersensitivity reaction being cell mediated.

Table 2. r values for the correlation between the skin test results and total Ig and CIC values / Coeficienţii de corelație între TCS și nivelele Ig totale și CIC

	Positive	Uncertain
$r_{\text{alergic test - CIC}}$	**1, p<0.001**	**0,334 , NS**
$r_{\text{alergic test– total Ig}}$	**1, p<0.001**	**0,133, NS**

NS- non significant

Following the results obtained, in the case of the first sampling, it is observed that the positive animals present an increased concentration of immunoglobulins, compared to the animals in the R and N groups, which are at the same level. The concentration of gammaglobulins in the positive animals from the first sampling is almost identical to the average of the positive lot at the second sampling. Although the differences are not statistically significant, it can be observed that at both samplings, the positive animals showed increased serum immune globulin concentrations compared to the R and N groups, which suggests that the infection with *M.tuberculosis* induces an increase in the level of total gammaglobulins that can be detected by the zinc sulfate precipitation test. This is also confirmed by the obtained correlation coefficient, but due to the very small number of individuals, a firm conclusion in this direction cannot be issued. For the uncertain (R) group, the obtained results indicate a slight increase in gammaglobulins compared to the negative group, and the calculation of r indicates a positive correlation compared to allergic reactivity, without being statistically significant.

c. Correlations between skin reactivity and phagocytic function in tuberculin-reactive cattle

The average values and standard deviations of the optical densities, for all variants, groups and samplings performed are presented in tables 3 and 4.

Table 3. Carbon particle inclusion in the presence ov bovine and avian PPD, 1st sampling / Activitatea de înglobare a particulelor de carbon in vitro în prezența tuberculinei bovine și aviare, recoltarea I

Variant	Time min	Positive	Uncertain	Negative
Control	**0**	0,826	0,673±0,409	0,776±0,281
	30	0,894	0,740±0,376	0,811±0,233
	75	0,949	0,806±0,435	0,906±0,227
Avian T.	**0**	1,019	0,595±0,320	0,640±0,352
	30	1,164	1,050±0,290	0,943±0,264
	75	1,299	1,152±0,121	0,990±0,255
Bovine T.	**0**	1,271	0,529±0,358	0,783±0,252
	30	1,208	0.902±0,431	1,127±0,218
	75	1,133	0,875±0,394	1,170±0,229

Polymorphonuclear phagocytes (granulocytes) and mononuclear phagocytes (macrophages) are the key element of non-specific immune protection, which opposes microbial aggression from the entrance gate by embedding and destruction (microphages) or by embedding and processing with surface expression of specific antigens (Kumar et al., 2019). The overcoming of this barrier by the aggressors leads to the installation of the infection and subsequently to the appearance of the symptoms of the disease.

In the pathogenesis of tuberculosis, due to their particular structure, the bacteria remain confined to macrophages, thus favoring their spread in the body (Maphasa et al., 2021, Queval et al., 2017, Bîlbîie and Pozsgi , 1985).

Analyzing the data obtained at the first sampling for the positive batch, it is observed that the optical densities of the experimental variants increased towards the final reading (75 minutes) except for those treated with bovine tuberculin, but without the difference being statistically significant. It seems that the presence of tuberculin, regardless of its origin, inhibits phagocytic activity. In the case of the uncertain group, the dynamics are similar, with the mention that the values are somewhat lower compared to those of the positive animal.

In the negative batch, the values of the control variants and those treated with avian tuberculin are lower, so that those of the samples treated with bovine tuberculin are higher. Bovine tuberculin appears to have a weak phagocytosis-stimulating effect in this batch towards the end of the reading period.

In the case of the second sampling, in the untreated samples from the positive batch UDO are higher and in the uncertain batch even higher than in the previous one. This fact may also be due to the increase in cell friability towards the end of the reading with the release of embedded carbon. Treatment with tuberculin does not cause an increase in phagocytic activity in the positive group, while in the control group a slight improvement is observed in the case of treatment with bovine tuberculin.

Table 4. Carbon particle inclusion in the presence ov bovine and avian PPD, 2nd sampling / Activitatea de înglobare a particulelor de carbon *in vitro* în prezența tuberculinei bovine și aviare, recoltarea II

Variant	Time min	Positiv	Uncertain	Negative
Control	**0**	1,172±0,161	0,844±0,338	0,444±0,258
	30	1,393±0,174	1,141±0,360	0,763±0,395
	75	1,365±0,104	1,169±0,333	0,698±0,395
Avian PPD	**0**	0,794±0,030	0,511±0,246	0,360±0,138
	30	1,159±0,047	0,978±0,286	0,721±0,315
	75	1,160±0,001	1,132±0,293	0,580±0,233
Bovine PPD	**0**	1,192±0,068	0,695±0,349	0,311±0,161
	30	1,473±0,009	1,137±0,311	0,652±0,259
	75	1,481±0,080	1,061±0,367	0,451±0204

In the negative group, on the other hand, towards the end of the reading period for each experimental variant, a decrease in optical densities is observed, which indicates an improvement in

phagocytic activity. All the values recorded in this batch are lower, proving that the cellular fragility is not so pronounced and that the carbon incorporation activity takes place much faster.

Comparing the values obtained in the two samplings, in the batches and the corresponding variants, it can be mentioned that in the positive batch in the second phase the values are much higher than in the first, perhaps due to the small size of the batches. No significant differences were recorded in the case of the uncertain category, with the exception of the variant treated with bovine tuberculin, where at the second sampling the data obtained indicate a very weak phagocytic activity. This phenomenon can be caused by the different time of infection in the animals from the two batches and the period that has passed since samplinging. The differences recorded between the two samplings for the negative group were statistically significant in the case of treatment with bovine tuberculin.

Table 5 shows the correlation coefficients between allergic reactivity and phagocytic activity in all three variants for the uncertain group. The obtained values indicate a positive correlation for all the variables calculated, without this benefiting from statistical assurance. The exception is the variant with avian tuberculin where the values are very close to 0, the time 75 having a negative value (correlation), the results not being statistically covered.

Table 5. r values for the correlation between the skin test results and phagocytosis in the uncertain group / Coeficienţii de corelaţie între TCS şi fogocitoză la grupul R

Variant	Time min	r
Control	**0**	0,399
	30	0,124
	75	0,040
Avian T.	**0**	0,087
	30	0,074
	75	-0,037
Bovine T.	**0**	0,367
	30	0,128
	75	0,207

Cellular reactivity in tuberculosis is one of the essential aspects of the immune response (Queval et al., 2017). The intervention of macrophages in the first stage of protection with sensitization and the subsequent intervention of lymphocytes, both antibody-secreting and those involved in delayed-type hypersensitivity, is known. These lymphocytes are the ones that determine the nodular or edematous reaction observable at the tuberculin inoculation site, when the allergen is inoculated into a sensitized animal (Kondratieva et al., 2022). The observation led to the idea of applying a test in which the presence of effectors can be monitored and which shows increased sensitivity. The *in vitro* lymphocyte blast transformation test is such a method.

Table 6 shows the average values and standard deviations of the stimulation indices, for each experimental variant, of the three lots in the two samplings. Following the results obtained during the first sampling, it is observed that the spontaneous cellular reactivity in the only positive animal is low compared to the average of the uncertain group (82.10 ± 9.84%) without the difference being statistically ensured. The obtained data correlate with those in the literature, which support the argumentation of the lymphocytic blast response in positive animals or uncertain for skin reactivity. It is also known that in positive animals there are fluctuations in immunity, so it is possible that the small difference between the positive batch and the uncertain, whose value exceeds the first, is given by such a situation.

The reactivity to PHA turns out to be the most intense in positive animals, following in order the average of the uncertain group (86.77 ± 16.80%) and negative (81.69 ± 15.78%). The observed response is the expected one.

The responses to the plant extracts and to avian and bovine tuberculin are variable. Thus, the presence of avian tuberculin stimulates the lymphocytes of the positive animal to the greatest extent, while bovine tuberculin acts with maximum intensity in the samples from the batch under uncertain. The

relatively weak reactivity to bovine tuberculin of the positive animals, less than that of the negative group, may be the indication of a habit in the animals in which the bacteria is a permanent "irritating thorn".

Table 6. Blast transformation indices by experimental variant: a comparison of the two samplings / Indici de stimulare (valori medii şi deviaţii standard): evaluare comparativă la cele două recoltări

	Positive		Uncertain		Negative	
Variant	*Rec. I*	*Rec. II*	*Rec. I*	*Rec. II*	*Rec. I*	*Rec. II*
M	76,63±9,31	81,17±10,35	82,10±9,84	84,02±13,39	74,9±9,53	81,04±18,08
PHA	98,45±10,22	88,28±10,94	86,77±16,8	88,30±15,12	81,69±15,78	87,01±14,48
Alcohol	61,76±8,35	88,49±12,42	85,89±15,35	88,30±14,35	81,0±10,86	85,12±12,88
Cal.	65,26±7,41	81,80±11,83	88,15±16,58	84,82±9,60	76,05±13,01	83,44±14,60
Ar.	56,51±8,99	93,51±14,79	69,85±8,64	77,13±14,08	77,70±13,23	68,13±9,97
Symph.	65,26±11,2	75,52±11,83	68,68±9,22	79,74±13,62	71,43±11,84	76,75±13,69
E. ang.	73,13±9,23	85,14±16,56	91,57±10,94	89,13±11,14	74,66±9,59	87,71±13,12
E. purp.	58,26±7,18	78,66±13,90	75,03±16,17	75,23±15,99	70,9±12,93	76,27±12,66
Avian PPD	92,96±10,11	93,51±24,26	85,59±10,8	89,65±17,12	77,99±10,85	84,64±14,22
Bovine PPD	70,80±8,67	91,21±18,04	92,37±7,45	93,16±11,61	78,38±8,14	85,92±11,51

Among the extracts, in the positive animal the maximum reactivity is induced by *E. purpurea*, known for its stimulating effects, while the rest of the extracts are weaker stimulants, but also strong enough (stimulation over 50%). The same situation is maintained for the group of animals under uncertain, with the mention that all the stimulation indices are increased. There is a difference of almost 20% between the biological effect of *E.angustifolia* (more intense) and *E.purpureea* (weaker). In the group of negative cattle, *Arnica montana* has the maximum effectiveness. In the case of this batch, the values are much more uniform than in the other batches.

In the case of the second sampling, the index of spontaneous blastogenic is somewhat higher than at the first sampling, without the difference being statistically significant. Both in the uncertain group and in the negative one, the spontaneous blastogenic indices were very close to those of the positive group. Even in the case of stimulated cultures, no differences were observed and comparing these values with those of the first sampling, a slight increase is observed. In the positive batch, in the case of the second sampling, the *Arnica* extract was the most effective (93.51 ± 14.79 %). Even alcohol in the dose used proves to be a stimulant. Both tuberculins acted stimulating, with a similar intensity to that of *Arnic*a. The blastogenic index at the second sampling in the presence of bovine tuberculin is much higher compared to the first sampling.

In the uncertain category, the bovine tuberculin had a more pronounced effect than the avian one, even more pronounced than in the positive group. This fact could be attributed to the small number of cases included in the positive group. Negative animals respond less well to in vitro tuberculin stimulation, regardless of type, than those in the positive or uncertain category. *E.angustifolia* was the extract with the maximum efficiency in the negative group, most of the extracts having less pronounced effects than in the positive or uncertain group, with lower stimulation indices.

CONCLUZII

1. Positive reactivity to the skin test was correlated with an increase in the level of circulating gammaglobulins, although they do not represent the essential mediator of the hypersensitivity reaction.

2. The precipitation test with PEG 4.2% allows detection of level differences in CIC concentration and although these are not statistically significant, the maximum values recorded in positive animals suggest an increase in immune complexes following infection, with diagnostic potential.

3. The carbon particle inclusion test proved to be useful in tracking the phagocytic activity in tuberculin-reactive cattle, with the detection of differences between categories. Although compared to the control, the phagocytic activity was less intense, the positive influence of bovine tuberculin was observed, especially in the case of the second sampling.

4. The blast transformation test allowd the investigation of sensitivity to tuberculin *in vitro*, blastogenic indices in its presence, regardless of type, being higher in the positive group, of a slightly reduced amplitude in the uncertain group and close to the value of the spontaneous index in the group negative.

5. The plant extracts used to verify their immunomodulatory effect prove to be effective stimulants with few exceptions (*Arnica* at the first sampling in the positive lot 69.85 ± 8.64% or *Symphytum* 68.68 ± 9.22%, the value below that of the spontaneous index).

6. *Echinacea purpureea* has an inhibitory effect on all samplings and batches, the induced blastogenic indices being lower than those from spontaneous transformation.

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CREATION OF A NEW BREED OF HIGH PERFORMANCE MEAT SHEEP WITH HIGH GROWTH RATE AT FATTENING OF MALE LAMBS, HIGH SLAUGHTER YIELD AND HIGH QUALITY CARCASSES

CREAREA UNEI NOI RASE DE OVINE DE CARNE PERFORMANTĂ CU VITEZĂ MARE DE CREȘTERE LA ÎNGRĂȘARE A MIEILOR MASCULI, RANDAMENT RIDICAT LA SACRIFICARE ȘI CARCASE DE CALITATE SUPERIOARĂ

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Abstract

*The research was carried out on fattened young male sheep of the **Palas Meat Breed**, the **Palas Prolific Breed** as well as on 2 hybrids F1 **Rouge de L'Ouest** x **Palas Meat Breed** and F1**Texel** x **Palas Meat Breed**. Weight gain to live weight of 40.0 kg/head, slaughter yield (R1 and R2), feed consumption efficiency (Ef), carcass tissue structure and carcass quality grading according to the EUROP grid were monitored. All data were statistically processed and interpreted.*

Key words: sheep, meat, fattening, slaughter, carcass

Rezumat

*Cercetările s-au efectuat pe tineret ovin mascul îngrășat din **Rasa de Carne Palas, Rasa Prolifică Palas** precum și pe 2 hibrizi F_1 **Rouge de L'Ouest** x **Rasa de Carne Palas** și F_1 **Texel** x **Rasa de Carne Palas**. S-au urmărit sporul de creștere în greutate până la greutatea vie de 40,0 kg/cap, randamentul la sacrificare (R_1 și R_2), eficiența consumului de furaje (E_f), structura tisulară a carcaselor și încadrarea în clase de calitate a acestora după grila EUROP. Toate datele au fost prelucrate și interpretate statistic.*

Cuvinte cheie: oi, carne, îngrășare, sacrificare, carcase

INTRODUCTION

The transformations that have taken place over the last decade in the field of sheep breeding and the alignment with existing practices at European level have led to a change of vision regarding local breeds and the application of crossbreeding with specialised breeds, and Romania can make an important contribution in this field due to the large number of sheep it has.

In the European Union there is a multitude of high performance meat sheep breeds with high growth rate at fattening, high yield at slaughter and high quality carcasses. Almost every country pays attention to the production of lambs that are marketed for meat on the domestic or foreign market, using the system of crossbreeding between two or more specialised breeds (Dickerson G.E). Slaughter weight performance varies with genotype, sex, age and fattening conditions (Martynuk E s.a). By combining and crossbreeding two breeds, higher slaughter lambs can be obtained due to the manifestation of the phenomenon of heterozys (Zupp W).

In Romania there is only one high performance meat breed, created at RDISGB Palas Constanta, comparable to the best meat breeds in the world. Research on the use of the **Rouge de L'Ouest** and **Texel**

breeds in crosses with the **Prolific Palas breed** has been reported by Vartic A. and collaborators, who reported average daily fattening gains of 302.47 g for F1 **Rouge de L'Ouest** x **Prolific Palas** hybrids and 304.78 g for F1 **Texel** x **Prolific Palas** hybrids compared to 235.47 g for **Prolific Palas** young. The two hybrid variants were also superior to the maternal breed in terms of thigh muscle index (TMI) which was 33.92% higher in the **Rouge de L'Ouest** hybrids and 28.43% higher in the **Texel** hybrids, the differences being highly significant compared to the maternal breed.

MATERIAL AND METHODS

The research was carried out on batches of fattened male lambs of the **Palas Meat Breed**, **Palas Prolific Breed**, F_1 **Rouge de L'Ouest** x **Palas Meat Breed** hybrids and F_1 **Texel** x **Palas Meat Breed** hybrids.

They were fattened to an average weight of 40 kg/head, followed by weighing, growth rate, specific feed consumption and feed efficiency expressed in grams of growth gain for 1 kg dry matter consumption.

Three animals were slaughtered from each variant and the slaughter yield, carcass quality indices and carcass quality classes were calculated according to the EUROP grid.

All data were processed and statistically interpreted (Snedecor G.W).

RESULTS AND DISCUSSION

Table no. 1 shows the values of daily weight gain

Table 1. The values of daily weight gain (g) depending of genotype / Valoarea sporului mediu zilnic (g) în funcție de genotip

Genotype	Daily weight gain (g)	± Differences between genotypes	
		g	%
Palas Prolific Breed	299.13 ± 15.6616	- 38.54	- 11.41
Palas Meat Breed	337.67 ± 4.4700	p < 0.001	
F_1 **Rouge de L'Ouest** x **Palas Meat Breed**	331.13 ± 12.7185	Between **Palas Meat Breed** and F_1 **Rouge de L'Ouest** x **Palas Meat Breed**	
		+ 6.54	+ 1.98
		p > 0.05	
F_1 **Texel** x **Palas Meat Breed**	335.20 ± 12.900	Between **Palas Meat Breed** and F_1 **Texel** x **Palas Meat Breed**	
		+ 2.47	+ 0.074
		p > 0.05	

It results that the highest yield was obtained by **Palas Meat Breed** lambs followed by F_1 **Texel** x **Palas Meat Breed** (fig.1) , F_1 **Rouge de L'Ouest** x **Palas Meat Breed** (fig. 2) and **Palas Prolific Breed**.

There were very significant differences ($p < 0.001$) between the **Palas Meat Breed** and the **Palas Prolific Breed**. Between the **Palas Meat Breed** and the 2 hybrids the differences were statistically insignificant ($p > 0.05$).



Figure 1. F_1 Texel x Palas Meat Breed / F_1 Texel x Rasa de carne Palas



Figure 2. F_1 Rouge de L'Ouest x Palas Meat Breed / F_1 Rouge de L'Ouest x Rasa de carne Palas

Feed conversion efficiency (E_f - g/kg S.U.) is shown in Table no. 2.

Table 2. Feed conversion efficiency / Eficiența de conversie a nutrețurilor

Genotype	Feed conversion efficiency (g/kg S.U.)	± Differences between genotypes	
		g	%
Palas Prolific Breed	260	+ 39	+ 13
Palas Meat Breed	299	p < 0.05	
F_1 Rouge de L'Ouest x Palas Meat Breed	287	Between Palas Meat Breed and F_1 Rouge de L'Ouest x Palas Meat Breed	
		+ 12	+ 4
		p > 0.05	
F_1 Texel x Palas Meat Breed	291	Between Palas Meat Breed and F_1 Texel x Rasa de Carne Palas	
		+ 8	+ 2.7
		p > 0.05	

There were significant differences ($p < 0.05$) only between the **Meat Breed** and the **Prolific Palas Breed**.

Table no.3 shows the slaughter yield.

Table 3. Slaughter yield depending on genotype / Randamentul la sacrificare în funcție de genotipul ovinelor

Genotype	R_1 (%)	R_2 (%)	± Differences between genotypes		
			R_1	R_2	Significance
Palas Prolific Breed	44.84±0.0084	54.36±0.0068	Palas Meat Breed and Palas Prolific Breed		
Palas Meat Breed	50.97±0.0115	58.46±0.0070	+ 6.33	+ 4.1	p < 0.05
F_1 Rouge de L'Ouest x Palas Meat Breed	49.46±0.0085	57.54±0.0022	Palas Meat Breed and F_1 Rouge		
			+ 1.51	+ 0.92	p > 0.05
F_1 Texel x Palas Meat Breed	46.13±0.0090	56.03±0.0121	Palas Meat Breed and F_1 Texel		
			+ 4.84	+ 2.43	p > 0.05

$$R_1 = \frac{\text{Weight of cooled carcass}}{\text{Live weight}} \text{ x } 100$$

$$R_2 = \frac{\text{Weight of cooled carcass}}{\text{Live empty weig ht}^{*}} \text{ x } 100$$

* Live weight from which the digestive tract content has been deducted

It can be seen that the **Palas meat breed** lambs had the highest yield, followed by the 2 hybrids. There were significant differences only between the **Palas Meat Breed** and the **Palas Prolific Breed**.

Table no. 4 shows the tissue structure of the carcasses.

Table 4. Tissue structure of the carcasses / Structura tisulară a carcaselor

Genotype	Muscles		Bones		Fat		Meat		± Differences between genotypes	
	g	%	g	%	g	%	g	%		
0	1	2	3	4	5	6	7	8	9	
Palas Prolific Breed	4663.33	55.2	2208	26.1	1586.47	18.8	6280	73.9	**Palas Meat Breed** and **Palas Prolific Breed**	
									g	%
Palas Meat Breed	5998.33	60.73	2075	21.00	1952.50	19.77	7950.83	80.50	+ 1701	+ 27.22 p<0.001
F_1 **Rouge de L'Ouest** x **Palas Meat Breed**	5835.00	62.19	2283.33	24.33	1265.00	13.48	7100	75.67	**Palas Meat Breed** and F_1 **Rouge**	
									+ 850.83	+ 11.98 p<0.01
F_1 **Texel** x **Palas Meat Breed**	5821.67	61.70	2203.33	23.35	1410.00	14.94	7231.67	76.65	**Palas Meat Breed** and F_1 **Texel**	
									+ 719.16	+ 11.98 p<0.01

It results that the breed with the highest amount of meat in the carcass was the **Palas Meat Breed** followed by the 2 hybrids.

There were very significant differences (p<0.001) between the **Palas Meat Breed** and the **Palas Prolific Breed**, respective distinct significant differences (p<0.01) between the **Palas Meat Breed** and the 2 hybrids.

Table 5 shows the quality grading of the carcasses.

Table 5. Carcass quality grading according to the EUROP grid / Calitatea carcaselor conform grilei EUROP

Genotype	Quality grid (%)				
	E_{2-3}	U_{2-3}	R_{2-3}	O_{2-3}	P_{2-3}
Palas Meat Breed	33.3	66.7	-	-	-
Palas Prolific Breed	-	-	100.00	-	-
F_1 Rouge de L'Ouest x Palas Meat Breed	-	100.0	-	-	-
F_1 Texel x Palas Meat Breed	-	100.00	-	-	-

It results that in the **Palas Meat Breed** one third of the carcasses were classified in class E_{2-3} (excellent, lean and medium-fatty carcasses, figure 3) and two thirds in class U_{2-3} (very good, lean and medium-fatty carcasses. The carcasses of the two hybrids fell entirely into class U_{2-3} (very good, poor and medium fat carcasses figure 5 – 6) and carcasses of the **Palas Prolific Breed** are entirely classified into class R_{2-3} (figure 4).



Figure 3. Palas Meat Breed Carcass E $_{2-3}$ class / Carcasă de Rasa de carne Palas clasa E 2-3



Figure 4. Palas Prolific Breed Carcass R $_{2-3}$ class / Carcasă de Rasa prolifică de Palas clasa R 2-3



Figure 5. F_1 Rouge de l Quest x Palas Meat Breed Carcass U $_{2-3}$ class / Carcasă de F_1 Rouge de l Quest x Rasa de carne Palas Clasa U 2-3



Figure 6. F_1 Texel x Palas Meat Breed Carcass U $_{2-3}$ class / Carcasă de F_1 Texel x Rasa de carne Palas Clasa U 2-3

Table no. 6 shows the thigh muscularity index (TMI) according to Purchas formulae .

$$TMI = \frac{\text{Weight of thigh muscles}}{\text{Length of femur}} \text{ x } 100$$

Table 6. Thigh Muscularity Index (TMI) / Indicele de muscularitate a coapsei

Genotype	**T.M.I**	**± Differences between genotypes**	
		Value	**%**
F_1 **Rouge de L'Ouest** x **Palas Meat Breed**	0.581 ± 0.0177	+ 0.005	+ 0. 9
F_1 **Texel** x **Palas Meat Breed**	0.567 ± 0.0084		
		p > 0.05	
Palas Meat Breed	0.592 ± 0.0078	**Palas Meat Breed** and F_1 **Rouge**	
		+ 0.011	+ 1.9
		p > 0.05	
		Palas Meat Breed and F_1 **Texel**	
		+ 0.016	+ 2.7
		p > 0.05	

From the table it can be seen that there were no significant differences (p > 0.05) between the values of the T.M.I index between the 3 genotypes.

CONCLUSION

The results obtained are encouraging in terms of creating the precursors of a new breed of high performance meat sheep comparable to the best breeds in the world.

It is estimated that the new meat breed could be homologated around 2036.

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